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HANDBOOK

OF

NUCLEAR MEDICINE PRACTICE

IN

DEVELOPING COUNTRIES

INTERNATIONAL ATOMIC ENERGY AGENCY

FOREWORD

This handbook entitled "Handbook of Nuclear Medicine Practice in Developing Countries" describes in general various aspects of Nuclear Medicine particularly for beginners. It is a multiauthor venture; most of the contributors are from developing countries where Nuclear Medicine has been part of medical practice for many years. This multi-authorship necessarily brings in a certain heterogeneity in the style and make up of chapters. The contents of the chapter reflect the personal experiences and bias of the author, which, may at time, be at variance with experiences elsewhere. No serious effort has been made to bring a uniform pattern in these chapters or to insist for a consensus of views in a given matter of practice, lest these stifle the originality in the chapters.

This handbook is by no means an exhaustive document in Nuclear Medicine practice; at best it may be considered as a companion to beginners in the speciality from developing countries, especially where detailed text books are not available. The present publication is an experiment, the results of which will be used for subsequent improvement of the handbook.

The authors and the sources of various chapters are gratefully acknowledged for their generous help and contribution to the birth of this document. Acknowledgement is also due to the staff of the Nuclear Medicine Section for their able assistance in the preparation of this handbook and particularly also to Ms. S. Clements for her unstinting help in bringing the same to its present form.

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HANDBOOK OF NUCLEAR MEDICAL PRACTICES IN THE DEVELOPING COUNTRIES

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Chapter 1

INTRODUCTION

This "Handbook of Nuclear Medicine Practices in the Developing Countries" is meant primarily for those, who intend to install and practice nuclear medicine in a developing country. By and large, the conventional Textbooks of nuclear medicine do not cater to the special problems and needs of these countries. The Handbook is not trying to replace these Textbooks, but supplement them with special information and guidance, necessary for making nuclear medicine cost-effective and useful in a hospital of a developing country. It is written mostly by those, who have made success in their careers in nuclear medicine, in one of these countries. One way to describe this Handbook will be that it represents the ways, in which, nuclear medicine is practised in the developing countries, described by those, who have a long and authentic experience of practising nuclear medicine in a developing country.

Nuclear medicine in the developing countries is patchy and non-uniform. Some areas are heavily ploughed, others are still as arid deserts. There are choices and compromises imposed by economic realities. Uneven distribution of talents and requisite facilities, in the hospitals of the developing countries, dictate the pattern of nuclear medicine practice in these countries.

But, then, why this Handbook? Will it help in any way to improve the existing situation?

One of the most satisfying part of the IAEA's efforts towards promotion of nuclear medicine in the developing countries is the "training activities", which comprises of training courses, provision of experts for *in situ* training and Fellowships for advanced training abroad. It is difficult to organize training in any of the above modes, which can meet fully the individual disparate needs of students from a variety of countries.

Notwithstanding all kinds of selection safeguards, it is not possible to select a homogenous group of trainees from different developing countries. The term "developing" is a dynamic term. Developing countries are at various stages of development. A group of trainees from these countries is bound to be heterogenous. It is difficult for a lecturer to pitch a lecture at a level appropriate for all the students. Some lectures are beyond the "capability to comprehend" of students, whose acquaintance with nuclear medicine, is only marginal. On the other hand, some students may find the lectures very elementary. A book is something different. You take what you need. You skip what you know. You omit what is not immediately relevant to your needs. This Handbook is designed to meet that kind of need, as and when a trainee from a developing country wants specific information, when he actually comes around to set up nuclear medicine in his (or her) own institution, which may be soon after a training course or may be several years later, when he is successful in assembling all the wherewithal for setting up nuclear medicine in his own hospital.

If one asks a member of the audience, after seeing a grand opera or a dare devil trapeze act, he might say it was divine and a privilege to hear or watch, but that is not something that he can do himself or expect to see in his own institution. A similar feeling is common in the minds of students at the end of a training course. "All the acrobatics were nice, but I can not set up any of those techniques. Nice to know about them, but not useful for me because

where am I going to get all those fancy instruments and costly radiopharmaceuticals, for my practice of nuclear medicine?!"

There is another worrying trend in the present day nuclear medicine, as practised in the advanced countries. Impact of other imaging modalities on the nuclear medicine imaging has been tremendous in recent years. Adversities have turned many of the experts to introspection to find out what are the limitations of nuclear medicine and what are its strong points. It is recognized, rightfully, that our forte is dynamic functional studies. However, the dynamic studies need sophisticated instruments and not-so-easy to get radiopharmaceuticals. A lecturer from an advanced country extols the virtues of SPECT and PET and immunoscintigraphy. A student from a developing country, who has just managed to acquire a simple gamma camera, may find that the gulf between what he can do and what he should be doing is wide and unbridgeable. What is the use of taking a first step on a road that is continuously stretching in front of him and the glorious dawn on the horizon is receding further and further away?

Most of the Chapters in this Handbook are written by experts practising, at present, in the developing countries. They all can be considered to have achieved success in their careers in nuclear medicine, in their respective countries. Their authentic experience and the prescription of the routes and routines, they have followed in their work should inspire confidence in the students from the developing countries that nuclear medicine has a significant role to play in the hospitals of these countries. In fact, nuclear medicine has more scope in the developing countries, as it is relatively inexpensive, as compared to some of the new imaging techniques.

We have tried to make the Handbook as practical as possible. Basic physics of nuclear medicine have not been dealt with in this book. Present day curriculum in schools and colleges would have provided the medical doctor with enough knowledge in nuclear physics to grasp what is necessary in the field of nuclear medicine. There are also many text books available, both old and new and not very expensive, which can be consulted by the more inquisitive. The concepts, facts and figures of basic physics in nuclear medicine have changed very little. Some of the newer areas in nuclear medicine, such as immunoscintigraphy, SPECT and PET, are described briefly as these and many others can be found in regular textbooks of nuclear medicine by those who wish to learn more about them.

It may not be assumed that the Handbook is meant to replace a training course. A good lecturer can make a world of difference in invoking the interest of the trainees in the subject being taught. A lecture has constraints of time and can not cover all aspects of a specific topic. A lecture has to be short enough to excite the interest and curiosity but long enough to cover essential aspects of the subject.

The Chapters in the Handbook are not expected to be as transient as handouts distributed at the time of the Course. The handouts are usually cryptic and may describe only those aspects of the subject that the lecturer plans to cover in his lectures.

INTRODUCTION

This Handbook covers some aspects of nuclear medicine extensively. For instance, the radioimmunoassays and all its ramifications are described at great length. This reflects the Agency's involvement in this field and Agency's input in training in this area. Agency has strongly advocated the use of bulk reagents for radioimmunoassays to reduce the cost of these assays in the developing countries, while at the same time, increasing the understanding of the quality control of the assays. Radioimmunoassays are within the realm of possibilities for the developing countries, with only a modest investment in money and men. Even if these assays are not used for a diagnostic service, they are one of the simplest and most inexpensive tools of clinical research. This kind of material is not normally found in conventional teaching and textbooks.

The IAEA realizes that training courses alone can not serve as a sole effective method for training. The advent of personal computers (PC) is revolutionizing training methods. A large data base that can be provided by a PC is unique. The interactive PC based tutorials can help a student to learn at his own pace in his own environment. The gradual integration of communication media like personal computers, books and videos are opening up new vistas in teaching. This Handbook, we hope, is one of the first step in developing new means of teaching and learning. This will be evident by the fact that parts of the Handbook can be obtained on computer diskettes, on request, but without any cost.

A task like this Handbook can not succeed in a large international organization like IAEA, unless there are perceptive promoters of such a task. It was the Director General, who endorsed the suggestion for a Handbook, made initially in a Travel Report, proving once again that there is someone up there who cares. Mr. Wojcik, Prof. Zifferero, Dr. Noramly, Dr. Ridwan, RCA co-ordinators, the staff of the Publishing Section and many others have made it possible to turn this endorsement into a practical proposition.

This Handbook was conceived and nurtured by Dr. R.D. Ganatra, the erstwhile Head of the Nuclear Medicine Section. Quintessence of his long experience of nearly three decades in nuclear medicine in the developing countries will be found in various Chapters that he has written or edited. In spite of his ill-health, he has put in herculean efforts to complete this task in a record time. This offering comes out of his suffering. He has been assisted in his task by his successor Dr. A. Cuaron, who has an equally wide experience of nuclear medicine, and by Dr. G. Nair from the Nuclear Medicine Section, as well as by me. Of course, a large part of the thanks are due to authors, who have contributed various Chapters at short notice, and has revised them several times. This shows their singular dedication towards amelioration of the practice of nuclear medicine in the developing countries. All these orbits around the nucleus of efforts of Dr. Ganatra has made a complete global atom.

I have great pleasure in presenting this "Handbook of Nuclear medicine practices in the developing countries" as first of the IAEA's efforts, in a new direction, for promotion of nuclear medicine in these countries.

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Chapter 2

SCOPE OF NUCLEAR MEDICINE IN THE DEVELOPING COUNTRIES

R.D. Ganatra

Many of the scientists in the developing countries recognize the value of medical applications of nuclear energy and try to introduce them in the premier hospitals of their countries. One has to ponder over few of the following questions before implementing such a plan.

Is it worthwhile to develop nuclear medicine in a country, where medical care is in its infancy, and public health problems paramount? Does nuclear medicine contribute to the care of the patients or is it mainly a luxury? Does it involve spending large sums of money for a small return? Does it play any role in resolving problems of population, pestilence or malnutrition?

There is a certain amount of glamour associated with nuclear medicine procedures, but are these procedures such that the information obtained through their use is not available by any other conventional means of investigations? What kind of a hospital in a developing country should have nuclear medicine? Should the size of the nuclear medicine unit depend on the size of the hospital? What are the basic minimum requirements for setting up a nuclear medicine department? Should there be less sophisticated equipment for the have-nots? What is the most economical radiopharmaceutical for the developing countries? Should they do their radioimmunoassays (RIA) with kits or with bulk reagents? How many radioimmunoassays they should carry out to make a laboratory economically viable?

Let us examine few of these questions to find out the scope and potential of nuclear medicine in developing countries?

Relevance of nuclear medicine to medical care.

Nuclear medicine is primarily diagnostic; its reputation is mainly because of its all-pervasive and non-invasive nature of its investigations. There is hardly any branch of medicine, in which there is not even one nuclear medicine application. A set of basic instruments can serve the needs of diverse disciplines, unlike something like an E.E.G., which would help only the neurologists and no one else.

This very wide spectrum of its applications should make us realize that these techniques, although sensitive would not be very specific. They may not establish a diagnosis conclusively but may exclude several diagnostic possibilities thereby eliminating the need for other costly investigations. For example, the presence of a space occupying lesion in the brain does not establish a histological diagnosis. A large number of other investigations will be necessary to arrive at an unequivocal diagnosis. On the other hand, a negative brain scan would remove the need for further neurological investigations, which may be expensive and occasionally even traumatic. Let us examine from this perspective some of the present nuclear medicine investigations:

1. <u>Investigations which establish a specific diagnosis:</u>

e.g. thyroid function investigations; RBC survival; some of the radioimmunoassays; lung scintigraphy etc.

2. <u>Investigations which exclude the possibility of a diagnosis of a certain kind:</u>

e.g. scintigraphy of the brain; renogram etc.

3. <u>Investigations which are helpful in the follow up of the patients:</u>

e.g. thyroid function tests; RIAs of certain hormones; imaging; Nuclear cardiology etc. Once a positive diagnosis is made and definitive therapy given, non-invasive character of the nuclear medicine procedures make them eminently suitable for the follow up of the patients.

Each laboratory test carries the responsibility that the diagnosis followed by appropriate therapy will alter the natural history of the disease. In these days of highly specific and potent therapeutic measures, precise diagnosis is essential before starting any treatment. Nuclear medicine screening tests helps in excluding many diagnostic possibilities and spares the patient unnecessary and misguided therapy. The intangible benefits of this role of the nuclear medicine are in terms of savings in time off work, lost earnings, medicinal expenses etc.

To justify nuclear medicine in a developing country, we have to see nuclear medicine in a new role. It is not for putting diagnostic labels, not for differential diagnosis as we have been conditioned to think so far. In a developing country it should be for differential management. How does it alter the management decision in respect to a particular patient? If management outcomes are restricted, there is no need for an investigation which does not help in any way the management of the patients. If there is no bypass surgery, what use is the thallium perfusion? Although primarily a diagnostic discipline, for its justification and survival in a developing country, nuclear medicine should lead to a sensible differential management.

Levels of medical care in developing countries.

The following pattern is prescribed by the WHO.

<u>A1: Teaching Hospital.</u>

(a) undergraduate and postgraduate training;

(b) speciality services e.g. cardiology, neurology etc.;

(c) competent radiology and other laboratory services;

(d) research;

(e) serves 5 to 10 million population.

A: Large urban general hospital.

(a) speciality services;

(b) competent Radiology and other Laboratory services;

(c) serves 2 to 5 million population.

B: Regional or provincial hospital.

(a) competent general medical care;

(b) good Radiology and other Laboratory backup;

(c) liaison with a large general hospital;

(d) serves ½ to 2 million population.

C: District or Rural Hospital.

(a) good out-patients care;

(b) competent in-patient care for the acutely ill patients;

(c) moderate Radiology and other Laboratory backup;

(d) arrangement for referral of patients for treatment or investigations to a larger hospital;

(e) serves 50 000 to 500 000.

D: Primary Health Centre.

(a) only out-patient care;

(b) arrangement for referral of patients for treatment or investigations to a larger hospital;

(c) elementary clinical pathology services;

(d) serves 10 000 to 100 000.

CHAPTER 2

Nuclear medicine facilities at different Levels of health care.

For setting up an effective and reliable nuclear medicine service in a hospital, following are the basic requirements:

(a) stable uninterrupted power supply;

(b) dust free and air-conditioned site;

(c) provision for initial capital cost of equipment;

(d) provision for recurring cost for maintenance of the equipment and for purchase of radiopharmaceuticals;

(e) reliable and frequent international and internal airline connections for delivery of radiopharmaceuticals;

(f) local maintenance and repair facilities;

(g) speciality services in the hospitals for rational referral of patients and subsequent management after diagnosis;

(h) reliable Radiology and clinical pathology services;

(i) personnel trained in nuclear medicine, medical physics, radiopharmacy, radioimmunoassays etc.

It should be recognized that nuclear medicine is a high technology medicine and it is not possible to set it up in all the hospitals. Certain infrastructure is required for successful institution of nuclear medicine in a developing country. It should be in a hospital where specialities like radiology and clinical pathology are well established, where at least some of the clinical specialities are flourishing; these specialities should have other ancillary investigational facilities; where it is possible to get well defined clinical material referred to nuclear medicine so that you are trying to answer specific rational questions and not trying to get results, which do not lead anywhere, as far as the management of the patient is concerned.

It is unquestionable that both A1 and A qualify for nuclear medicine installations. Category D obviously can not have it. Same is probably true for C, although hospitals of this type may be able to collect blood samples for radioimmunoassays and send them to larger hospitals. Category B is doubtful. If it satisfied most of the above criteria, it should have a modest nuclear medicine set-up, where not only in vitro studies are done but also few of the in vivo investigations can also be carried out.

Requirements for the growth and development of nuclear medicine in developing countries.

What should a developing country do to promote nuclear medicine? Practice of nuclear medicine requires sophisticated electronic instruments and a variety of radiopharmaceuticals. Ideal situation would be when both are obtainable from local sources. It is not an easy task for developing countries to produce these electronic marvels locally. It anticipates a widespread electronics industry in a country so that various components which go in the big machines are also made locally. One, who has worked in a developing country would realize how exasperating a task it is to maintain, service and repair imported instruments. They break down often in the tropics, are difficult to service due to lack of spare parts and their down-time is unusually long. Many of the modern instruments have lots of "frills and laces" and as a policy, it is prudent to purchase something which is "bare to bones" and simple to use but still capable of providing the essential range of applications.

There are two ways in which a new health care technology can succeed in a developing country: either it is better than the existing technology in terms of sensitivity and specificity or it provides the hospital a status symbol. The latter type of "ornamental technology", very few developing countries can afford, but it is very difficult to convince the public, who want nothing less than the best, that new is not necessarily the best. Many imaging devices are installed because they give a good image of the hospital and not because they offer better diagnostic images of the patient.The questions that need to be asked before buying new equipment are: Does the new technology lead to better care of the patients? Is it more cost effective than the existing technology? Is the new machine likely to be viable in a hospital of the developing countries?

Problems with radiopharmaceuticals are similar. Unless there is a broad based Nuclear Energy program in a country, it is not economical to set up a reactor, just for production of the radiopharmaceuticals. It is necessary for most of the developing countries to purchase the radiopharmaceuticals from abroad. Coordinating their supply, commensurate with varying needs of the department is a complicated task, invariably resulting in unutilized material and economic waste. The present trend of using short half life radiopharmaceuticals makes it still more difficult.

One thing which can be profitably done by all developing countries is to avoid import of kits for radiopharmaceuticals and radioimmunoassays. It is possible to buy bulk reagents and prepare kits on a national or a regional basis. This brings down the cost for some of the kits to one-tenth of the original cost. The technique of preparing kits from the bulk reagents is not difficult. Quite a few of the Agency's present programs are geared towards cultivating this kind of regional self-sufficiency.

For practice of nuclear medicine in a hospital of a developing country, it is necessary to create several amenities at a national level and also to have good international links for supply of radiopharmaceuticals and for repair of instruments. This is possible only if institution has a generous allotment of foreign exchange. From the viewpoint of the planners and

economists, the whole national effort and all the international alignments are justified only if the nuclear medicine facilities are available in as many hospitals of the country as possible. As physicians, we may accept the relevance of nuclear medicine in total medical care of a sick person but for setting up nuclear medicine in the hospitals of the country, there needs to be a decision about the national priorities and for more than one reason such a decision should not be based on political and economical considerations.

Nuclear medicine at different levels of health care.

How to set up nuclear medicine in developing countries, what equipment to order, how much space should be allotted in a hospital and all other similar questions are dealt with separately in different chapters.

It will be realized from the foregoing arguments that different levels of nuclear medicine facilities are envisaged for different kinds of hospitals. For A1, it is a comprehensive range of in vitro and in vivo investigations; for A and B, the range will be somewhat restricted; for C, it will be mostly in vitro studies. What each hospital should or can do depends largely on the interests of the attending physicians and health problems of the community. For any hospital, it may be wise to start with a limited range of services and then to expand it gradually *pari passu* with increase in awareness of nuclear medicine in medical staff. Without being too specific, it can be said that there will be three classes of nuclear medicine laboratories: the large one, the modest one and the small one.

Radioisotope therapy is possible only in a large A1 hospital. Thyroid cancer therapy involves radiation protection problems of a speciality nature, and it is in the best interests of the community that this kind of treatment is handled at a central large hospital with appropriate facilities for radiation protection and radioactive waste disposal.

Conventionally, thyrotoxic patients are not kept as in-patients for their radioiodine therapy because their individual doses are less than 15 mCi. In advanced countries, it is taken for granted that every patient would have adequate toilet facilities at home. This is not the case in developing countries. Many poor patients have no toilets and urine is passed in a washing area in the kitchen where utensils are cleaned and children bathed. It may be desirable to keep this category of patients in the hospital for at least three days in developing countries for radioiodine therapy.

Scintigraphy.

There used to be a lot of argument as to which instrument is better for which kind of hospitals for carrying out imaging of various organs. Still few years ago, the rectilinear scanner was considered as a more suitable choice as an imaging device than a scintillation camera for the developing countries. It was simple, rugged and reliable. However, market pressures of demand and supply have left only the scintillation camera as a choice. A decent dual head scanner is no longer in the market. If a hospital decides to have imaging facility, the question they have to decide is whether they should have it along with the dedicated

computer and whether the whole system should be in a rotating mode or in a stationary phase.

The main forte of nuclear medicine is dynamic functional studies and for that, purchase of a computer is unavoidable. The rotating mode of SPECT promises advantages with the newer radiopharmaceuticals, especially in investigations of brain and heart. The cost difference between the rotating and the non-rotating gamma camera is not that great. The SPECT option requires stringent quality control, and if the department is capable of coping with these problems, there is nothing wrong in trying to go for the best available.

Non-imaging nuclear medicine instruments.

Although "imaging" is the main preoccupation of any modern nuclear medicine department, there are quite a few other things which can be done with simpler instruments and which are of greater relevance to the health problems of developing countries. A scintillation probe along with a spectrometer is always useful for various sundry jobs ranging from thyroid uptake to nuclear cardiology. A dual probe unit (or even more than two probes) along with the spectrometers and recorders is handy for doing renograms, which take away an unduly long time of the gamma camera.

Any nuclear medicine unit in a developing country will be doing a large number of radioimmunoassays. The unit may start with a manual well scintillation counter, but will have to switch over to an automatic counter sooner or later. A multicrystal non-mechanical type of a counter has a good record of trouble-free service. A personal computer attached to such a counter is useful for data processing for quality control of assays.

A dose calibrator is absolutely essential in a nuclear medicine department. Several types are available in the market. One of the robust types with less of the fancy features is an appropriate choice.

A variety of radiation protection monitor probes are inevitable in nuclear medicine work.

Ultra-sound imaging device is a non-nuclear instrument but having it in a nuclear medicine department is of great help. Most of the nuclear medicine units have only one gamma camera which is continuously busy. The ultrasound spares the camera for those essential things which can be done by nuclear techniques only. Both these imaging modes supplement each other in terms of diagnostic information. When radiopharmaceuticals are not received or when the gamma camera is out of order the imaging can still be continued for few of the organs with the help of ultrasound scanner.

Radiopharmaceuticals.

$^{99}Tc^{m}$ generator is a work-horse in a modern nuclear medicine department. Source of supply should be reliable and capable of delivering the generator unfailingly. The stable kits are quite expensive and as many of them as possible should be made locally. It will need

initial setting up of the radiopharmacy facilities but in the long run, the local production would reduce the cost considerably.

Radioiodine ^{131}I is needed if therapy of the thyroid diseases is undertaken. Few other radioisotopes are needed for specialized work, e.g. ^{51}Cr, ^{58}Co B_{12}, ^{59}Fe etc.

Radioimmunoassay kits of the commoner assays should be made from the bulk reagents locally. The kits for the assays which are done infrequently may have to be imported periodically. It is economical to import them when sufficient number of samples are collected.

Indium generator was heralded at one stage as the generator for developing countries because it would be required to be imported once in 6 months. However, its energy characteristics are not suitable for gamma camera, and with decreasing use of scanners, the popularity of Indium generator has faded.

Conclusion.

Elaborate equipment plays an important part in nuclear medicine of today but we should not forget that the most important instrument in nuclear medicine, as in all other sciences, is still the human mind. Xerox copy of a nuclear medicine laboratory of an advanced country in the surroundings of a developing country leads to versatile and glamorous incapacity. Transplant of technology can not be attempted in a climate of jeopardy, adversity and uncertainty. Innovation, and not imitation, is required for a successful transplant. Many recipes are described in this Handbook for learning to do "more with less". It is still possible for a nuclear medicine department in a hospital of developing country to be an island of excellence in a sea of mediocrity, because there is hardly any other branch of medicine, where so many of the basic sciences have come together in the service of a patient.

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Chapter 3

RADIONUCLIDES AS TRACERS

R.D. Ganatra

Nuclear Medicine is usually defined as a "clinical speciality devoted to diagnostic, therapeutic and research applications of internally administered radionuclides.". Diagnostic implies both in vivo and in vitro uses. In modern times, there is hardly any medical research, where a radioactive tracer is not used in some form or other. Normally basic medical research is not considered as nuclear medicine, but clinical research applications of radioisotopes are considered as an integral part of this speciality.

Radioisotopes are elements having the same atomic number but different atomic weights. For example, ^{131}I, ^{125}I, ^{123}I are all isotopes of the same element. Their chemical and biological behaviours are expected to be identical. The slight differences in the weights, that they have, is due to differences in the number of particles that they hold inside the nucleus. Some isotopes are perturbed by this kind of change in the nuclear structure. They become unstable, and emit radiation till they reach stable state. These are called radioisotopes. Radioisotopes have few immutable characteristics: they are unstable, they all disintegrate at a specific rate, and they all emit radiations, which have a specific energy pattern.

Importance of radioisotopes in medicine is because of their two characteristics: their biological behaviour is identical to their stable counterparts, and because they are radioactive their emissions can be detected by a suitable instrument. All isotopes of iodine will behave in the same way and will concentrate in the thyroid gland. There is no way of detecting the stable, natural iodine in the thyroid gland, but the presence of radioactive iodine can be detected externally in vivo by a detector. Thus, the radioactive iodine becomes a tracer, a sort of a spy, which mimics the behaviour of natural iodine and relays information to a detector.

The radioactive tracers are popular because of the ease with which they can be detected in vivo and the fact that the measurement of their presence in the body can be in quantitative terms. The measurement can be very accurate and sensitive. Whenever the measurements can be done in vivo, the information is obtained in dynamic terms, as it is happening, as if the physiological events become transparent.

The radioisotopes are physical entities and their radiations and measurements are characterized by laws of physics. It is, therefore, essential to learn some nuclear physics for practising Nuclear Medicine. It is no more or no less than learning the morphological and biochemical characteristics of microorganisms. Doctors are used to learn as much as possible about the tools of their trade.

The older textbooks of nuclear medicine started with a heavy bitter dose of nuclear physics. Now that the nuclear medicine has developed as a clinical discipline, the amount of physics in a nuclear medicine textbook is becoming less and less and is mostly on the need-to-know basis. It is like learning anatomy. One can memorize a large number of facts,

but use only very few of them in clinical practice. Same thing holds true for nuclear physics in relation to the practice of nuclear medicine. The approach in this Handbook is also to give few salient facts, which one needs to know, in actual day-to-day practice of nuclear medicine.

The radioactive tracers can be of three types:

(a) where a radionuclide itself is a biologically important substance e.g. iodine,

(b) where a radionuclide is an integral part of a biological molecule, e.g. radio-cobalt in Vitamin B_{12}, and

(c) a radionuclide is welded chemically to a substance of biological interest e.g. radio-iodinated albumin.

An ideal tracer should have the following properties:

(a) the radionuclide should be firmly attached to the biological substance. It should not get dissociated in transit through the body.

(b) the biological behaviour of the tracer should be identical to its stable counterpart.

(c) the tracer should mix intimately with the tracee in all body compartments.

(d) radioactive tracer by itself should not pose any hazard, because of its radioactivity, nor should its chemical nature produce any pharmacological effect.

One other concept should also be understood when working with radionuclides. They emit radioactivity till they transmute themselves to a stable element. Their radioactivity has a life span and it is measured in terms of half life. This is the time period in which the radioactivity is reduced to half. For example, 100 units becomes 50 in eight days, in case of ^{131}I; it will become 25 after another eight days and so on. It is not a linear progression, but an exponential decay. The half life is a physical property of an element, which can not be altered by any external factors. There are some formulas and mathematical expressions to describe this function. They will be described in a later Chapter.

RADIONUCLIDES AS TRACERS

The way the radionuclides are produced in the reactor, every atom does not become radioactive. Non-radioactive tracer component is called a carrier. One must be conscious of the carrier amount in any radioisotopic preparation. Ideally the tracer should be carrier-free. The amount of carrier, if too large, can upset the physiological balance, and may even produce pharmacological effects.

When we detect radioactivity, there is some component of it, which is arising from the background radiation. This comes from cosmic rays, naturally occurring radioactivity in building material, radioactive ^{40}K in the human body. etc. The signal produced by the tracer should be several folds higher than this background noise.

When we have an ideal tracer and a well defined physiological concept, it is possible to ask several basic questions:

Where is the tracer going?

How much of the tracer is going where?

At what rate it is going there, and at what rate it is leaving the organ?

Procedures designed to answer these questions with the use of radioisotopes form the basis of Nuclear Medicine.

CHAPTER 3

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Chapter 4

DECISION ABOUT BUYING A GAMMA CAMERA

R.D. Ganatra

A large part of the referral to a nuclear medicine department is usually for imaging studies. Sooner or later, the nuclear medicine specialist will be called upon to make a decision about when and what type of gamma camera to buy. There is no longer an option of choosing between a rectilinear scanner and a gamma camera as the former is virtually out of the market. The decision that one has to make is when to invest in a gamma camera, and then on what basis to select the gamma camera?

Basically gamma camera is a devise to depict the distribution of a radionuclide in an organ. The detector is a scintillation crystal (thallium activated sodium iodide crystal), a flat circular disc of 30 to 40 cm diameter, with thickness varying from ", ¼" and ½". Behind the crystal there are 37 or more photomultipliers arranged in a specific way to detect the position signal, and in front of the crystal is a heavy collimator with a large number of holes to channelize the path of the gamma photons. Whenever a gamma photon interacts with the detector, it produces three signals: X, Y and Z. The first two are the position signals and defines the precise location of the interaction. The third Z signal specifies the photopeak energy of the interaction. Scatter photons degrade the position information and the attempt is made to eliminate all scatter signals by rigorous spectrometry. These signals are depicted on a persistence oscilloscope, so that a large number of them will show a kind of a map of the distribution of the radiopharmaceutical in the organ. The duration of the dots on an ordinary scope is as short as 10^{-6} seconds, but their intensity is sufficient enough to expose a photographic or a X-ray film to produce a hard copy of the image. A schematic diagram of a gamma camera is shown in **Fig. 4.1.**

The following are the principal components of a gamma camera:

(a) mechanism to move the detector head vertically;

(b) mechanism to rotate the detector head by 180°;

(c) mechanism to rotate the yoke by ± 90°;

(d) safety features to control the movement of the detector head, so that it doesn't injure the patient in any way;

(e) Radionuclide energy selection on a spectrometry;

(f) display of the image on a persistence scope. This image is mostly useful for positioning of the patient. Rotation or transposition of the scope image is also possible;

(g) a camera scope, which allows intensity adjustments;

(h) adjustments on the camera scope for getting multiple rapid sequence images;

(i) pre-set time or pre-set count adjustments of the camera scope.

All the above features are standard on all the gamma cameras and the user has no choice in its selection. However, the user can select some of the following components:

Collimators

(a) collimators; Low energy parallel hole (must be selected because the principal radiopharmaceutical is $^{99}Tc^{m}$);

(b) Medium energy parallel hole (necessary, if occasionally radioiodine compounds are used for imaging);

(c) slant hole (optional for Nuclear cardiology);

(d) pin-hole (optional for magnifying small organs like thyroid);

(e) diverging (optional for including a large field of view);

(f) converging (optional for its magnifying effect);

(g) biplanar (optional for nuclear cardiology);.

Collimators are quite expensive. It is always troublesome to change them from patient to patient. Most of the studies are done with $^{99}Tc^{m}$, and there are very few occasions where the change of the collimator is essential.

A considerable amount of money can be saved by a judicious choice of one or two from the above list.

Scintillation crystal

(a) Crystal size; usually 40 cm. The cost of the larger crystal is quite high. There is no strong reason for buying a size larger than 40 cm;

(b) Crystal thickness; ¼", ⅜" and ½". Usually the thin crystal is adequate, because of the low gamma energy of $^{99}Tc^{m}$, the most commonly used radioisotope;

(c) number of photomultipliers, usually 37. More than that can enhance the resolution, but the cost goes up and the troubles of keeping them all properly tuned and working in unison are too mettlesome to justify a small increase in the resolution;

(d) energy resolution should be better than 12 %;

(e) energy range, at least 60 to 600 kev. The spectrometric calibration should be simple;

(f) Two display scopes;

(i) monitor scope with variable persistence and

(ii) camera scope with minimum persistence.

The display scopes are attached to various devices to get a hard copy of the image.

(a) **polaroid film**, although commonly available, is a poor choice because of the poor contrast qualities. Moreover, these films are not usually available in the developing countries.

(b) **multiformater** is an electronic device which can provide multiple images, usually on a 8 x 10" X-rays film. This makes it convenient to see several views or several time - lapse views together. Most of the current cameras have this kind of formatter as a built-in facility. Buying them as an accessory is quite expensive, somewhere around US $20 000. A manual version is available. They are quite ingenious, inexpensive and worth considering, if funds are low. A manual system is less liable to break down than an electronic system.

In some countries, even X-rays films are scarce and a possibility of an adapter for 35 mm camera should be explored. The disadvantage of the camera system is that the film can be developed only when the whole roll of 36 exposures is over. Unless such a camera is motorized, it is difficult to do fast sequential scintigraphy.

All camera devices should provide rotation of the image by 90 and 180 degrees.

Arrangement for marking anatomical landmarks and patient identification on the film display are always desirable.

System performance characteristics

Since the ultimate quality of the image depends on the overall performance of the system, it is natural to study the following important system parameters which affect the quality of the image, in choosing a camera.

(a) intrinsic spatial resolution.

(b) intrinsic energy resolution.

(c) intrinsic spatial linearity.

(d) intrinsic count - rate performance.

(e) System spatial resolution.

(f) system sensitivity.

The standardized tests have been described by NEMA and IEC to determine all the above parameters quantitatively, which sets some performance standards for the industry and makes it easier for the customer to compare one instrument with another. (NEMA = National Electrical Manufacturer's association. IEC = International Electrotechnical Commission).

The following Table describes briefly the tests prescribed by these international organizations, how to do them and what information to expect from them. As a result of this kind of standardization, which exists in the industry for more than a decade, one can now safely assume that five or six gamma cameras, which are currently available commercially have comparable performance ratings. Selection is no longer on the basis of the quality of performance, but more on the basis of maintenance and services, and whatever de luxe features the user fancies.

NEMA envisages the use of few phantoms for system check.

1. **Flood field phantom**

   (a) The energy of ^{57}Co is similar to that of $^{99}Tc^{m}$ but has a longer half life of two years. Flood sources are commercially available, which contain uniformly dispersed 2 - 3 mCi of ^{57}Co in a round flat hollow disk. Such disks are available commercially for US $400. They are convenient and are not likely to leak. They need to be replaced every two to three years.

   (b) A sealable, empty hollow plastic disk is available at a cost which is about four times less than the above type. This needs to be filled and sealed every morning just prior to its use with about 3 to 5 mCi of $^{99}Tc^{m}$. This is time consuming and there is a high probability of leakage and contamination during this preparation. A careless preparation can also leave a large air bubble inside the phantom, which can mimic a space occupying lesion in the calibration image. Simplest in this genre is a trough of water containing 5 mCi of $^{99}Tc^{m}$. However, the above mentioned risks multiply and

they are likely to keep the radiation protection officer busy. The greatest hazard of this kind of open or sealable Flood sources is the possibility of contaminating the camera crystal.

(c) small sealed 30 ml vial containing gelatin embedded ^{57}Co are also available for use as a Flood source but they also are not cheap.

2. **Bar phantom.** This is a transmission phantom consisting of lead strips parallel to each other in four arrays of different widths and spacings.

3. **PLES Bar phantom** consisting of an array of lead bars with equal spacing between them.

These are the basic phantoms and they should be purchased along with the camera. Fashions in phantoms change as frequently as fashion ware, but one should remember that they are not to be acquired as museum pieces but are meant for daily wear.

In a developing country, most of the nuclear medicine units have a single gamma camera and it cannot be for ever preoccupied with the task of defining and refining the performance of the gamma camera.

After the initial decision about buying the gamma camera, the next important decision is whether to buy one with the computer or one without it. The decision needs to be made less frequently now, because most of the gamma cameras are sold as an integrated camera - computer systems and it is increasingly difficult to buy a camera without a computer.

The computer is absolutely essential for dynamic functional studies, but what kind of dynamic studies are commonly needed in a developing country? Nuclear cardiology cannot be done without a computer, but doing these studies are worthwhile only, if a hospital has a well organized cardiology and cardiovascular surgery as specialities. SPECT is impossible without a fairly hefty computer. Renograms are too time consuming in a department, where there is only one gamma camera. Cerebral blood flow is usually done as sequential images. Albeit a computer provides a capability of manipulating a gamma camera image in many ways such as background cut-off, smoothing, masking a non-uniform area, etc. These are mostly cosmetic corrections, and the question is whether it is desirable to disguise the raw, naked analogue image in fancy clothes.

Computer helps in many quality control tasks so that they can be done automatically. Image archiving and retrieval are easy with the digitized data. Image recall later is possible so that many data processing functions can be done as and when desired.

Some of the trouble shooting and diagnostic functions are gradually being taken over by the computer. Day is not far away, when many of the computer based studies can be done by a personal computer with an appropriate software.

On the other hand, decision to postpone buying the computer at a later date is not particularly sanguine because the marriage of an old camera with a new computer is not very salubrious. Instead of improving the image, it may increase the troubles. It is better to buy the computer along with the camera, and that too from the same company to avoid mismatch and simplify the problems of maintenance.

Whether to buy the computer and with what attachments depends on the clinical needs of the hospital. It should always be taken by a nuclear medicine specialist and not by a hospital administrator. In a developing country, buying of an instrument is a monogamous act and it is always difficult to replace an old or even a dead instrument. Built-in obsolence of the modern equipment and rapid improvements that are taking place in the design and functions of the instruments require change or upgrading of the equipment after every five years. Financial provision for this should be provided in the budget planning, otherwise the pioneer premier department of nuclear medicine ends up as a museum of old unusable equipment.

Another major purchasing decision that a nuclear medicine specialist is called upon to make is the choice between a stationary gamma camera and a rotating tomographic type (SPECT). First step should be to evaluate the actual clinical needs of the department, and not to be lured away by the mystique of the new technology and the advanced equipment. The environmental factors like the air-conditioning, dust and the supply of electricity become more critical in case of complex instruments. Shopping for the best that is available in the market and buying better than the other sister institutions are strong temptations, but also a road to disaster. Compared with the cameras of the past, the performance of today's cameras is superb, and all of them can produce pretty pictures. All the gamma cameras offer similar software for routine clinical needs. In 1987, NEMA also established standards for SPECT performance and all the commercial vendors provide performance standards, which match the NEMA criteria. If a convincing need for SPECT is established and the environmental conditions are not too unfavourable, another overriding feature is the service facilities and the kind of support available from the manufacturers. A recent survey in one of the advanced countries showed that a large number of SPECT gamma cameras are used for planer imaging.

<u>What does SPECT offer in terms of improvement in diagnosis?</u>

(a) images in tomographic format which is comparable to CT and MRI.

(b) Because it sees multiple planes of a large solid organ, the contrast factors can be better in some planes between the target and the non - target regions. This would lead to some improvement in detection of small space occupying lesions (SOL).

(c) Positioning the detector head is easier.

(d) SPECT needs better count rates, which are usually possible with $^{99}Tc^{m}$ radiopharmaceuticals. New brain and myocardial imaging compounds improve the diagnostic efficiency of SPECT imaging.

What are the limitations of SPECT imaging?

(a) A SPECT system is much more sensitive to variations in electrical supply than an ordinary gamma camera. An excellent technical support is needed to avoid frequent breakdowns.

(b) Minor defect in the quality control, such as field non-uniformity can magnify the defect and may lead to a false positive diagnosis.

(c) SPECT studies need more time than planar imaging.

(d) SPECT images are more difficult to interpret and a special experience is needed before embarking upon tomographic imaging.

The usual expedient out of the dilemma of whether to buy SPECT or not, is to postpone the decision and buy a system which can be upgraded later. When considering upgrading after few years, one must realize that the camera images would have already degraded and chances of getting better images with SPECT are slim at that stage. Moreover upgrading later is costlier than buying the system *de novo*.

At the present stage, proven usefulness of SPECT is for brain and myocardial imaging, but both these imaging procedures need new radiopharmaceuticals, which are quite expensive. If a hospital has special interest in these subjects, it may be worthwhile to invest in SPECT. Another field where SPECT is likely to be useful is oncology, where detection of metastases is one of the major tasks. Detecting small space occupying lesions is something, which SPECT does well.

Although good quality control is required for SPECT, one should not forget that many of the quality control tasks are becoming automated and becoming in-built functions of the "new generation" gamma cameras, but these additional electronic marvels in a developing country are likely to increase the maintenance and repair problems, besides, sometimes the instruments which have automated functions have no alternative standby ways of correcting the itinerant function.

CHAPTER 4

Following is a specimen request for a gamma camera:

PROCUREMENT REQUEST FOR GAMMA CAMERA.

1. **Gamma Camera:**
   - 1.1. camera components
     - 1.1.1. large field crystal, e.g. ⅜"
     - 1.1.2. detector stand upgradable for SPECT.
     - 1.1.3. uniformity /Z correction
     - 1.1.4. dual peak analyzer with spectrum display
     - 1.1.5. persistence monitor
   - 1.2. collimators:
     - 1.2.1. high resolution low energy
     - 1.2.2. medium energy (^{131}I)
     - 1.2.3. Pinhole
     - 1.2.4. collimator carts and rack
   - 1.3. image formatter
     (if computer is not purchased simultaneously)

2. **Computer:** (preferably integrated with gamma camera)
   - 2.1. Specifications:
     - 2.1.1. image display resolution:
       > = 256 x 256, > 8 bit deep
     - 2.1.2. list mode capability
     - 2.1.3. floating point hardware
   - 2.2. Disks:
     - 2.2.1. hard disk(s): e.g. Winchester
     - 2.2.2. (dual) floppy unit
   - 2.3. camera - computer interface
     (suitable for SPECT later)
   - 2.4. peripherals:
     - 2.4.1. matrix printer (NLQ)
     - 2.4.2. second terminal station (monitor)
     - 2.4.3. multi-formatter: (at least 1:4 & 1:25 formats)

2.5. clinical software:
- 2.5.1. brain, kidney
- 2.5.2. lung ventilation / perfusion
- 2.5.3. renal function
- 2.5.4. nuclear cardiology
  - first pass
  - gated blood pool
  - myocard (TI)
  - Fourrier phase analysis

2.6. other software:
- 2.6.1. QC test programs
- 2.6.2. programming language: choice of:
  - Basic interpreter
  - Fortran compiler
  - Pascal compiler
- 2.6.3. [data base: optional]
- 2.6.4. communications
  - data transfer

3. Accessories: (to be quoted separately)

3.1. cardiac accessories:
- 3.1.1. ECG monitor cables, electrodes
- 3.1.2. ECG trigger (synchronizer)
- 3.1.3. defibrillator
- 3.1.4. cardiac stress system:
  - bicycle ergometer,
  - treadmill

3.2. Patient accessories
- 3.2.1. patient positioning bed mattress
- 3.2.2. head immobilizer / support
- 3.2.3. injection arm rest

3.3. consumables
- 3.3.1. boxes of ECG paper
- 3.3.2. polaroid film packs
- 3.3.3. 8 x 10" film cassettes
- 3.3.4. 8 x 10" film packs

3.4. computer accessories
- 3.4.1. floppy disks
- 3.4.2. printer ink ribbon
- 3.4.3. computer paper

4. Additional items:
to be quoted separately.

4.1. power conditioning:
- 4.1.1. constant voltage stabilizer
  suitable for (peak) power consumption of above
- 4.1.2. drop-out relay:

4.2. test phantoms:
- 4.2.1. quadrant bar (3 - 4.5 mm)
- 4.2.2. orthogonal hole
  (hole dimension related to intrinsic resolution)
- 4.2.3. $_{57}$Co test source for PMT tuning.
- 4.2.4. $_{57}$Co patient marker pen

4.3. installation / training:
- 4.3.1. site preparation
- 4.3.2. installation
- 4.3.3. application training (on site)
- 4.3.4. service training for hospital physicist/engineer
- 4.3.5. user / service manuals: two sets

4.4. service:
- 4.4.1. spare parts (to be specified)
  PM Tubes
  extender boards
  specialized tools
- 4.4.2. maintenance: choice of:
  preventive maintenance
  emergency calls
  computer maintenance
- 4.4.3. software upgrade policy?
- 4.4.4. warranty

TABLE I.

Performance measurements of gamma camera

No.	Performance Parameter	Definition
1	Intrinsic spatial resolution.	Intrinsic = without collimator; Spatial = accuracy in determining the location of the gamma ray interaction in the X-Y plane.
2	Intrinsic energy resolution	ability to identify accurately the photopeak events
3	Intrinsic flood field uniformity	variability of observed count rate density with a homogenous flux
4	Intrinsic spatial linearity	amount of positional distortion caused by the gamma camera
5	Intrinsic count rate performance	max. count rate at which the gamma camera can function accurately
6	Multiple window spatial registration	positional deviations in the image at different energies
7	System spatial resolution with and without scatter	system = includes collimator; ability to detect accurately the location of the gamma camera interaction on a X-Y plane
8	System sensitivity	efficiency of detecting incident gamma rays

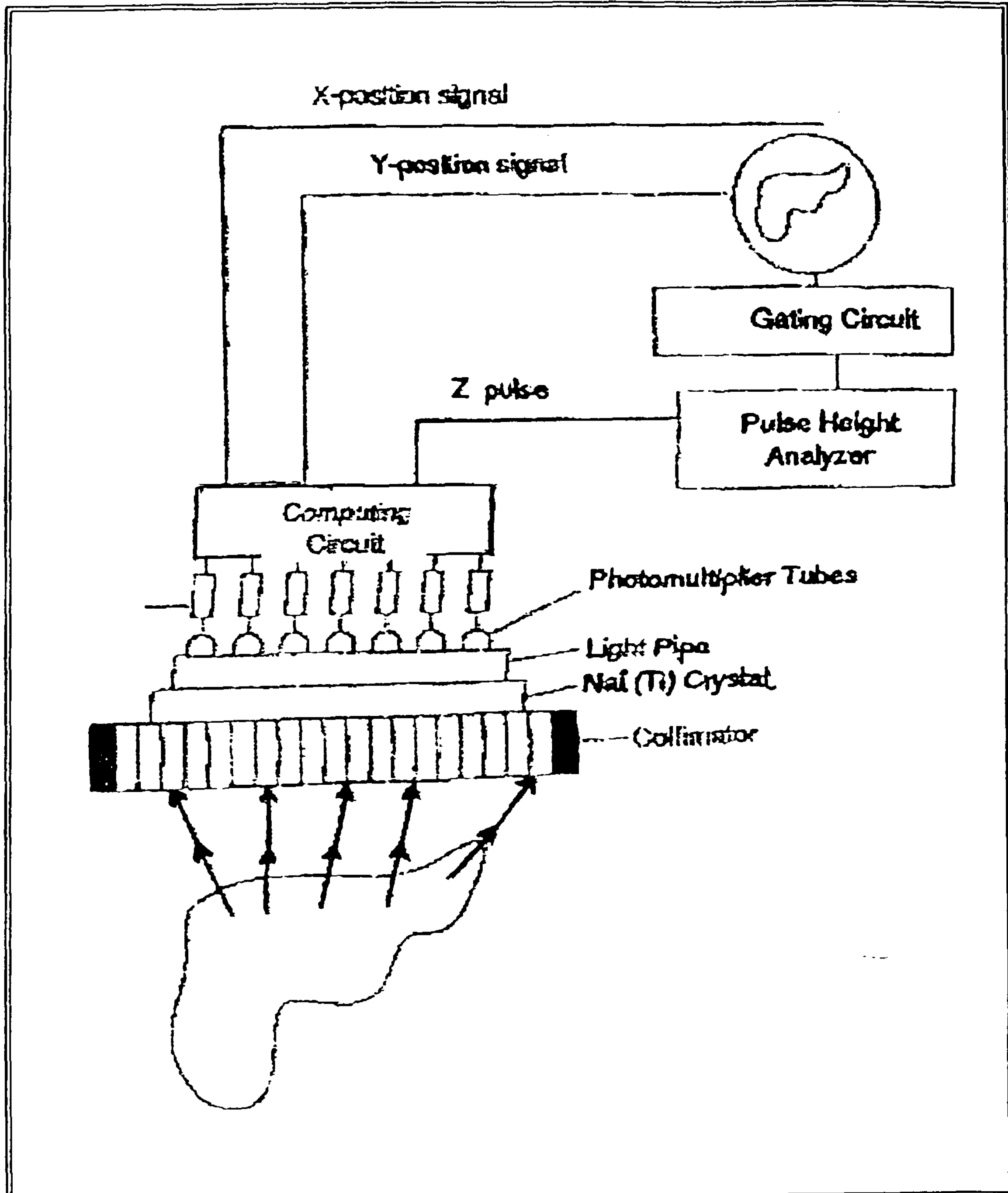

Fig. 4.1 Schematic diagram of a gamma camera. Gamma photons emitted from an organ pass through a lead parallel-hole collimator before interacting with the scintillation crystal. Photons that strike the collimator perpendicular to the face of the crystal will be detected; whereas all other photons are absorbed in the collimator. Interactions in the crystal are channelled through the light pipe and are converted to electric signals and amplified in the preamplifiers. These signals are processed to determine the spatial location (x, y coordinates) and absorbed energy (z pulse) of the scintillation event.

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Chapter 5

SCINTILLATION CAMERA-COMPUTER SYSTEMS
(General principles of Quality Control)

R.D. Ganatra

Introduction

Scintillation camera-computer systems are designed to allow the collection, digital analysis and display of the image data from a scintillation camera. The components of the computer in such a system are essentially the same as those of a computer used in any other application, i.e. a central processing unit (CPU), memory and magnetic storage. Additional hardware items necessary for nuclear medicine applications are an analogue-to-digital converter (ADC), which converts the analogue signals from the camera to digital numbers, and an image display.

The analogue image information produced by the scintillation camera consists of three signals - the X and Y signals representing the position of the photon interaction in the crystal, and the Z signal, which indicates that the energy of the interaction falls within the pulse-height analyzer (PHA) energy window set for the radionuclide in use. If the camera is an all-digital one, the image data may be transferred to the computer through a direct digital interface.

It is possible that the transfer of data from camera to computer degrades the information to some extent. The computer can generate the image for display, but it also provides the capability of manipulating the primary data to improve the display of the image. The first function of conversion from analogue to digital mode is not within the control of the operator, but the second type of manipulation is in the control of the operator. These type of manipulations should be done carefully without sacrificing the integrity of the incoming information.

Components of a Camera-computer System

Analogue-to-digital conversion

Special line driver circuits are commonly used to drive the low-power scintillation camera signals to the computer. The line drivers may also alter the voltage levels of the signals so that they are suitable for the computer interface. Failures in these circuits may produce artefacts in the digital image, but usually will not affect the analogue operation of the camera. If the analogue and digital images differ, these circuits should be considered as potential sources of the problem. The X and Y position signals must be converted to digital numbers to be processed by the computer.

Data processing

The data processor includes the CPU and the memory of the computer. The CPU in a conventional computer is the section that controls the timing and operation of the overall

system. It also includes the arithmetic processing unit which performs the calculations and makes logical decisions.

The computer memory consists of a series of storage locations, or bins, into which data can be placed as words for later retrieval and manipulation. Memory is characterized by the number of storage locations and the size of the individual word. The number of locations determines the amount of data and the size of programs that can be present at any given moment. The size of the memory word determines the magnitude of the number, which can be stored at a given location as a binary number. Some word sizes have been given special names. The most common is the byte, which refers to a group of eight binary digits or bits.

In general, the size of the memory word determines the counts that can be collected in a digital image. Some computers allow the user to select the size that will be used for image collection. Use of an 8-bit storage element allows the collection of a count of 255 per image element (or pixel). Use of a 16 bit storage element accommodates numbers of up to 65 535 or $\pm < 32\ 767$ per pixel, depending on the particular computer.

The use of an 8-bit storage element for nuclear medicine imaging is a limitation and a potential source of error. In imaging procedures in which the radiopharmaceutical is concentrated in a small anatomical area, the pixels corresponding to this area quickly become saturated. This may cause distortion of the quantitative data unless the system is capable of performing a suitable correction. It is important for the user to understand the clinical significance of such limitations and to choose the data collection mode appropriate to the clinical study to be performed.

Image formation

The output from the ADC is used in one of two ways by the computer during data acquisition - list mode and frame mode. In list mode, the digital data representing the co-ordinates of photon interactions in the crystal are simply stored as lists in memory analogous to those that a person would record as numbers on a sheet of paper. In frame, or histogram mode, the digital data are used to identify the address of a specific memory location corresponding to the location of the interaction. Frame-mode collection constructs an image in memory buffers during collection, while list-mode only generates a list of interaction co-ordinates. Dynamic flow studies can be performed in frame mode by periodically writing the images to disk and restarting the collection in memory. A modified form of frame mode, called ECG-gated acquisition, is often used for cardiological studies. In this mode, the data acquisition is synchronized by the patient's electrocardiogram. In such gated acquisition, a series of frames are generated, each one representing a small segment of the cardiac cycle.

The number of pixels in the array or matrix, into which the digital image is divided determines the ability of the computer to retain the spatial resolution provided by the scintillation camera. A camera with a larger field-of-view requires a larger matrix to provide the same spatial resolution in the final digital image. The choice of matrix size for a

particular clinical study should be based on the analytic requirements of the study. A study which is performed primarily to perceive fine detail in the final image requires a finer matrix than one performed simply for the generation of time-activity curves from large regions-of-interest. Aside from the question of spatial resolution, the choice of matrix size has an impact of the expected count per pixel. For a given imaging situation, a change from one matrix size to the next higher, e.g., from 64 x 64 to 128 x 128, reduces the count per pixel by a factor of four, since the image is distributed over four times as many pixels. The use of a finer matrix can thus sometimes be used to prevent pixel saturation.

It is necessary to provide supplementary storage other than that provided by the memory of the computer. The nuclear medicine imaging procedures generate more data than what can be contained in a reasonably sized computer memory and the data must be stored for later retrieval and analysis. Magnetic storage is achieved by the use of two types of media: magnetic disk and magnetic tape. Disks are used for rapid storage and retrieval, while tape is more often used for long-term storage.

Data are recorded on disk by a read/write head which passes over the surface of the disk in prescribed circular tracks and creates small magnetized zones on the disk. The disk surface is logically divided into a number of storage blocks into which the image data and programs are placed by the computer. The number and size of the data blocks are dependent on the particular disk design. Small flexible disks, called floppy disks or diskettes, may have a total capacity as low as 128 000 bytes, while large disk systems may have a total capacity of more than 300 million bytes. Data transfer rates for disk systems can be as high as 250 000 bytes per second. Such high transfer rates may be required in high count rate studies in which counts are written to disk during collection. It is important to understand that the modern computer disk unit is a precision electromechanical device which must be properly cared for. Without appropriate preventive maintenance and careful handling the disk unit will soon be out of order.

Data transfer rates for tape systems are significantly lower than for disk systems. Magnetic tape storage is used primarily for long-term storage of data. With appropriate handling of the data, tape can be used for transferring data from one computer to another. With disks this is possible only between similar computers.

Image display

The image display is presented to the user on a cathode-ray tube display, the intensity or the colour at a point on the screen being related to the count of the corresponding pixel in the array. Most displays allow to alter the contrast, brightness or grey scale of the display without modifying the actual image data.

User interaction

User interaction with the display is achieved through a device such as a light pen, joystick or mouse. A light pen is a light-sensitive pointer aimed directly by the user at the

selected part of the image. A joystick is a small resistive device adjusted by the user. The computer continually monitors the position of the joystick in both the X and the Y direction and places a cursor on the display screen at a point having co-ordinates proportional to the position indicated by the joystick. A mouse similarly places a cursor on the display screen at a point under its control. These devices may be used to indicate regions-of-interest, single points or anatomical landmarks.

Performance Characteristics

Only by proper testing can it be determined that a camera-computer system is operating as it should. Those of concern in acceptance and routine testing are identified below, along with the major design and operational factors that influence them.

(a) Energy resolution;

(b) flood-field uniformity;

(c) spatial linearity

These parameters are the same as those for a gamma camera.

Integral and differential ADC linearity

ADC linearity is a performance characteristic of an ADC that describe its ability to convert accurately an analogue position signal to a digital address or location.

An ideal system should give a linear relationship between the location of an interaction in the crystal and the corresponding address in the digital image. This should be true for both the X and the Y direction. Poor integral linearity in an ADC causes the relationship between distance on the camera face and distance on the digital image to vary across the image. It is difficult to detect without precise quantitative measurements.

The major factors that degrade integral linearity in a camera-computer system are a poorly calibrated analogue amplifier or a failure in the camera itself. Differential non-linearity may be present in an ADC as a result of faulty power supply, which allow transients to affect the conversion process. Another possible cause of differential non-linearity is improper matching of circuits in the analogue part of the computer-camera interface.

Spatial resolution

Spatial resolution is a performance characteristic of a camera-computer system that describes its ability to resolve two separate point or line sources of radiation as separate entities.

The spatial resolution of a camera-computer system is conventionally quantified in the same manner as for a camera alone, the full width at half-maximum (FWHM) being determined from the digital image of a line source by linear interpolation between pixel counts.

A major factor that changes spatial resolution in a camera-computer system is the sampling of the image, i.e. the number of digital picture elements. Increasing the area of the camera face corresponding to the digital image without a corresponding increase in the matrix size degrades the spatial resolution. This is an operational characteristic of digital systems and should not be considered as a system failure. Other than this, the spatial resolution of a camera-computer system is subject to all the factors which affect the gamma camera primarily.

Count-rate performance

The count-rate performance of a camera-computer system describes the relationship between the observed count rate and the number of incident photons.

The complexity of the camera-computer system and its response to changing count rate make it impossible to describe the count-rate response by a single parameter. The usual method of quantifying system count-rate response is to produce a curve of observed count rate versus input count rate over the range of count rates expected in clinical operation. This curve can be generated in a scatter-free configuration corresponding to manufacturer's specifications or it can be generated under conditions more closely simulating the scatter characteristics of a patient. The former method may be used for acceptance testing, but the latter provides results that are clinically relevant.

The major factors that degrade count-rate response in a camera-computer system are extended ADC conversion time, maladjusted sample-and-hold circuits, poor scintillation camera performance and delays due to other data-processing which the computer may be performing at the time of data collection. In systems which are able to perform simultaneous analysis and collection or simultaneous collection from two cameras, the possibility of degraded count-rate response due to delays originating in software, especially at high count rates or high frame rates, must be seriously considered by the user.

Timing accuracy of data collection

Timing accuracy of data collection of a camera-computer system describes the ability of the system to partition accurately data into the desired temporal segments or frames.

Timing accuracy may be quantified by performing a simulated clinical study and comparing the apparent frame time, as deduced from the count in each frame, with the requested frame time and by comparing the apparent collection time, as deduced from the sum of the apparent frame times, individual frame durations with the requested collection time. Another timing consideration is the ability of the system to perform properly a gated

study and to divide accurately the cardiac cycle into the desired number of segments without undesired delays or variations in collection times.

The major factors degrading accuracy in gated studies are uncertainties in timing of signals generated by the electrocardiograph and delays between generation of the gate signal and its receipt by the computer, due to intervening electronics.

Operational Considerations

General operating conditions

It is important that the computer in a camera-computer system be used in an optimum environment. Power failures, as well as poorly conditioned power supplies, may not only render clinical results useless, but may also physically damage the equipment, which should be properly protected against such hazards. Suitable isolating transformers and drop-out relays (DORs) are essential in localities where electrical power has a tendency to be erratic. Although not all manufacturers agree, it is usually best to switch off a computer at night or when it is not in use, though not the associated scintillation camera.

The temperature and humidity ranges within which a computer will operate are very limited and care must be taken that these are not exceeded. High temperature and humidity can result in expensive failures.

Documentation

The complexity and versatility of a camera-computer system make it imperative that adequate documentation be obtained at the time of installation. Documentation for both the hardware and the software should be provided. It is strongly recommended that the buyer obtain sufficient documentation about the hardware to allow repairs to be made by a competent electronics technician.

Preventive maintenance

The room in which a camera-computer system is installed should be kept scrupulously clean. Although protected by air filters, disks can be destroyed by high levels of dust and smoke. The filters should be cleaned at regular intervals. Where appropriate, manufacturers also specify regular cleaning of disk packs, disk heads and magnetic tape heads. Such cleaning, although advisable, should be carried out only by properly trained staff. Improper cleaning of disks can be much more damaging than no cleaning at all. More specialized computer maintenance should be performed by a qualified service engineer at regular intervals.

Software

The software does not come installed in the computer, but normally accompanies it on magnetic media. These distribution media should never be used for routine operation of the computer. They should always be copied and the originals stored in a safe location away from the computer itself. The importance of this policy cannot be over emphasized. If the contents of the distribution media are accidentally destroyed in the absence of back-up copies, the entire system is useless until new copies are obtained from the manufacturer. This may entail significant expense in both time and money.

Record keeping

It is essential that a log book be kept with the system at all times. Unexpected events tend to happen and they should be recorded in the log book in as much detail as possible. The user should also try to find out why the unexpected event occurred. Examination of small, seemingly inconsequential, failures may allow the prevention of major failures at some later time. It is also useful to find out where similar systems are in use (preferably before the system is purchased) so that when problems arise other users can be contacted and advice obtained.

Test conditions

The following specific test conditions are assumed to hold during all testing procedures on a camera-computer system.

(a) No electrical or mechanical modifications to the system should be made prior to testing.

(b) The PHA should be adjusted before any tests are carried out, so that the specified window is used, centred on the appropriate photopeak.

(c) Background radiation levels should be reduced to a minimum by removing extraneous radiation sources, including patients to whom radiopharmaceuticals have been administered.

(d) The count rate in any test, unless otherwise specified, should not exceed 10 000 c/s in cameras manufactured before 1978 and 20 000 c/s in newer cameras.

(e) The radionuclide, source configuration, collimator, instrument settings, imaging parameters and test results should be recorded in the instrument log book, accompanied by the images whenever possible.

(f) At acceptance testing, a representative of the manufacturer should be present.

(g) Rotation of the analogue image by use of the scintillation camera orientation switches should cause a corresponding rotation of the digital image.

(h) The camera-computer interface should be adjusted so that the useful field-of-view of the camera without zoom, i.e. the field-of-view defined by the collimator, is entirely contained in the digital image.

(i) Any zoom and image displacement switches on the scintillation camera should be switched off.

Tests to be carried out

Many of the tests for a camera-computer system are similar to those for a scintillation camera alone. Most deal with the ability of the computer to reproduce faithfully the information provided by the camera. Such systems being still in evolution, additional or alternative tests may be required in the future. The tests described in the next chapter provide a basic evaluation of the system relevant to nuclear medicine applications. They do not represent a complete and exhaustive test of the computer. It is assumed that the computer will have been subjected to, and passed, all the routine tests provided by the manufacturer for evaluation of the proper operation of its hardware. Further, the distinction between cameras and computers as separate pieces of equipment is becoming less clearly defined as scintillation cameras become more sophisticated and incorporate more digital electronics. Most systems, whether integrated or not, can be tested using the procedures presented in the subsequent chapter.

The tests described may be considered in three categories:

(a) Static tests of the camera-computer system.

(b) Dynamic (timing) tests of the camera-computer system.

(c) Tests of the software for data collection and processing.

COMMENTS ON QUALITY CONTROL OF SOFTWARE

The software of a camera-computer system is as much a part of the overall system as is the hardware and it must be properly monitored to ensure correct operation. This monitoring must include acceptance and routine testing.

Since the software available with such systems changes rapidly, it is not possible to define a set of protocols to enable users to test their own software. The potential of a system to produce errors is almost unlimited, and care must be taken at all times to ensure that the results are reasonable. The following guidelines may be helpful in limiting operator errors, and checking a system to see that it is reasonably well-behaved.

If the basic checks of the system produce satisfactory results, and the tests of computer timing in dynamic - and ECG-gated - acquisition are satisfactory, the next set of tests to be performed should check arithmetic data manipulation.

In general there are three useful types of data which serve to validate computer programs for camera-computer systems:

(a) validated clinical data,

(b) data generated from physical phantoms, and

(c) data from mathematical simulations.

With each, data are provided so that the values determined by any analytical procedure can be checked against the expected results. This type of test is especially useful when a software upgrade has occurred allowing values determined previously to be checked against values with the new software.

Validated clinical data in this context are data from a patient, which have been processed by a number of reputable institutions and are accompanied by the numerical values found by these institutions, which serve as expected values when the data are analyzed. Confirmatory clinical information obtained from other sources should be available to verify these expected values. Suitable data may also be generated from carefully designed physical phantoms to simulate certain aspects of patient studies.

It is recommended that sets of reference clinical data be obtained for each clinical procedure in regular use. These may be either validated clinical data provided by other laboratories or the results of clinical studies performed in the institution itself which serve for reference purposes. Whenever the software is modified in a significant manner, the new software can then be tested on these reference data. There should be at least one normal and one abnormal case for each procedure.

Data from mathematical simulations are data of known form and statistical noise distribution generated by a computer. More complex simulations can be based on physiological models and can be helpful, for example, in testing cardiac software. It is strongly recommended that a program for generating simulated tomographic acquisitions be used for testing tomographic software.

The analysis protocol for each clinical procedure likely to be used should be tested. It is often helpful if a knowledgeable representative of the manufacturer is present during the tests. Any discrepancy in the documentation should be noted. If a set of validated clinical data exists, the programs should be tested using these data and the results examined; if differences are noted, reasons should be sought.

Complete software evaluation is not possible unless software documentation is available. It is important to ensure that a complete set of documentation exists for software in use, and that this is updated as necessary. Errors, including errors of documentation, should be noted in the log book as they are found.

The above material is based on the IAEA-TECDOC-317, which is undergoing revision at present. The actual Quality control procedures are described in a subsequent chapter in this book.

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Chapter 6

SINGLE PHOTON EMISSION COMPUTERIZED TOMOGRAPHY (SPECT)

R.D. Ganatra

Introduction

Tomography in nuclear medicine did not originate after the introduction of X-ray computerized tomography (CT). Even in the days of rectilinear scanner, tomography was attempted with multiple detector heads rotating around the patient, but the counts at each plane were never very high to obtain a satisfactory image. A high resolution focusing collimator can look at different depths but taking several slices in one projection was a time consuming process. Rectilinear scanners lose lot of counts in the collimator to look at one point, at one time, in one plane. It is true that attempts to do tomography with gamma camera really got a boost after the success of CT. By that time, algorithms for doing reconstruction of images also were highly refined and far advanced.

Operational principles

The SPECT system comprises of a conventional gamma camera mounted on a special gantry and connected to an appropriate computer system. A series of planar images are collected, while the camera is rotated through either 180° or 360°, around the patient. These planar images are called projection images and are used to create transaxial slice images by filtered back projection of the data into a transaxial plane. The last sentence is sufficient to give rotational dizziness to a simple minded physician. There is no point in understanding the intricacies of data processing that the computer does for generating tomographic images. Suffice it to say that each projection image consists of counts in each pixel. Each pixel counts are corrected for attenuation, because the gamma photons arising from pixels at different depths undergo different degrees of attenuation. Image quality depends on the integrity of the counts in each pixel in each view.

Apart from the usual problems of uniformity, linearity and count rate capability that exist with the gamma camera, the rotation creates a few additional plights. When a powerful algorithm uses a series of images for the reconstruction of one image of a slice, any artefact of the planar image gets multiplied several fold. Moreover, an artefact may not be recognized as an artefact, because the final image is generated by manipulation of a large number of images, some good and some bad.

The following are the main components of a SPECT system.

(a) gamma camera with 37 or more photomultipliers; self-correcting mechanism for correction of uniformity,

(b) patient couch, which can be conveniently introduced into the scanning gantry. It is made from a special material to minimize attenuation. It should be possible to align the long axis of the bed with the axis of rotation of the camera.

(c) gantry. Controlled by microprocessor interfaced to main computer.

(d) rotation controller

(e) emergency stop and other patient safety devices

(f) position read-out devices.

Advantages of SPECT

What advantages SPECT offers, which are not there in gamma camera? Cost of SPECT is higher than the cost of an ordinary gamma camera. Is the extra cost justified? The stationary gamma camera sees a whole chunk of a solid mass of an organ in each projection. If there is a space occupying lesion in that organ, contributions to the overall count rate from the overlying and underlying tissues cannot be avoided in a planar gamma camera. A reconstructed single plane in SPECT has a sharp definition of a space occupying lesion, because of the better target to non-target ratio.

SPECT is not without problems. The count rates are low and is no where near to what one gets in CT. Having more than one detector head increases the cost considerably. A rotating detector head can not be too close to the patient, as the camera's resolution becomes inferior, further away it is from the object. Camera's uniformity, linearity, spatial resolution become worse in the rotating mode. A slight non-uniformity is amplified in the rotating mode leading to a high rate of false positives for the unwary. Dynamic studies where count rates change rapidly are not possible with the SPECT. Notwithstanding, the industry has done a remarkable job of improving the quality of the images obtained on the SPECT by improving the system uniformity and linearity, introducing on the fly corrections and refining the reconstruction software. In fact, the SPECT produces better images, where the lesion is of the positive type and not a cold area. As the tendency is to design more and more of

lesion specific and function specific radiopharmaceuticals, the type of situations where it is possible to get positive images are increasing and the use of SPECT is increasing along with it.

Quality Control of SPECT

SPECT images can define a lesion better than planar image, but for that, it is necessary to calibrate (an old fashioned term but can be exchanged for quality control to appear erudite) the gamma camera perfectly, choose the right radiopharmaceutical, and get statistically reliable data, which can be processed for image reconstruction. The latter task needs a special kind of computer which, in turn, also requires attention to its quality control.

The following quality control evaluations need to be done for the SPECT regularly.

For the gamma camera part

field uniformity
energy resolution,
spatial resolution,
linearity,
count rate capability.

For the SPECT part

slice thickness,
tomographic contrast,
tomographic uniformity,
tomographic resolution,
tomographic linearity,
centre of rotation,
sensitivity - slice and volume.

SPECT in a developing country

The developing countries usually have only one gamma camera in the nuclear medicine department and this single instrument can not do bone imaging, nuclear cardiology and SPECT, in addition to the already heavy load of liver and the thyroid. Even if the SPECT is available, it would be mainly used for planar imaging.

Of course, the SPECT will like to have more counts but apart from the quantity of counts, there is something like the quality of counts. Counts can be increased by widening the window, by slowing down the speed of rotation or using a low resolution collimator but this type of increase in the counts is going to degrade the image and not improve its quality.

One does not buy SPECT with the hope that it will provide images better than planar gamma camera NOW; one buys it with a hope that in a couple of years, there will be radiopharmaceuticals, which would need SPECT for satisfactory images. Just as the gamma camera got a boost after the introduction of $^{99}Tc^m$. The SPECT has still to find a match which will make the SPECT images almost obligatory for worthwhile diagnostic information. This is beginning to happen in the case of brain and heart.

Applications and potential

Clinical application of SPECT has become widespread now, because of the development of suitable radiopharmaceuticals and improvement in instrumentation. The SPECT provides a direct measure of regional organ function and is performed with nuclides such as ^{123}I and $^{99}Tc^m$ that emit a mono-image photon during their decay. SPECT is far less expensive than positron emission tomography.

Central nervous system

A number of radiopharmaceuticals have been developed recently that cross the blood-brain barrier, distribute in the brain proportional to the regional blood flow and remain in the brain long enough to permit cross sectional imaging with SPECT. These radiopharmaceuticals have revived the interest in brain imaging, which had dwindled after the advent of CT and MRI. Now, nuclear imaging is an investigation of choice for several clinical situations. The new cerebral perfusion agents are ^{123}I labelled amines and $^{99}Tc^m$ labelled HMPAO. The latter is likely to be available in the developing countries in near future.

In acute cerebral infarction, changes in blood flow are seen earlier than CT and MRI. The SPECT studies are better than other investigations to predict outcome and to plan treatment accordingly.

Perfusion SPECT is an accurate diagnostic test in patients with suspected Alzheimer's disease, allowing accurate separation of patients with Alzheimer's disease from those with multiple infarct dementia.

SPECT has proved a valuable and easily available tool for identifying the epileptic focus.

It can be used like PET to study neuro-receptors with receptor binding radiotracers in the diagnosis and understanding of a variety of neurological and psychiatric disorders.

Cardiovascular system

Many of the problems associated with planar myocardial scintigraphy, such as superimposition of one portion of the myocardium on another are reduced with emission tomography. Perfusion SPECT, after the injection of ^{201}Tl, during stress is more accurate than planar imaging for diagnosing the presence of coronary artery disease, for determining the location of the stenosed vessels, and for assessing the functional significance of coronary artery stenosis before and after angioplasty.

^{99}Tc^{m} labelled perfusion tracers have resulted in improved image quality, since myocardial washout of the tracer is slower and photon flux is considerably greater for the same radiation dose to the patient.

Early assessment of the size of the infarct is also possible with SPECT. This parameter is useful in prognosis and planning of the treatment.

Skeletal system

Bone scintigraphy is an important part of the diagnostic strategy in patients with both malignant and benign disease. Regions of increased osseous metabolism appear as scintigraphic abnormalities before morphological changes can be detected by other imaging modalities. Planar scintigraphy often fails when there are overlapping osseous structures. Bone scintigraphy is very sensitive for the detection of subtle changes in bone metabolism. SPECT is more sensitive than planar imaging (85% vs 65%) for identifying increased metabolism at the site of the pathology.

Other applications

Hepatic SPECT using radiotracers, that are selectively extracted by the reticuloendothelial system, is more accurate than planar imaging for the identification of focal lesions but cannot compete with either CT or MRI for detection of small lesions
< 1 cm in diameter, for the identification of the total number of lesions. Single photon emission computed tomography is more accurate than planar imaging for detecting small (<3 cm) and deeply seated lesions. SPECT with labelled monoclonal antibodies may further improve tumour detection if current problems of specificity and poor image quality, can be overcome.

In its early years, SPECT was principally a research tool. Today its availability in many nuclear medicine facilities, its applications in dementia, stroke, and epilepsy; and its increased accuracy over standard planar techniques for heart, skeletal, and tumour imaging have made SPECT one of the major contributors to the current revolution in medical imaging.

CHAPTER 6

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Chapter 7

POSITRON EMISSION TOMOGRAPHY (PET)

R.D. Ganatra

Positron emission tomography (PET), Magnetic resonance imaging (MRI) and Computerized tomography (CT) are not competitive imaging modalities but are complementary techniques. PET has the potential of visualizing the biochemical events in vivo, in a non-invasive and a quantitative way. As in the case of other nuclear imaging devices, it is basically a counting equipment, which maps out the distribution of radioactivity in an organ. Its success or failure as a diagnostic tool depends largely on the radiopharmaceutical used for tracing a specific metabolic pathway.

All radiopharmaceuticals for PET have to be positron emitters. The common radionuclides used for labelling are ^{11}C, ^{13}N, ^{15}O and ^{18}F. The first three are integral part of many biologically active molecules. There is no suitable radionuclide of H, but F acts as a good substitute for H in many chemical reactions. As the half-lives of all these compounds are extremely short, it does not permit lengthy labelling procedures. Moreover, the cyclotron has to be available in close vicinity of the hospital. Such hospital based cyclotrons are available commercially but they cost about a million US dollars. A similar amount will be the cost of the PET instrument. If one adds the cost of site preparation to these costs it will be apparent that PET will need initial investment of about four million US dollars. It would also be necessary to have trained professional staff for the physical and chemical parts of the production of radiopharmaceuticals in a cyclotron. Is all this expense justified in a developing country?

The following Table lists some of the commonly used radiopharmaceuticals for PET studies and the type of the information that they provide. Most of the time the metabolic information is based on quantitation of the uptake which depends on the blood flow and receptor binding. Beyond these initial metabolic studies, detailed temporal studies are not possible, because of the short half life of the radionuclides. Nevertheless, PET has provided a lot of interesting information on several brain and heart disorders, although the information can not yet be called as diagnostic.

Cyclotron designs are becoming standard but to make the cyclotron relate more to radionuclides of proven diagnostic worth, it is necessary to have cyclotrons of higher energy beam to produce ^{123}I, ^{201}Tl, ^{67}Ga and ^{111}In. This would increase the cost of the cyclotron and its operation. Few generator produced positron emitting radionuclides are available but they are costly and difficult to obtain.

On the whole, PET is at present mostly a research tool and does not provide a wealth of information that would improve the management of the patient. For a developing country, it may be better to leave this costly venture by the side and concentrate on conventional nuclear medicine imaging and hope that there also newer radiopharmaceuticals, which are true tracers of metabolic events, will be available in the years to come

TABLE I. USES OF POSITRON EMITTERS WITH PET

RADIOPHARMACEUTICAL	USES.
2-Deoxy-2[^{18}F]fluoro-D-glucose and [1-^{11}C]-2-deoxy-D-glucose	= Brain glucose metabolism = myocardial glucose metabolism = tumour metabolism
Fluorine 18-labelled fluoro-DOPA	= Brain dopamine metabolism
Carbon 11- and ^{18}F-labelled butyrophenones	= Brain dopamine receptor
^{11}C-labelled amino acids	= Protein synthesis = Tumour metabolism
^{11}C-labelled fatty acids (1-^{11}C-palmitic acid)	= Myocardial metabolism
^{11}C-labelled dimethyloxazolidinedione	= Brain pH
Oxygen 15-labelled tracers (oxygen gas, water and carbon monoxide)	= Oxygen utilization = Blood flow = Blood Volume
Radionuclide generators (strontium-82-rubidium 82 and germanium 68-gallium 68)	= Blood Brain barrier disruption = Myocardial blood flow and metabolism = Miscellaneous

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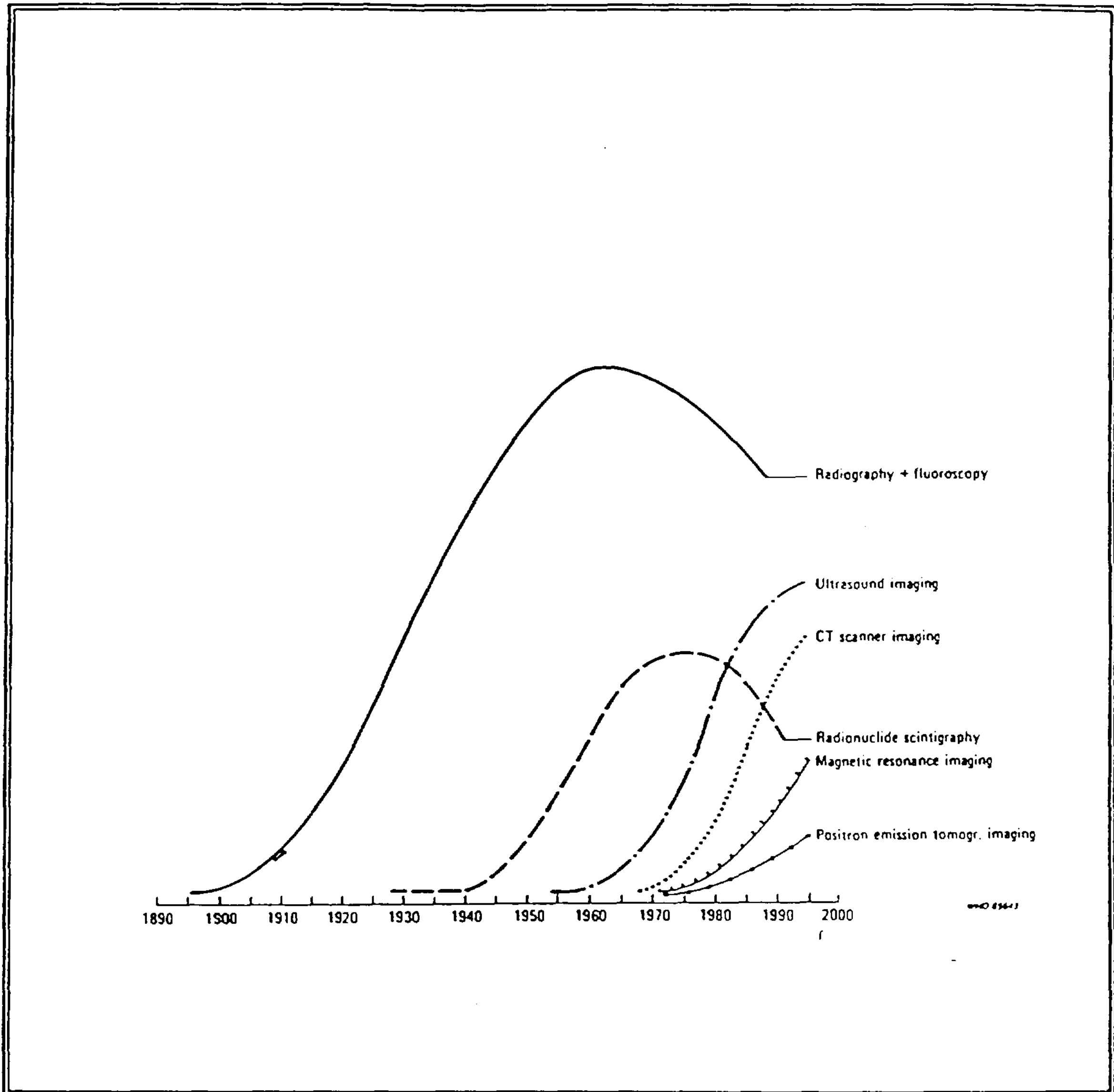

Fig. 7.1 Diagnostic imaging technologies - comparative presentation of their evolution (introduction, adoption, decay) on time scale.

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Chapter 8

QUALITY CONTROL OF IMAGING DEVICES

P.S. Soni

Quality Assurance and Quality Control in Nuclear Medicine

Quality assurance in nuclear medicine refers collectively to all aspects of a nuclear medicine service. It would include patient scheduling, radiopharmaceutical preparation and dispensing, radiation protection of patients, staff and general public, preventive maintenance and the care of instruments, methodology, data interpretation and records keeping, and many other small things which contribute directly or indirectly to the overall quality of a nuclear medicine service in a hospital.

Quality Control, on the other hand, refers to a single component of the system and is usually applied in relation to a specific instrument and its performance.

Quality Control of Scintillation Camera

The scintillation gamma camera is a standard device available in every nuclear medicine department to obtain various organ images of patients. The image quality depends upon various factors, some of them are operator dependent and others are design variants. The operator dependent parameters are kept optimum, at the hospital level, by using standard protocols and appropriate imaging techniques. The design dependent parameters vary from manufacturer to manufacturer. There were no standard performance definitions and protocols available till NEMA (National Electrical Manufacturers Association, U.S.A.) laid down some standards in this respect in 1980. Afterwards, it was possible for the manufacturers and customers to do intercomparison studies between different gamma cameras at the time of quotation and after installation.

Why Quality Control?

Assessment of the performance of an instrument is of paramount importance, not only at the time of installation, but also at regular intervals during its use, particularly after major repairs. Quality control measurements at regular intervals are also of great help in detecting deterioration in the performance characteristics of an instrument as it gets older. Initially, upon completion of installation, it is of utmost importance to evaluate and record optimal performance parameters of the instrument as a baseline for future reference. When establishing these base line parameters, protocols should be developed that can be accomplished with available in-house facilities and can be completed within a reasonable time. The need for quality control tests for acceptance at the time of installation and at subsequent follow-up during its usage is much more significant in developing countries due to the variations in the performance caused by frequent fluctuations in temperature, humidity and

power supply. Moreover, the service and maintenance back-up available in a developing country could be either very poor or at times non-existent.

In short, quality control is important - at acceptance level, during routine operation as a check on maintenance measures, and for intercomparison of different systems.

For scintillation gamma cameras, the most important performance parameters are flood field uniformity, spatial resolution, spatial linearity, energy resolution, count rate capability, multiple window spatial registration and sensitivity. Minimum tests which should be done periodically in each department are listed below:

Tests to be carried out

Daily Care and Preventive Maintenance for Scintillation Camera

(a) Note the room temperature daily and plot the readings on a graph paper.

(b) Use air filter for clean air environment and keep room humidity low (approx. 50%) because high humidity especially at higher temperature, changes the working characteristics of electronic components due to fungal growth which causes leakage of current. Presence of dust enhances this effect.

(c) Do not direct air blow of air-conditioner directly towards the instruments.

(d) Use voltage stabilizer for stable electric supply. Fluctuation in power supply may change gain of the photomultiplier tubes.

(e) Turn oscilloscope intensity to minimum when not in use.

(f) Turn persistence oscilloscope off at night.

(g) Do not leave the crystal open and unshielded. Always keep a collimator attached to the detector.

(h) Do not keep any radioactive material on top of the collimator. Check for contamination on it before attaching it to the detector.

(i) Calibrate the Pulse Height Analysis (PHA) for the energy of radionuclide in use for the clinical work.

(j) Check proper dot intensity on the formatter before exposing the film.

(k) Do not touch optic lens and clean photographic camera lenses regularly with a soft hairbrush.

(l) Perform quality control tests regularly and record the results in a logbook.

(m) Maintain a service record by recording service calls and repairs undertaken in the service maintenance logbook.

(n) Check that the collimator in use is appropriate for a patient's study and ensure its proper mounting.

Acceptance reference tests

The acceptance tests to be carried out are highly dependent upon manufacturer's recommendations but the following should be invariably done for future reference.

(a) Intrinsic/System flood field uniformity,

(b) Sensitivity,

(c) Spatial resolution and spatial distortion,

(d) Test of counting performance viz. Maximum count rate capability, Counting losses and dead time,

(e) Test of multiple-window spatial registration.

Routine Tests

(a) Check the integrity of detector head and collimator mounting,

(b) Check analyzer peak and window settings for radionuclide in use,

(c) System uniformity,

(d) Sensitivity,

(e) Check the function of camera scope and hard-copy device,

(f) Background check under operating conditions for radionuclide in use,

(g) Check the temperature of film developer and its concentration to control developing time.

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Periodical Tests

(a) *Weekly:*

(i) Test of sensitivity,

(ii) Test of spatial resolution and spatial distortion,

(iii) Test of contrast by step wedge phantom,

(iv) Intrinsic/System uniformity at various intensities,

(v) Test of total performance.

(b) *Quarterly:*

(i) Confirmation of analyzer energy peak and window settings for specific radionuclides,

(ii) Test of system count rate performances,

(iii) Sensitivity,

(iv) Test of Intrinsic and System flood field uniformity,

(v) Spatial resolution.

(c) *Half Yearly:*

(i) Test of multiple-window spatial registration, if more than one PHA is used simultaneously to produce a composite image, as in ^{67}Ga imaging,

(ii) Counting performance,

(iii) Sensitivity,

(iv) Test of preset and manual PHA window settings,

(v) Test of flood field (Intrinsic/System) uniformity,

(vi) Test of intrinsic spatial resolution,

(vii) Test of flood field uniformity at energies other than 140 Kev.

Flood Field Uniformity

The flood field uniformity of a scintillation camera is the ability of the camera to produce an uniform image when exposed to a homogeneous spatial distribution. Flood field uniformity of a detector, without collimator, is called "Intrinsic Uniformity", whereas with collimator it is called "System Uniformity".

The non-uniformity of response has many interrelated causes including drift in photomultiplier tube (PMT) gain, cracked crystal, improper calibration of the energy peak, accumulated dirt and dust on the cathode ray tube (CRT) or the optic system, phosphor burn on CRT, failure of one or more PMT, spatial non-linearities, deterioration of the crystal and high count rate.

<u>Procedure for Intrinsic Uniformity</u>

(a) Remove collimator, place the lead ring on the detector.

(b) Place $^{99}Tc^{m}$ point source in a disposable plastic syringe. Adjust the activity to have a count rate of 25 000 to 30 000 per second (cps) on 20% PHA window at a distance of five times the diameter of the crystal from the face of the detector.

(c) Peak the instrument for $^{99}Tc^{m}$ (Peak 140 Kev, 20% window).

(d) Choose intensity settings used for liver scan.

(e) Take flood field pictures in format four on 8 in. x 10 in. X-ray film, at a preset count of two to five million.

(f) Change the intensity below and above the setting used in step (d); repeat step (e).

(g) Develop the film.

(h) Compare the flood field images with earlier reference images.

(i) Any non-uniformity should be reported to the maintenance engineer.

(j) On digital camera, acquire flood image on 64 x 64 matrix and total count per pixel of about 4000. Take four images on a hard copy by varying the grey scale. Calculate integral/differential (CFOV/UFOV) uniformity [for details please refer to IAEA-TECDOC-317] using quality control software supplied by the manufacturer.

(k) A value of 10% or more above the manufacturer's worst case value would call for corrective action.

Action to be taken in case of non-uniformity

(a) Check photo peak calibration.

(b) Change the orientation setting to 90° and take a flood field picture. If the non-uniformity does not change position on the film, then, the fault is in the Hard Copier or the camera scope and camera lenses.

(c) Clean CRT of the hard copier or camera scope and its optic lenses (in Analogue camera). Persistent non-uniformity indicates oscilloscope malfunction e.g. burnt areas in the CRT screen phosphor can produce dark spots on the image.

(d) If non-uniformity spots do rotate with the image, then the fault is in the detector, possibly a drift in PMT gain.

(e) Obtain two flood fields, above and below the photopeak (approx. 25% of photopeak width). If the areas that are bright in one image are dark in the other, or vice versa, - then, all the PMT's are not properly balanced. If one area remains dark in both images, call the service engineer either for a defective PMT, crystal or optical coupling of the light guide.

Measurement of System Sensitivity and Uniformity

The sensitivity of a camera is measured as the number of detected counts per unit time per unit source activity for a specified energy window and geometry of measurement. Since sensitivity varies from collimator to collimator, it is important to know the relative sensitivities of different collimators.

(a) Place flood source (^{57}Co) on collimated detector.

(b) Adjust the intensity to liver scan setting.

(c) Take flood field picture by collecting two million counts and record the time (secs).

(d) Find out the activity of the ^{57}Co source on the day of experiment (in Bq).

(e) Calculate the sensitivity:

$$\frac{\textbf{2 000 000 counts}}{\textbf{(- sec) X (- Bq)}} = \textbf{Counts / Sec/ Bq}$$

(f) In a worst case, system sensitivity should not be less than 340 counts/min/μCi.

Intrinsic Spatial Linearity and Resolution

Spatial linearity: It is one of the performance parameters of the gamma camera that describes the amount of spatial distortion of the image. Spatial linearity can be quantified in terms of degree of linearity from the image of linear bars on the 90° bar phantom or line phantom described by NEMA. Spatial distortion and flood field uniformity are closely related. If severe non-linearity is there, the non-uniformity will also be there in the same area.

Spatial resolution: It is the minimum distance between two point sources that can be resolved separately. Intrinsic resolution is affected by the energy of gamma ray, crystal thickness, light guide, PHA window width and count rate. Whereas, system resolution is affected by the type of collimator used and source-to-collimator distance.

The minimum perceptible bar spacing in an image of 90° quadrant bar phantom is used as an index of camera spatial resolution. Intrinsic spatial resolution can be quantified from the full width at half maximum (FWHM) of the line-spread function of a line source or from the 90° quadrant bar phantom image, using the following relationship.

$$\mathbf{FWHM = 1.75\ B}$$

where B is the width of the smallest bar that the camera can resolve.

Intrinsic spatial linearity and resolution (Half Yearly Test)

(a) Remove collimator, turn the detector up side down.

(b) Place a bar phantom on top of the detector, with the bars carefully aligned with the X and Y axis of the detector.

(c) Place a source of approximately 1 mCi (37 MBq) of $^{99}Tc^{m}$ source at a distance, five times the diameter of the crystal.

(d) Set a 20% window and peak the instrument for $^{99}Tc^{m}$.

(e) Collect two million counts at liver scan intensity.

(f) Rotate the bar phantom by 90°, 180°, 270° and take the pictures and record the counting time.

(g) Assess image for spatial linearity and resolution.

Quantification of spatial resolution

(a) Analogue image method:

(i) Find out the width of the bar in a 90° bar quadrant phantom which the camera can resolve (say 'B').

(ii) Resolution; FWHM = 1.75 B.

(iii) Average the FWHM values in the X and Y directions.

(b) On Digital Camera-Computer System:

Instead of bar phantom, Intrinsic resolution phantom having two line parallel collimated line source can be used [for more details please refer to IAEA-TECDOC-317].

System spatial linearity and resolution

(a) For system linearity and resolution the collimator is not removed. Otherwise, the steps (a) and (b) are the same as above.

(b) Place ^{57}Co flood source having 185 MBq (5 mCi) on the top of a 90° quadrant bar phantom. If a ^{57}Co flood source is not available, then use a flood phantom containing about 185 MBq (5 mCi) $^{99}Tc^{m}$.

(c) Set a 20% window and peak the camera.

(d) Repeat steps (e) to (g) and quantitation steps as described above.

Quantitation of performance parameters of gamma camera by densitometer

An IAEA Research Project (RB-3356) on Quality Camera was initiated in 1983, for carrying out quality control studies of gamma cameras installed in various places in India. During the survey it was felt that the standard performance measurement tests were not suitable for the following reasons:

(a) The recommended protocol involves the use of computer or a multichannel analyzer which are not available in some of the nuclear medicine departments,

(b) The standard performance measurement tests recommended by NEMA are simple for those, who are well trained but are too complicated for an average technologist,

(c) Quality control protocols suggested by NEMA and IAEA-TECDOC-317 are primarily based on digitised data from Anger Camera and by-passes the camera scope and photographic imaging system which are usually used to obtain hard copies of the clinical images on X-ray film.

We have developed a densitometric technique for evaluation of gamma camera's performance. It is a modification of NEMA protocols and also based on recommendations made by IAEA [IAEA-TECDOC-317]. Most important aspect of this technique is that it evaluates total performance, inclusive of the display imager and photographic hard copier, - a total performance evaluation, which is more meaningful to the user.

Technique

The technique essentially consists of obtaining images on X-ray films. Further quantitation of various performance parameters is done in terms of film densities and not in terms of count rates per pixel obtained from the computer. In our method, point by point analysis of the image is carried out with a densitometer. An automatic scanning micro-densitometer, interfaced to PDP-11/34 computer, is used for scanning the film densities on quality control X-ray films. The film density distribution histogram represents the uniformity response of the detector. Various performance parameters, which can be assessed by this densitometric technique, are uniformity, spatial distortion and system spatial resolution.

Uniformity: The central field of view (CFOV) and useful field of view (UFOV) are expressed as percent variation (PV; SD/Mean x 100) of film density values obtained by scanning (scan increment in x and y direction and aperture size 100 micron) the flood image on microdensitometer.

The second parameter for the intrinsic uniformity which describes the asymmetry (AS) of the frequency distribution of film densities (FD) values. Asymmetry (AS) is determined by estimating the deviation of mid-points of total range of FD from the median values in the distribution. The following relationship is used to determine the asymmetry, which is also a useful parameter for intrinsic uniformity.

$$AS = (L + R/2) - M$$

where L is the lowest value of FD, R is the range of FD and M is the median value.

Longer tail or low FD values will result in negative value of 'AS' (shows localised cold area) and the positive value of 'AS' represents a higher end of the FD spectrum (shows focal hot area). Flood field images of five different gamma cameras and their film density frequency distribution curves are shown in **Figs. 8.1 and 8.2** respectively. It is obvious that broader the distribution, poorer is the uniformity of the flood field. Thus the entire curve represents the extent of non-uniformity.

These two parameters, percent variation 'PV' and asymmetry 'AS' are complimentary to each other, the former being more sensitive to generalised non-uniformity and the latter more sensitive to focal non-uniformity (e.g. PMT defective or crystal crack). Special programs are written for analysis of the flood field images.

Spatial Linearity: The parameters used for measuring non-linearity are the same as recommended in IAEA-TECDOC-317 manual. Absolute linearity, which measures the maximum deviation from the object grid of the pattern, is obtained from the maximum

displacement of the line of object grid on the image. This can be done on the densitometer by scanning the image of the pattern of parallel lines (3 cm apart) equal spacing (2.5 mm) phantom on a densitometer. The density (FD) values recorded on scan line are analyzed by a special software written for absolute linearity measurement. Distance between the consecutive peaks is measured and from all such distances, the mean peak to peak distance and its standard deviation (SD) are computed and printed out. From the print out, the maximum peak to peak distance is found, converted to real distance on the detector by using conversion factor and subtracted from the actual distance between the two grids on the phantom.

Other parameter that can be evaluated with this technique is differential linearity, which measures the short range deviations from the straight line and is given by the SD in mms, for adjacent peak separations between all peaks in a specified field of view. The SD obtained in mm, converted to real distance, is the differential linearity.

System Spatial Resolution: System spatial resolution is measured as full width at half maximum (FWHM) of the line spread function obtained with two parallel line sources (5 cm apart). Image of the two line sources is obtained on X-ray film and scanned on a densitometer. The density values on the peaks is converted to intensity setting values (from the characteristic curve of the film) and the LSF is plotted with respect to intensity values in arbitrary units. The new LSF curves are then used to calculate FWHM in mm which is converted to absolute distance by using a conversion factor.

The quantitative method of analyzing densitometric data for defining the performance parameters of scintillation camera as described above is very simple. The basic flood field images required for further quantitative analysis can be obtained in any hospital without difficulty by following a standard protocol. Analysis of these images can be done at a centralised institution on a service basis, a system similar to that of a film badge service. The potential of this method is:

(a) in survey of large number of scintillation cameras;

(b) in acceptance testing after installation of the system and

(c) in a long term follow-up of scintillation camera performance as described below.

Sequential evaluation of Performance Characteristics of Gamma Cameras by Densitometric Techniques

One of the application of densitometric technique is sequential study of the performance characteristics of scintillation camera at regular intervals. We had carried out such studies on two gamma cameras. Camera A, installed in 1978, did not have uniformity correction circuit and Camera B, installed in 1984, had built-in uniformity as well as linearity correction circuits. From the log books, old data and flood field films from 1982 to 1987 of both gamma cameras were taken out. Quantitation of various performance parameters were carried out on these films by microdensitometry, by the methods described above.

The deterioration of intrinsic uniformity (PV and AS) of the two gamma cameras over a period of six and four years respectively, are shown in **Figs. 8.3 to 8.6**. Camera A (without uniformity correction circuit) showed better CFOV than UFOV values **(Fig. 8.3)**. Average PV (CFOV) value is approximately 5%, whereas PV (UFOV) value varies for 4.0% to 23%. In the beginning of 1984, the PV value of this camera was very high; 16% (CFOV) and 23.0 (UFOV). There was improvement in the performance as a result of tuning of the photomultiplier tubes **(Fig. 8.3)**. Improvement in uniformity did not last for a long time.

Camera B, with uniformity and linearity correction circuit, showed constant intrinsic uniformity in terms of PV (CFOV) with time **(Fig. 8.5)** and the average PV value was less than 4.0. In 1986, PV (UFOV) value was very high, but it became normal after tuning.

Asymmetry values of camera B **(Fig. 8.6)** were constant till June 1986, but worsened afterwards. On the other hand, Camera A showed continuously a cold area (negative AS value) in the flood field films **(Fig. 8.4)**.

In conclusion, Camera A without uniformity correction circuit showed poorer uniformity than camera B which had automatic uniformity - linearity correction.

Count rate performance tests

The ability of a camera system to operate successfully at high count rates can be evaluated in terms of:

(a) Maximum count rate capability

(b) Dead time (T) and

(c) count rate at 20% counting losses.

<u>Maximum count rate capability</u>

(a) Remove collimator. Place the ring to restrict the field to useful field of view (UFOV).

(b) Bring a point source (approximately 800 μCi) of $^{99}Tc^{m}$ from a certain distance, to the detector face in steps, along an axis. Measure the count rate at every step and find out the maximum acceptable count rate.

(c) Compare this value with the manufacturer's specifications. In a worst situation, it should not be less than 60 000 cps.

Dead Time (T)

It is the time interval for which the system is insensitive after every recorded event in the electronic system. It can be categorised either as paralysable or non-paralysable system depending upon electronics involved in it.

Paralysable System is unable to record a second event unless there is a time interval of at least Tp between the two successive events. The period of insensitivity is further extended by any additional true event taking place before full recovery.

Non-Paralysable System is insensitive after each observed event. The period of insensitivity is not affected by any additional true counts.

A technique for measuring the dead time of the camera by a two source method is described in TECDOC-317, under the assumption that the system is fully paralysable in the range where a 20% data loss is expected.

Measurement of dead time (Tp) by two source method

(a) Use two sources A and B, with 2 MBq (54 μCi) of $^{99}Tc^{m}$. Remove all other radioactive sources from the room.

(b) Place both sources (A + B) at a distance greater than 1 meter, along the axis in front of a bare crystal with a lead ring. The count rate should be 20 000 cps.

(c) At this distance, measure A, A+B, B, B+A, A in sequence (20% window counting).

(d) Find the mean of A (R1), B(R2) and A+B (R12).

(e) Calculate the Tp at different count rates by using the formula:

$$Tp = \frac{2 R_{12}}{(R_1 + R_2)^2} \ln \frac{R_1 + R_2}{R_{12}}$$

where R_1, R_2 and R_{12} are count rates from source A, source B, and Source A and B together, respectively.

(f) Calculate the input count rate for a 20% loss ($R_{-20\%}$), by

$$R_{-20\%} = \frac{1}{Tp} \ln \frac{10}{8} = \frac{0.2231}{Tp}$$

(g) Calculate the observed count rate for a 20% count loss $C_{20\%}$, by

$$C_{20\%} = 0.8 \times R_{-20\%} = 0.8 \times \frac{0.2231}{Tp} = \frac{0.17848}{Tp}$$

(h) At routine testing, a change in the value of $R_{-20\%}$ by more than ± 20% from the reference value would call for follow-up action.

Alternative Method

Measurement of counting losses (copper absorber plates method):

(a) Remove the collimator and place the ring to mask the camera to UFOV.

(b) Calibrate the instrument for $^{99}Tc^{m}$ (Peak 140 Kev, 20% window).

(c) Take background count for a preset time of 100 seconds and note the background count rate.

(d) Take approximately, 5 mCi (185 MBq) of $^{99}Tc^{m}$ in a collimated vial (shield the source with a lead pot having thickness of 6 mm and fill the vial with water so that there are no air bubbles) and place it at a distance greater than 1.5 m along the detector axis. The count rate should be approximately 75 000 cps.

(e) Take the counts by increasing number of absorber plates in front of the source till background counts are recorded, approximately 2000 ± 500 (N_1).

(f) Assuming that at this low count rate, counting losses are negligible, the incident count rate, N_o, given out by the source and falling on the absorber having total thickness 'X' (no. of plates x thickness of single plate) is obtained from the formula:

$$N_1 = N_o^{-ux}$$

where u is absorption coefficient of copper at 140 Kev energy.

(g) Using the value of N_o and u; the true count rate (N_i) emerging at different thicknesses (X_i) of the copper absorber and coming to the detector, are estimated from the formula:

$$N_i = N_o e^{-uxi}$$

where i = 1,2 plates.

(h) Plot a graph of true counts vs observed counts (on linear graph paper).

(i) From the curve, find out maximum true counts Rt^{max} and maximum observed counts Ro^{max}.

Calculate paralysable and non-paralysable dead time by using the formula:

$$Tp = \frac{1}{Rt^{max}}$$

$$Tn = \frac{1}{Rt^{max}} + \frac{1}{Ro^{max}} - \frac{e}{Rt^{max}}$$

(j) Determine from the graph the count rate corresponding to a 20% count loss by using the formula:

$$\% \text{ counting losses} = \frac{Rt - Ro}{Rt} \times 100$$

QUALITY CONTROL OF SPECT SYSTEM

Planar imaging provides only a two-dimensional image of a three-dimensional distribution of activity within the body, whereas single photon emission computerized tomography (SPECT), gives 3-D information about an organ, by removing the super-imposition of overlying structures. Unlike conventional planar imaging, SPECT imaging is quite intolerant of poor camera performance and bad patient positioning. Image defects present in the planar views are amplified by the reconstruction process. A good quality assurance program is essential to minimize the adverse effects of various artefacts on the quality of SPECT studies.

Flood Uniformity

Any gamma camera, even when properly tuned, exhibits some non-uniformities, while these variations in field uniformity are acceptable for planar images, they can cause severe artefacts in reconstructed SPECT images due to variations caused by local magnetic field influences on photomultiplier tubes during detector rotation (which is not a serious problem with improved version of PMT - shielding designs), slippage of PM tubes, shift of the collimator, and differential non-linearities in the computer interface (ADC). Defects in the collimator, even minor ones, may introduce further non-uniformities due to regional sensitivity variations. During the reconstruction of an image, any non-uniformity defect is back projected throughout the 360° radius of rotation, and will produce a circular pattern around the centre of rotation which is known as a "ring artefact" or "bull's eye". If the non-uniformity is due to "hot area", the pattern formed will be a ring of increased activity. If the non-uniformity is due to a photopenic area, the outcome will be a ring of decreased activity.

The ring artefact depends on:

(a) the amplitude and location of non-uniformity (e.g. non-uniformity defects close to the centre of rotation produce small and intense ring artefacts and those at the periphery of the field of view, large and diffuse ring artefacts) and

(b) the size of the object (proportional to the ratio of size of object to size of non-uniformity) and

(c) image counting statistics. For that reason, ring artefacts are more prominent in SPECT liver or lung imaging than in heart imaging, since a small organ will create less problems than large objects.

CHAPTER 8

Uniformity Correction

Non-uniformity can be corrected by acquiring a flood field with infinite statistics, e.g. for a matrix 64, the total number of counts required is 30 million counts. For the matrix size 128, 120 million counts are required. The general rule is that the standard deviation per pixel of the flood should be around 1.0%.

Procedure for Uniformity Correction

(a) The SPECT software available on the system enables the user to collect and retrieve a flood-correction matrix.

(b) Fill the commercially available refillable transmission source phantom with approximately 25 mCi $^{99}Tc^{m}$.

(c) Mix the activity uniformly; care should be taken to avoid air bubbles and bulging of the phantom as this would introduce a non-uniformity across the field of view.

(d) Clamp the flood source to the collimator face.

(e) Acquire a flood field correction matrix with acquisition parameters - 360° rotation, 180 number of views, radius of rotation of LEAP collimator and total counts per study 30 million for 64 x 64 matrix or 120 million for 128 x 128 matrix. In general, the rule of the thumb is to have at least 10 000 counts/pixel so that statistical error is around 1.0%.

(f) If necessary, an overnight collection of the flood counts can be done.

(g) Store flood correction matrix coefficients in save area as described in manufacturer's operating manuals.

(h) Flood correction can be applied either to each projection frame during acquisition of a clinical study or to the complete data projection at the end of the study.

(i) Repeat step (a) to (d) for various collimators.

Centre of Rotation (COR)

Back projections of a point source, located on the axis of rotation (mechanical COR) of the detector, will be at the centre of each of image matrix at all angles (electrical COR). If mechanical and electrical centre of rotation are well adjusted the reconstructed image represents accurately a point source without any distortion. In practice, it is very difficult to align perfectly the centre of rotation of the camera detector with the centre of the image matrix. With any misalignment or offset, the SPECT image of the point source is a ring

rather than a point. This offset is not important for planar imaging but can degrade the quality of the reconstructed SPECT images. A ring, produced by a small offset, may not be resolved, but will blur the reconstructed image. In the case of 180° acquisition (e.g. myocardial imaging) the back projection lines will intersect at a semi-circle instead of a full circle, as is the case for a 360° acquisition and the point source reconstructed image will produce a crescent pattern.

Causes of COR Artefacts

COR artefacts, or misalignments, may be the result of gradual wear and tear of the gears of the rotating gantry, discrete changes in crystal packing or change in the ADC gain etc. Software available in the system can calculate the value of offset correction to the data acquisition matrix before back projecting to the image matrix.

The COR correction is performed after the uniformity correction; otherwise the sensitivity map would not match the shifted images.

Procedure (To measure the offset of the COR - Weekly)

(a) Place a point source or line source near the centre of rotation.

(b) Acquire a SPECT study with 60 projections (every 6°), with a matrix of 64 and if necessary with matrix 128 (as suggested by the manufacturer's operating manuals) to improve the resolution for small offset values.

(c) Display the data in cine mode and examine the quality of sinogram. Calculate the COR factors by the software.

(d) Store the value of offset correction for later acquisitions.

(e) If a significant change in the COR plot is observed, then remount the collimator and repeat the above procedure.

(f) The offset value should be less than ¼ pixel. A deviation of more than ½ pixel would require a call to the service engineer to inspect ADC and gantry.

(g) Repeat the study with different collimators.

Alignment of Detector/Table with the axis of Rotation

The detector must be parallel to the axis of rotation (i.e. collimator holes are perpendicular to the detector and thus the axis of rotation). Since reconstructed images are made up of data from other planes, images will be blurred if the camera head is not parallel

to the axis of rotation. Check built-in bubble level prior to acquisition, to make sure that the detector head is perfectly levelled.

Adjustment of X-Y Gains

The gains over the two axis of the detector should be matched so that pixel cross-section areas are square.

QUALITY CONTROL OF NUCLEAR MEDICINE COMPUTER

Quality Control of Computer Hardware

Computer used in Nuclear Medicine has several important components viz. camera-computer interface (ADC); central processing unit (CPU); memory devices; display unit; operating system etc.

Analogue digital converter (ADC) is the one which interfaces gamma camera to the computer and converts the analogue signals from the camera to digital numbers. The output of the camera normally consists of three analogue signals. The X and Y position signals carry the position information of the detected event and Z unblank signal indicates that a valid event has been detected. If the camera is an "all-digit" type, the image data may be transferred to the computer through a direct digital interface.

The X, Y and Z (unblanking) signals must arrive at the camera scope in exactly the right sequence. Most computers allow adjustment of the Z unblank pulse length to ensure that X and Y signals are converted into digital form at an optimum time. If conversion is carried out too early, position signals will not have sufficient time to settle and hence loss of resolution will occur. Very long unblank pulse will also cause invalid position signal resulting in loss of resolution and sometimes image distortion. By adjusting the pulse length of unblank signal and simultaneously observing clinical and quality control images will alert the user to this problem.

Z-pulse length adjustment of ADC

(a) Remove collimator and place a resolution phantom on the detector.

(b) Place a point source ($^{99}Tc^{m}$) at a distance five times the diameter of the crystal.

(c) Adjust activity in such a way as to have count rates of 25 000 to 30 000 cps with 20% PHA window width.

(d) Locate ADC and Z control boards.

(e) Connect channel A of scope (CRO) to 'X' test point and channel B to Z test point on the board.

(f) Adjust trigger level of channel A and B, on the scope, for a stable display.

(g) Monitor the image of bar lines on the display.

(h) Observe the X signal on the scope and adjust Z pulse length to the optimum.

(i) The X signal must be perfectly flat during the unblanking pulse length. Adjust the Z pulse length in such a way so that its falling edge occurs before any change in the X signal.

(j) During adjustment of the unblanking pulse length, observe the image of the phantom on the display. The optimum unblanking pulse length is the one where image shows best resolution.

(k) Repeat steps (f) to (j) by connecting channel A of the scope to 'Y' test point.

X/Y Position signal gain adjustment

In ADC, the gain of X and Y position signals should be adjusted properly to give equal amplification so that object to image relationship remains constant in both X and Y directions. In that case a round object will give a round image. A drift in one of the amplifier will show a round object as an oval image. To ensure that the position signals cover the full field of view of the computer, it is common to provide gain and offset adjustments in the ADC.

(a) Remove collimator and place a protective cover on the crystal.

(b) Repeat step (b) to (f) as described above.

(c) Locate the X gain and X offset adjustments in ADC board.

(d) Draw a square region of interest (RoI) of maximum size to define the field of view of the computer.

(e) Adjust X gain and offsets in such a way that the flood image touches the box generated by RoI horizontally left and right.

(f) Repeat step (c) to (e) for Y gain and offset.

(g) Check that the flood image touches the box on the top, bottom, left and right side. If not, then readjustments of X/Y gains and offsets are required.

(h) If the gains over two axis of the detector are not equal, pixel cross-section areas over the matrix will not be square, which is a pre-requisite for accurate reconstruction of a SPECT image.

Note: It is recommended that the above tests are performed in the presence of a service engineer.

Quality Control of Clinical Application Software

Computer software: A nuclear medicine computer system consists of hardware components with software elements to form a functioning unit. The software components in a nuclear medicine computer system are:

(a) Acquisition;

(b) Display;

(c) Analysis;

(d) Reporting programs;

(e) Operating system;

(f) Quality assurance programs to assess the validity of information received, the system calibration and finally quality control of application software and

(g) application programs written by the user.

All manufacturers of nuclear medicine camera-computer systems offer a wide range of clinical application software. In addition, a large number of user-written programs are also in clinical use. It has been found that the results obtained with various programs on the same study differ considerably. It is important to evaluate all new software obtained from the vendors or other users. Even a so called perfect software may not be free of bugs, which may be subtle and may occur only under specific circumstances. In short, apart from routine quality control of imaging devices, it is of utmost importance to do quality control of clinical application software.

Software validation: The term quality assurance or quality control in the context of nuclear medicine should be restricted to the performance of the hardware and the same term should not be extended to software. Instead of quality control of software, it is more appropriate to call it 'software validation' since there is no hardware breakdown associated with this. For example, non-uniformity of a gamma camera could be due to drift in the gain of the PMT or breakdown of the electronic circuits. In contrast to this, software 'failure' may occur if the input data is improper.

Efforts have been made to define the quality control protocol for the software. However, no standardized methods are yet available, due to the dissimilarities between the hardware and software of nuclear medicine computers. Recently, an attempt has been made in Europe by the establishment of a project within the frame work of COST (Co-operation in Science and Technology) entitled "quality control of nuclear medicine software".

Software Phantoms: A Software Phantom is simply a typical set of studies (static or dynamic) of a particular type, which can be analyzed on computer systems having different hardware and software packages. The results obtained with different computers should be comparable. Once a software phantom is established, it can serve as a "gold standard" to validate different software to ensure that the results obtained match the expected results. The main objectives of software phantoms are:

(a) To check the performance of the data analysis software of different computer systems i.e. software validation.

(b) To check the intra-variation results of institutions, and operators.

(c) To familiarize the new users to a wide range of analysis software.

Development of software phantoms: A software phantom can be developed from three types of data:

(a) Validated clinical patient data (real).

(b) Data generated from hardware phantom i.e. Pseudo patient data.

(c) Mathematical phantoms.

First two software phantoms i.e. software phantoms derived from real clinical data and pseudo patients are based on the assumption that the acquisition is performed without any error. In contrast to this, a mathematical phantom does not depend upon the performance of a scintillation gamma camera and acquisition software.

Validated clinical patient data: It is a typical clinical study in a patient with a confirmed diagnosis. These studies are usually collected from a reputed institution and the diagnostic confirmation is by other "gold standard" techniques. A set of clinical studies (having normal and abnormal physiological conditions) are used as input to the software system to be evaluated and the results compared with what would be expected.

A major difficulty in the development of this type of software phantom is to ensure that the results obtained from it do meet expectations. One has to be absolutely sure of the diagnostic validation. For example, a gated cardiac study (MUGA) that demonstrates an EF of 20.0% is included as a software phantom, the question arise is how sure one can say that the EF is 20.0%? It is difficult to comment on the accuracy of the result, unless it is confirmed by some other modality or "gold standard". Also, sometimes it is difficult to collect data that will test the software for a variety of conditions which may be expected to occur in clinical practice.

Hardware Phantom (Pseudo patient data): A pseudo patient data can be obtained from some simple physical model or hardware phantom, something similar to the 90° bar quadrant

phantom used to assess the resolution of gamma camera. For dynamic studies, more complex software phantoms may be derived from data collected from hardware phantoms with mechanical motion simulating physiological conditions such as cardiac motion. One of this type of phantom is a cardiac phantom used to evaluate EF software.

Such a phantom, not only assesses analysis software but also acquisition software, whereas "real patient data phantom" is independent of acquisition software. At the same time, the drawback of this type of phantom is that if acquisition of data is not perfect, the result derived from it may be bizarre. Also, the phantom may not simulate all conditions likely to be encountered in clinical practice e.g. arrhythmia or premature heart beats.

Mathematical Phantoms: This type of phantom is derived from an algorithm having the desired input characteristics i.e. patient study is made from a set of mathematical equations e.g. left ventricular curve generated from cosine wave equation. Such a phantom is free from possible errors imposed by data acquisition. Recently, several mathematical phantoms have been tested for validating the EF and Fourier phase analysis software packages but these software phantoms have been found lacking in several respects.

Problems encountered in the Development of Software Phantoms: Software phantoms are very useful for validating analysis software, especially centres in developing countries would have an opportunity to compare their results directly with those obtained in more developed centres. Though, the idea seems to be very simple but the testing of a software, is in general, a complicated task and a number of problems encountered during validation of a software are:

(a) File structure of patient files varies from system to system, which imposes limitations on the free exchange of clinical data between institutions,

(b) The non-standard recording of administrative data on different computers is another problem e.g one system may record the data and may increment the image array in a matrix from top left to bottom right, whereas another system may increment from bottom left to top right. Similarly, one computer records the ECG R-R interval and another system records only the frame time.

Solution to this problem is either, all manufacturers should change over to a common image data file format or they provide software for conversion of one format to a common format. Recently, American Association of Physicists in Medicine (AAPM) has recommended a standard format for image data exchange on magnetic tape. Using this as a file structure for intermediary storage and distribute of software phantoms may be the solution to the problems that have been encountered to date.

Software phantom for Dynamic Cardiac Phantoms:

Recently, IAEA has initiated a number of projects to develop a software phantom for gated blood pool study with the objective that the latter can be used in developing countries

for validation of their EF analysis software. In one of the project, a random set of MUGA studies consisting of normal and several abnormal cardiac situations were collected. The original studies were in DEC-GAMMA-11 file format and later on efforts were made to convert these studies to five different computer systems. The software phantoms were then analyzed on each of the six computer systems using the available EF analysis software. There were number of initial problems encountered during transfer of file from one to another magnetic media and reformatting the file structure for image and administrative records in order to make the studies readable as patient files on different systems. Later on, the analysis software either failed to analyze the studies or could only provide incomplete results. The non-standard file structure and administrative data are some of the initial teething problems causing hindrance in achieving perfect software phantoms which are totally compatible for different computer systems.

Test of Computer acquisition timing:

In static mode:

(a) Remove the collimator and place 300 μCi (10 MBq) ^{99}Tc^{m} point source in front of the open crystal in such a way as to have count rate of 5000 cps.

(b) Set the real-time clock of the computer to the correct time of the day.

(c) Acquire a static image in the computer for 1000 seconds and simultaneously start stop-watch.

(d) Record the stop-watch time at the end of the data acquisition.

(e) Record the elapsed time recorded by the computer (difference of real time clock at the end and beginning of the study).

(f) Repeat step (c) to (e) with a higher count rate (40 000 cps).

(g) Compare the requested collection time (1000 sec in this case) with the times indicated by the stop-watch and the real time clock of the computer.

(h) In ideal conditions, there should not be any difference in time and an error of more than 1.0% is not acceptable.

In dynamic mode:

(a) Place 300 μCi (10 MBq) ^{99}Tc^{m} point source in front of the open crystal in such a way as to give a count-rate of 25 000 to 30 000 cps,

(b) Acquire a static image in normal mode for 20 sec.

(c) Also, set up a dynamic acquisition study with the largest frame rate available (say 500 to 1000 frames per sec).

(d) Acquire the data and start stop-watch simultaneously.

(e) Record the elapsed stop-watch time at the end of the acquisition.

(f) Repeat steps (c) to (e) for different matrix (e.g. 32 x 32 word, 64 x 64 byte, 64 x 64 word etc.) and also for list mode acquisition.

Data Analysis:

(a) Determine total count in the static image (say Cs) and in each frame of the dynamic (say Cf).

(b) For each frame, calculate the apparent frame time, tf, by the formula:

$$\frac{C_f}{t_f} = \frac{Cs}{t_s}$$

where t_s is acquisition time for static image (in this case 20 sec).

(c) Calculate total apparent collection time for the dynamic study i.e. t_f, where n is the total number of frames.

In dynamic study

(a) Calculate the percentage error in acquisition time, i.e.

$$\text{Time lost} = \frac{(\,Cf\,) - Cs}{Cs} \times 100$$

where i = 1, 2...n (frame no.)

(b) Time lost between frames should not be more than 5.0%. Any increase in time loss, could be due to problem with the computer interface or with the camera itself (e.g. photopeak drift).

Counting precision

(a) Perform counting precision (X^2 test) on the Cf values by using the relationship

$$X^2 = [(Cf)i - Cf]^2 \; / \; Cf$$

where Cf is an individual frame count and Cf is the mean of different frames.

(b) Check whether the variation in counts is within that expected by counting statistics.

Check of ECG-Gated Acquisition:

In gated studies, it is necessary to ensure that the timing of the ECG R wave is correct. Gating requires a constant heart rate and cannot be applied effectively in the presence of an arrhythmia or false ECG triggered R wave. With the result, the End Systolic (ES) point will be shifted on the LV time activity curve. IAEA-TECDOC-317 has described a simple test to check the performance of ECG gating system, as given below:

Procedure:

(a) Place the $^{99}Tc^{m}$ point source (approximately 3 mCi) in front of the detector.

(b) Connect ECG leads to a normal volunteer (ensure constant heart rate).

(c) Acquire data in computer according to MUGA frame mode protocol.

(d) Repeat the acquisition in list mode.

(e) Repeat the study with the volunteer occasionally moving arm, to produce spikes on the ECG to check the beat rejection system.

(f) Select region of interest and plot time-activity curve (TAC). In perfect ECG gating signal the time activity curve will be a horizontal line.

(g) Calculate the mean $\pm$ S.D. of the data points in the first three quarters of the TAC.

(h) Any deviation of more than three S.D. of the TAC curve from a horizontal line may indicate a failure in ECG gating system and should be investigated further.

QUALITY CONTROL OF RECTILINEAR SCANNER

Introduction

Since rectilinear scanner is a slow imaging device, it is better to keep a number of performance parameters for quality control to a minimum, so that the time involved in doing quality control will not interfere with the normal routine of the department. Many of the components of a scanner are not subject to change during operation. These include collimator, motor speed of scanner, rate meter time constant and linearity of spectrometer.

Few quick daily/weekly tests to ensure that the scanner components are operating correctly, are given below:

(a) Test of function of scaler-timer/parameter.

(b) Photopeak-to-total count ratio.

(c) Step-wedge response.

(d) Density calibration.

Test of function of scaler-timer ratemeter (Daily)

(a) Switch on the scanner and put on 'test' position, so that 50 cycles per second external signal is counted for 60 sec.

(b) If 3000 (50 x 60) counts are registered, proper functioning of the timer-scaler is indicated.

(c) If electric line frequency is 60 cycles per sec, then the rate meter should show 3600 counts per min.

(d) Presence of noise in the electrical line will increase the count rate.

Photopeak-to-total count ratio (Weekly)

(a) Remove collimator.

(b) Place a sealed ^{57}Co (122 Kev) disc source at a distance of three feet from the crystal surface. Always use the same distance for follow-up studies.

(c) Calibrate the PHA by keeping one volt window.

(d) Open three volts window above the peak (lower base line 107, upper base line 125 Kev).

(e) Collect minimum of 10 000 counts. Note the time and record the count rate (counts/min).

(f) Open the upper window to maximum and lower window to 60 Kev (Integral counting).

(g) Again collect 10 000 counts and record the count rate.

(h) Calculate photopeak-to-total count ratio

$$\frac{\textbf{Peak Counts/min (Step e)}}{\textbf{Total counts/min (Step g)}}$$

(i) Any change in photopeak-to-total count ratio indicates loss of resolution and sensitivity.

(j) For comparison, the test should be carried out weekly under identical conditions i.e. source to collimator distance, window width, gain and H.V.

Test of contrast enhancement/film density of photodisplay/ background subtraction by step-wedge phantom

Contrast enhancement (Weekly)

(a) Take 2.5 mCi of $^{99}Tc^{m}$ in a Emission-type step wedge phantom [Please, refer to IAEA-TECDOC-317].

(b) Place the phantom horizontally under the scanner and perform scanning using a commonly employed routine setting.

(c) Repeat step (b) using different contrast enhancements.

(d) Compare the images with the reference images.

(e) A change in the step-wedge image is an indication of change in contrast enhancement or intensity.

Film Density of Photo Display (Weekly)

(a) If a film densitometer is available, measure film density (FD) at the centre of each section of the image of step-wedge phantom.

(b) Plot a graph of film density vs wedge thickness for various settings of contrast enhancement.

(c) Compare results with reference curves.

(d) File curves in the log book.

Alternative Method:

If step-wedge phantom is not available, Film density of photodisplay can be calibrated by a simple method as given below:

(a) Invert the detector head and place a container with radioactive source on top of the collimator and fix it with adhesive tape.

(b) Adjust the detector voltage and switch on the integral mode.

(c) Select proper range and adjust the discriminator level in such a way that the pointer in a ratemeter shows 100% deflection.

(d) Set range differential at 100%.

(e) Keep high time constant (approx. 1 sec) and select one way mode on the scanner.

(f) Start the scan motor and obtain a full line corresponding to 100% density on a photographic film. During the return of a scanner, when it does not record (since it is scanning one way mode), adjust the discriminator level in such a way that the count rate is reduced to 90%. In the next line, when the scanner will record, 90% line will be obtained. In a similar way obtain 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% and 0% lines.

(g) By varying light source voltage and film density obtain various sets of ten lines tests.

(h) From these various sets, decide the optimum light source voltage and film density.

Test of Background Subtraction

(a) Repeat step (a) and (b) using different settings of the background subtraction control.

(b) Compare the image with the reference image.

Note: **It must be emphasized that the above simple tests are adequate only for assurance of correct operation or identification of gross malfunction.**

CHAPTER 8

QUALITY CONTROL OF DOSE CALIBRATOR

Dose calibrator is not an imaging device, but it is still included here because every dose that is injected into a patient or used for filling a phantom is measured in a dose calibrator prior to its use. If the dose calibrator is faulty, none of the calibration studies or the clinical investigations would give reliable results.

The majority of dose calibrators consist of ionization chambers coupled to circuits that convert the ionization current produced by radioactive sources, and display it in a digital form in units of activity. The accuracy and working performance of dose calibrator depend upon many factors and regular quality control parameters, viz. precision, accuracy and linearity of its response to activity and operational checks of reproducibility and background needs to be done daily.

Materials required for Quality Control

(a) Sealed low, medium and high energy gamma radiation sources calibrated to $\pm$ 5% overall accuracy or less, e.g. ^{57}Co (approx. 1 mCi; 37 Bq), ^{137}Cs (approximately 100 μCi; 3.7 Bq) and ^{60}Co (approx. 50 μCi; 1.85 Bq).

(b) Unsealed radionuclides e.g. ^{99}Tc^{m}, ^{113}In^{m}, ^{131}I in solution.

(c) Sample vials, syringes, pipettes, source holder and remote handling device for sources.

Test of Precision and Accuracy (Quarterly)

This test will provide information about random errors or loss of precision due to background changes, scatter (due to extra shielding), change in the pressure of the chamber gas and slow electronic drift, etc.

Procedure

(a) Note the background reading.

(b) Measure the activity and record the background subtracted reading.

(c) Perform to replicate readings (Ai), where i=1,2..n

(d) Calculate Mean, S.D. and percentage of variation by using the following formula:

$$\% \text{ PV} = \frac{\text{SD}}{\text{Mean}} \times 100$$

(e) Repeat step (a) to (d) for each gamma-radiation source.

(f) % PV value should be ± 5%.

(g) A quality control chart on which the % PV readings are plotted will highlight the trend in instrument performance.

Accuracy

To assess accuracy, calculate for each source the percentage difference between the mean measured activity 'A', and the activity of the source after applying decay correction on the day of measurement, A i.e.:

$$\text{Accuracy} = \frac{(\text{A} - \text{'A'})}{\text{A}} \times 100$$

(a) Repeat tests of accuracy for a wide energy range by using different radionuclides, such as ^{57}Co, ^{137}Cs and ^{60}Co.

(b) Accuracy for each source should be within ± 5%.

Measurement of Linearity of Dose Calibration (Quarterly)

(a) Elute ^{99}Tc^{m} from the generator.

(b) Transfer 100 mCi or more ^{99}Tc^{m} activity (initial activity should be equal to or greater than the highest activity for which the instrument is being used) in a sample vial and cap it firmly.

(c) Insert the sample vial into the dose calibrator.

(d) Measure and record the net activity (after subtracting the background) at one hour intervals up to 36 hours or more.

(e) Draw the line of decay of ^{99}Tc^{m} on a semi-log graph paper.

(f) Fit the line by least square method.

(g) Also plot net activity on a semi-log paper readings obtained in Step (d) for every hour.

(h) Compare the data points plotted to a constructed straight line. In case, the instrument is linear, all these points (Step d) should fall on this straight line.

(i) Any discrepancies indicate non-linearity of the activity response of the instrument.

(j) All individual activities measured in the test should be within ± 10% of the values corresponding to the straight line fitted to the data points.

Note: Care should be taken that $^{99}Tc^{m}$ solution is not contaminated with other radionuclides and there is no ^{99}Mo breakthrough.

Operational checks

(1) Background Response:

Record the background reading in activity units of a low gamma radiation K factor; Keep the K factor same every day and take reading. Any increase in the background response of more than 20% requires necessary action.

(2) Reproducibility of Performance of a Calibrator:

(a) Measure the background corrected activity of a long lived sealed medium energy gamma ray source (e.g. ^{137}Cs, ^{57}Co etc. daily.

(b) Draw the line of decay of a standard source.

(c) Draw the limits of acceptability (± 5% of expected activity) by two other straight lines parallel to the above line.

(d) Plot readings obtained in Step (a).

(e) If any individual reading lies outside these limits, it may be due to the faulty performance of the instrument.

SUGGESTED READINGS

[1] NATIONAL ELECTRICAL MANUFACTURER'S ASSOCIATION, NEMA 1: Performance measurements of scintillation cameras, Standards publication No. NUI-1980, Washington (1980).

[2] INTERNATIONAL ATOMIC ENERGY AGENCY, Quality Control of Nuclear Medicine Instruments, IAEA-TECDOC-317, Vienna (1984).

[3] SORENSON J.A., Dead time characteristics of Anger Cameras, JNM, Vol.16, No.4, (1974) 534-535.

[4] RAIKAR U.R., DESHPANDE S.M., SONI P.S., GANATRA R.D., Dead time of scintillation cameras with and without computer, International Journal of Nuclear Medicine and Biology, Vol.6 (1979) 181-182.

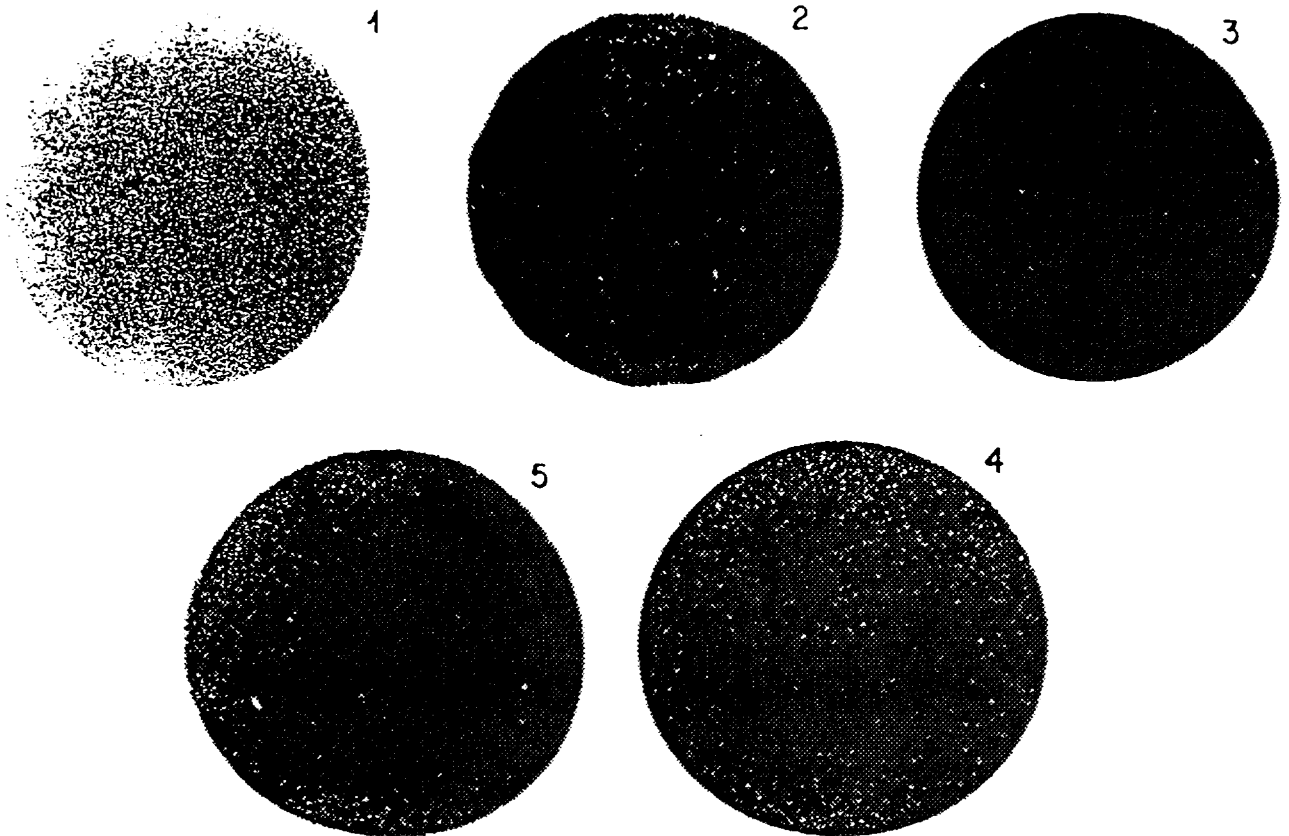

Fig. 8.1 Flood field images of five different gamma cameras.

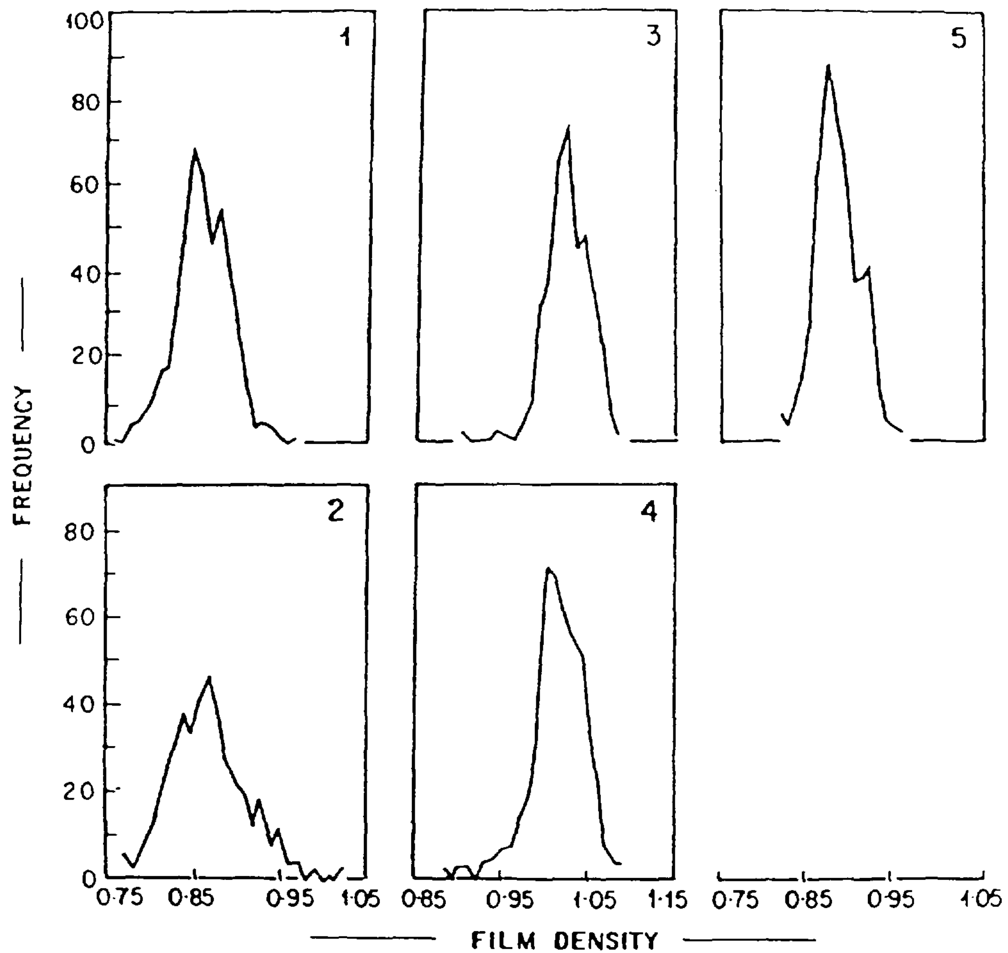

Fig. 8.2 The film density frequency distribution curves of the flood films shown in Fig. 8.1. It is obvious that broader the distribution, poorer is the uniformity of the flood field. Thus the entire curve represents the extent of non-uniformity.

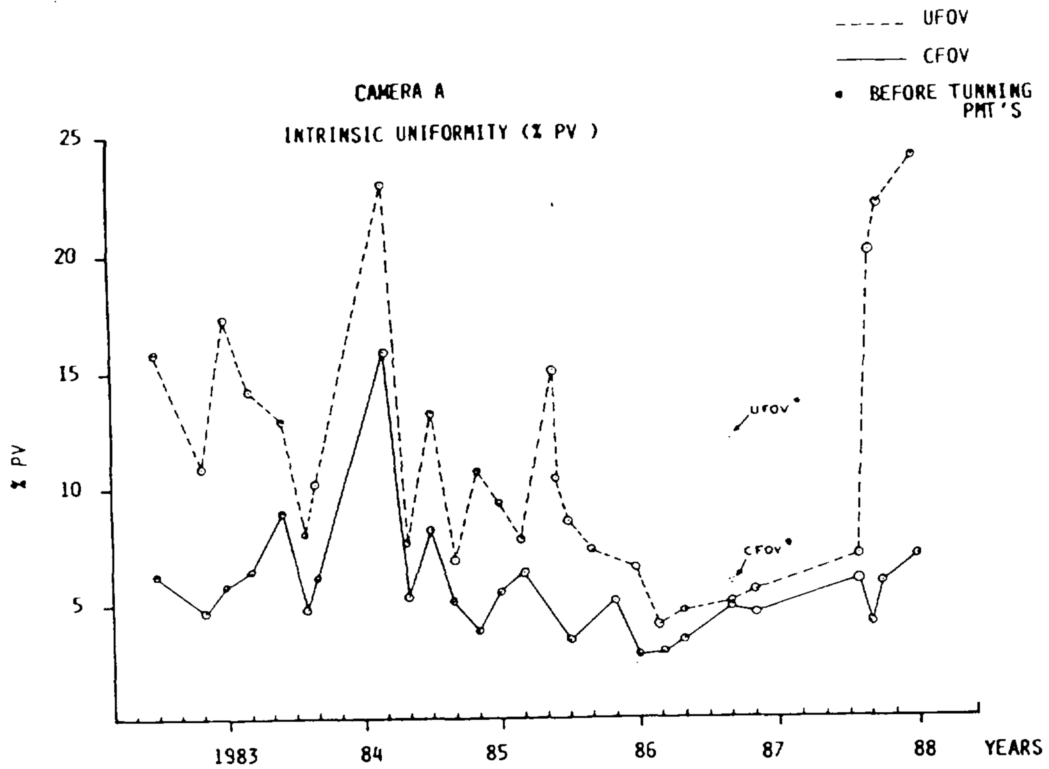

Fig. 8.3 The deterioration of intrinsic uniformity (PV) of a gamma camera over a period of six years. Camera A (without uniformity correction circuit) showed better CFOV than UFOV values. Average PV (CFOV) value is approx. 5%, whereas PV (UFOV) value varies for 4% to 23%. In the beginning of 1984, the PV value of this camera was very high; 16% (CFOV) and 23% (UFOV). There was improvement in the performance as a result of tuning of the photomultiplier tubes. Improvement in uniformity did not last for a long time.

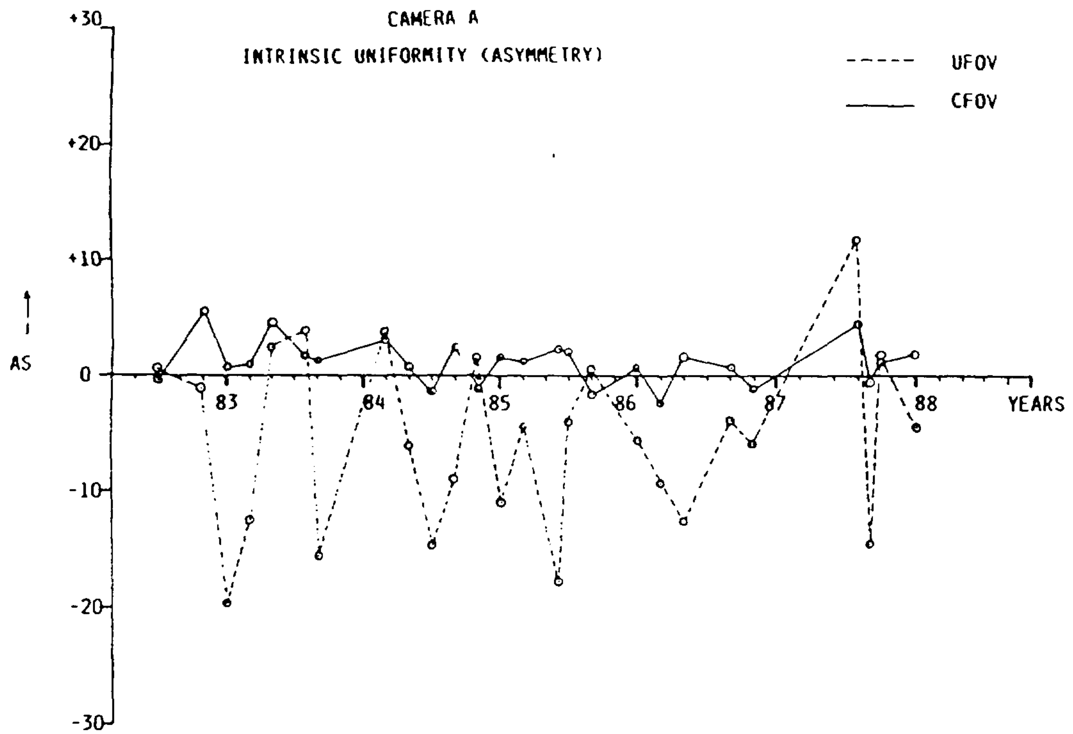

Fig. 8.4 Intrinsic uniformity of the camera A in terms of asymmetry. This Camera showed continuously a cold area (negative AS value) in the flood field films.

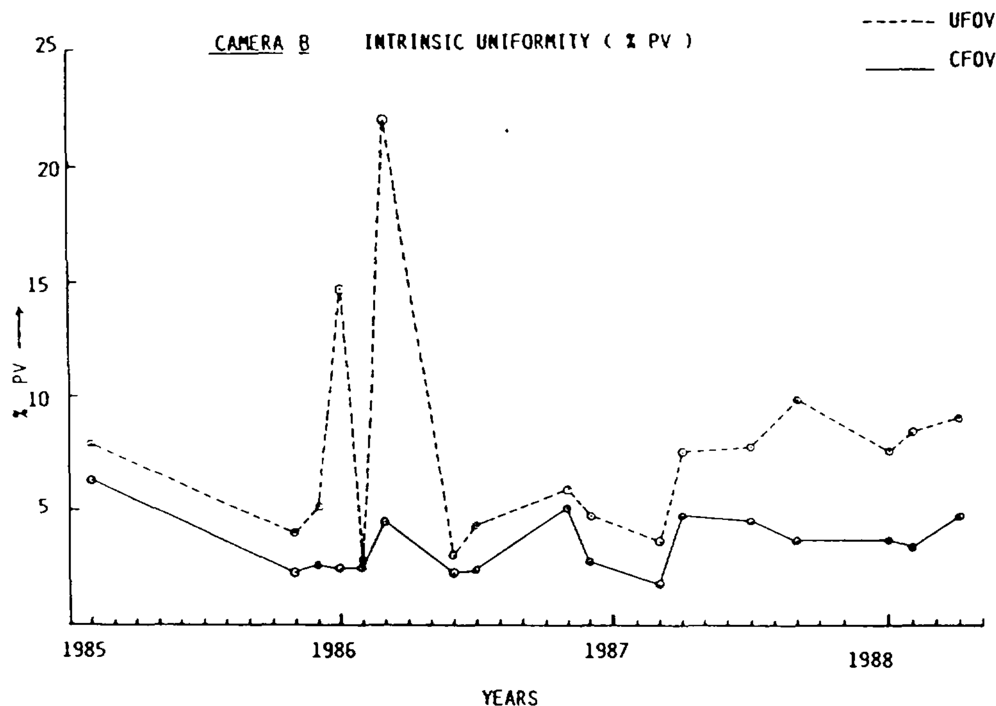

Fig. 8.5 Intrinsic uniformity (PV) of a gamma camera B over a period of four years. This Camera had uniformity and linearity correction circuits, and showed constant intrinsic uniformity in terms of PV (CFOV) with time. The average PV value was less than 4.0. In 1986, PV (UFOV) value was very high, but it became normal after tuning.

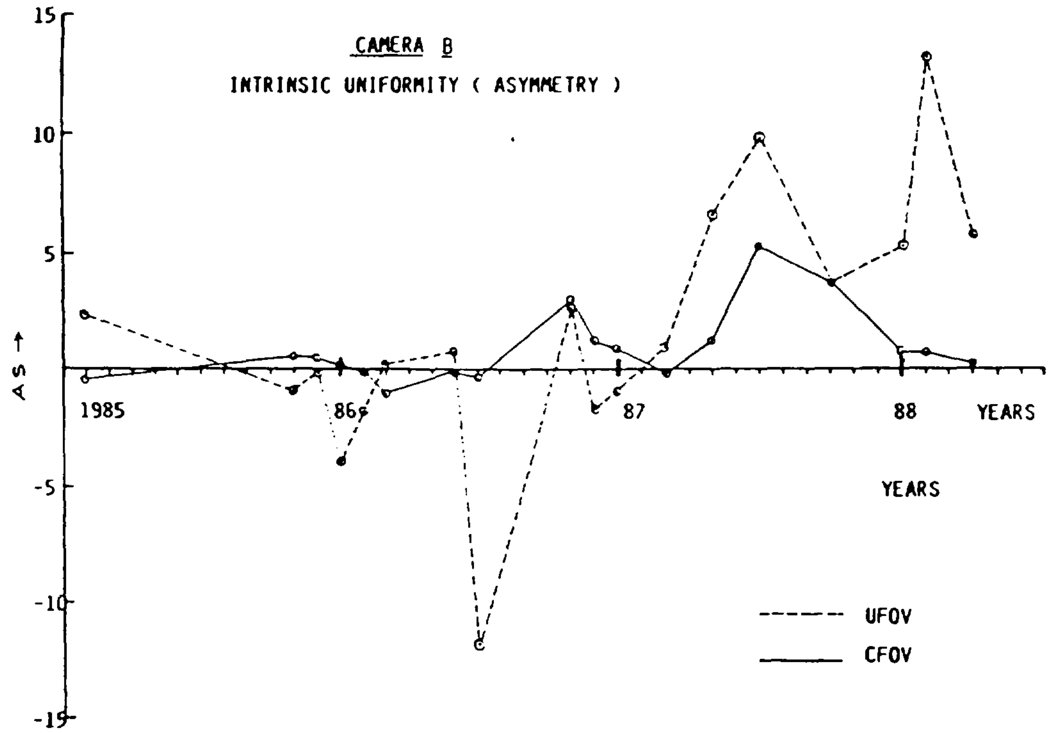

Fig. 8.6 Asymmetry values of camera B were constant till June 1986, but worsened afterwards.

CHAPTER 8

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Chapter 9

MAINTENANCE OF NUCLEAR MEDICINE INSTRUMENTS

P. Ambro

Introduction

Maintenance of instruments is generally of two kinds:

(a) corrective maintenance, on a non-scheduled basis, to restore equipment to a functional status by repairs;

(b) preventive maintenance, to keep equipment in a specified functional condition by providing systematic inspection, quality control, detection and correction of early malfunctions.

Most of the instruments used in nuclear medicine are rather complex systems built from mechanical, electrical and electronic parts. Any one of these components is liable to fail at some time or other. Repair could be done only by a specialist who is able to evaluate the condition of the various parts ranging from cables to connectors, from scintillators to photomultipliers, from microprocessors to microswitches. The knowledge of the intricacies of the various electronic components required for their repairs is quite wide and varied. The electronics industry turns out more and more multi-purpose chips which can carry out the functions of many parts used in the instruments of the earlier generation. This provides protection against unauthorized copying of the circuits but it serves another purpose as well of inhibiting repairs by non-factory personnel. The situation is further complicated by the fact that most of the manufacturers do not supply manuals required for the repairs with the instrument. This practice dictates the present state-of-the-art of repair technology. The factory engineer usually repairs the instruments by board-swapping, where the faulty board is replaced by a factory tested one. Board diagnostics are done with a computerized tool. This style of repairs is undoubtedly quicker and efficient than the old style of on-site manual trouble shooting and component replacement. On the other hand, the redeeming feature of the new instruments is the fact that the reliability of the newer electronic components is far superior to that of the earlier parts. This means longer periods between failures and less calls for repairs.

These trends of the instrument design should be taken into consideration when a policy has to be developed for the repairs of the hospital based equipment.

The term **preventive maintenance** is not so well defined. Ask any ten maintenance engineers, to describe preventative maintenance, and you will get ten different meanings, because it varies widely in scope and intensity of application. Some think of preventative maintenance only in terms of periodic inspections to prevent breakdowns, some add repetitive servicing upkeep and overhaul to it. Further along the way are those who study the maintainability, safety, and fitness of the equipment for the intended task before it is purchased and installed. It is well established that prevention is better then cure and that a well designed preventative maintenance program will yield benefits far in excess of its cost.

The main reasons for establishing a planned preventative maintenance can be summarized as follows:

(a) to protect investment in equipment, through regular and adequate maintenance, thereby ensuring a long life expectancy of the instrument.

(b) to safeguard return on investment by maximizing equipment utilization with minimum downtime,

(c) to ensure performance quality,

(d) to prevent waste of materials, tools and spares,

(e) to guide and activate maintenance personnel,

(f) to help in cost containment.

Engineering demands

Before determining a policy for the maintenance of equipment, one should take an overview of the needs of the institution or existing system for services. These will be dependent upon many factors and may differ widely between hospitals and countries.

Maintenance requirements are dictated by the number of instruments, their use (and abuse), environmental conditions that may accelerate degradation and failure. The number and type of instruments depend on the size of the hospital, the nature of its speciality services, local health problems, the extent of liaison with other nearby medical facilities and economic resources.

The number and the nature of existing instruments are good primary indices of service requirements. Climatic conditions will influence service demands. Electrical and electronic components are vulnerable to damage from high humidity. Solid-state circuits are sensitive to excessive heat. Scintillators might crack due to quick temperature changes. Dust or similar airborne pollution can deteriorate equipment.

A large nuclear medical centre has unique demands for maintenance expertise, depending upon the services it offers. Generally the consequence of equipment failure must be considered in assessment of technical service requirement. The question can be put whether a delay in repairing an instrument would endanger lives by delaying diagnosis and treatment and increasing the period of the patient's stay in the hospital. If so, in-house trained technical staff or quick access to external help must be considered as a basic requirement.

The quality of public utilities (such as electric power supply) also affects the overall maintenance demands. Nuclear medicine instruments are vulnerable to transient voltage fluctuations beyond the normal limits of tolerance.

What kind of service facilities are required depends on what kind of work the nuclear medicine unit is set up to do. The nuclear medicine units are classified as follows:

Type C: only in vitro assays are done. No in vivo work.

Type B: In vitro plus a limited amount of in vivo work such as thyroid uptakes, renogram, and imaging with rectilinear scanner.

Type A: In vitro plus comprehensive in vivo nuclear medicine facilities including gamma camera and a computer.

Table I
A summary of services usually associated with various types of nuclear medicine units described above

Services	Type of nuclear medicine unit		
	A	B	C
Radiation safety inspection	-	X	X
Instrument calibration	X	X	X
In-house repairs	-	-	X
Emergency repairs	-	X	X
Preventive maintenance	X	X	X
Purchase evaluation	-	X	X
Site preparation	-	X	X
Acceptance testing	X	X	X
User instructions	X	X	X
Physicist, part-time	-	X	-
Physicist, full-time	-	-	X
Engineer,full-time	-	-	X
Technician, mechanic	-	-	X
Technician, electronics	-	X	X
Coordinated training for staff on quality control	X	X	X

In all units, there is a need for daily routine radiation safety inspection, instrument calibration/quality control and periodical technical instruction on the use of instruments. The Type C Unit is primarily an in vitro diagnostic centre, where a failure of the instrument does not require emergency repairs. Often Type B Units are unable to keep an in-house staff for repairs. But in small units the medical, paramedical and the nursing staff traditionally accept some responsibility for the basic maintenance and safe use of their instruments.

In most countries, inspection service for new equipment is not available and purchase evaluation is done only for major investments like computers and cameras.

What is the best maintenance policy?

The dependence of modern health care upon technology, makes maintenance of all medical instrumentation a vital necessity. Obviously, equipment will need maintenance and medical institutions at all levels must consider the establishment of a service structure for this purpose.

The determination of a maintenance service must take into accounts the restraints imposed by socio-economic conditions, geographic location, the environment, the level of medical expertise, the available technical know-how, and even political factors.

The economic aspects of hospital equipment maintenance are based on the standard managerial concepts. Unfortunately, it is not usually realized that it may cost more to maintain equipment than to buy it, and that such continuing expenditures must be considered as part of the total investment.The additional costs are incurred at breakdown because of disruption of normal procedures and possible non-utilization of the radiopharmaceuticals. In economic terms, poor maintenance will lead eventually and inevitably to a waste of the nuclear medicine unit's overall assets.

An effective program for management of nuclear medicine equipment must be based on a realistic appraisal of its cost and available financial resources. It will be always dependent on the money available. One must therefore eliminate areas of service that are out of economic reach.

If a local health care authority chooses to establish its own service agency, it should examine the cost of staff, training, testing and repair facilities, spare parts, communication and transport etc. It should then compare these costs with those incurred in hiring commercial services plus occasional dispatching of defective equipment for repairs to an outside agency. Sometimes it is practical to utilize the maintenance services of government departments, research laboratories, or universities, but the consequence of inadequate repair or excessive delay must also be considered in deciding on such an alternative. The effectiveness of maintenance and repair may be influenced by accessibility, in terms of time rather then distance.

A comprehensive maintenance policy may include both in-house and contracted services. It is realistic to use the latter where available and concentrate the in-house facilities for services that are less accessible in the region. Hospital directors mostly support the contracted services concept on the basis that it often provides better services for less cost. The general recommendation expressed in simple words is "do not compete with outside services, collaborate with them".

MAINTENANCE OF NUCLEAR MEDICINE INSTRUMENTS

It is an almost inevitable conclusion that some in-house technical maintenance will be required in Type A Units. It is rather important that it should not be merged with the regular hospital maintenance services. Such form of organization has been tried in many centres all over the world with little success.

The central service agency concept works well if provision is made for prompt response to the needs of more remote parts of the system. It may be supplemented by regional representatives whose function is to provide routine preventative maintenance and minor repairs.

Within the framework of combined Co-ordinated Research and Technical Co-operation Projects in South-East Asia and Latin America, the International Atomic Energy Agency helped the participating Member States in the development and implementation of national quality control and preventative maintenance programs.

The development policy recommended by the IAEA is rather straightforward. In all nuclear medicine units, the quality control of the instruments should be introduced. Preventive maintenance programs were suggested and supported under the first phase of the project to overcome environmental and power-line related hazards on the equipment. During the second phase, the IAEA helped to improve the skills in local repair facilities and provided the necessary tools needed for such a work.

Some facts: The national program on instrument quality control in Asia was initiated in Thailand and India in 1983, the Republic of Korea in 1984, and in Sri Lanka and Pakistan in 1986. Now in Asia over 120 Nuclear Medicine Centres participate and every year 3 - 5 more laboratories join it.

In Latin America nearly 200 laboratories were surveyed by the national project supervisors. Now in more then 150 laboratories quality control and preventative maintenance are regularly practised using the IAEA-TECDOC-317 as a guide book.

Personnel

Most of the instruments used in nuclear medicine laboratories are rather expensive. They are complex devices with thousands of components in a maze of interrelated circuits. The development of a new gamma camera electronics system might take years of work of a team of ten to fifteen highly skilled specialists.

It is understandable that no matter how highly skilled an engineer may be, he will not be competent to carry out repairs on a highly complex proprietary instrument, like a camera, unless he or she has had a training in the factory of the manufacturer. The problem is that most designs contain patented solutions to certain problems. Unless specifically trained by the manufacturer, one has to nearly re-invent the maintenance procedure; for example, the fine tuning procedure of a linearity correction. The situation might be further complicated if even the circuit diagrams are not available. Under such conditions a capability is required

from the engineer to evaluate his/her limits in knowledge on the "least replaceable part" basis in specific cases.

Factories may authorize their trainees to conduct repairs on their instruments but only after acquiring the requisite skills. It is a general rule in industrial countries to allow repairs on cameras only by factory authorized personnel. Translating the concept into medical terms: one might not expect successful brain surgery from a dentist, although both might do drilling of some sort.

What training should the technical staff have in a nuclear medicine laboratory and where can they obtain it?

Since nobody is allowed to work in a radioisotope laboratory without a training on radiation safety this should be the first item on the list. Local courses for this purpose are available in most countries.

The entrance criteria for the technical staff should be set to a standard of at least a BTEC National or City and Guilds Part II Certificate or equivalent. Technical High schools can provide information on the compatibility of their degrees to those mentioned here.

For an in-plant camera course, a BTEC Higher National or City and Guilds Part III Certificate, a minimum of three years of practical experience and a successful language test are necessary.

According to experience the Part II Certificate holders can be effectively trained for the quality control tests but they should be provided with a technology guidance in a local language.

Some remarks: The curricula of the physicists in most countries do not contain the practical trouble shooting skills which are included in the Part II Certificate training. Do not expect such skills from physicists. You can be sure that the certificate level personnel will learn the quality control procedures in half a day. Strangely enough, most of the university graduates in electronics have similar problems, they are effective only in a team with a technician. Only thing, one can hopefully expect from an engineer or a physicist is an ability to read the instrument manuals and TECDOCS in their original versions!

It is worthwhile to list here the knowledge required from the maintenance staff for adequate servicing.

- Personal safety know-how in quality control, preventative maintenance and repairs (radiation, electrical, and mechanical)
- Quality control and operation of the instruments
- Test instrument selection and operational know-how
- Component tests and evaluation
- Circuit operation, expected signals and voltages in a good system
- Capability to understand operation and repair manuals
- Parts removal and replacement skills
- Safety of valuable components during testing and repair
- Diagnosis of faulty instruments
- Environmental hazards and protection measures
- Preventive maintenance planning
- Parts procurement, inventories, budget, costing.
- Self-evaluation on "least replaceable part" level.

What services can be expected from a Part III Certificate holder technician in a nuclear medicine laboratory?

He or she can reduce the number of faults traceable to environmental effects. They can repair most of the power-supply breakdowns. They can effectively run a preventative maintenance program. But they are unable to repair a computer or a camera without specialized training.

Selection of equipment

Choice of correct equipment to satisfy a specific clinical need is a complex medical, technical and a financial task. If coordination between specialists is poor, efficiency of the

desired medical service will be impaired. It is often said that commitment to Quality Assurance starts from the purchase decision. One should join an ante-natal clinic soon after conception and not after delivery of the instrument.

Too often hospital administration is guided by the promotional literature, offered by manufacturers or agents, or on the opinion of a specialist who has just returned from a tour abroad. Often the technical guidance of the people likely to be involved in maintenance is omitted from the decision making process.

Ideally, the local technical staff can and should evaluate maintenance needs, and the general maintainability of the available instruments in the local milieu.

It may come as a surprise to many that initial investment in equipment may be smaller than the overall cost of its maintenance, site preparation and the installation. Often a relatively small savings, due to procurement on lowest tender basis, does not take into account the life long maintenance cost. Ultimately it may turn out to be a case of penny wise and pound foolish!

The wise purchaser should examine the after-sales policy of the seller, are they prepared to provide good services at reasonable price, do they have the proper number of factory trained engineers, is their spare parts backup from the factory is well organized, are they financially sound to survive market fluctuations.

One should try to collect as much information as is possible about the manufacturers from as many sources as possible. Sometimes it may be wiser to buy the same brand which is in use at other institutions and where the manufacturer has built up a good reputation for maintenance. In such cases, it is much easier to solve the problems of maintenance, personnel training, spare part stock keeping and, since other users are nearby, one can gain from their experience.

A few words on donated instruments. It could be rather frustrating to receive an expensive system which is not fitting into the local infrastructure. One should examine these cases in the same way as for a fresh purchase because we have to take care of it latter on. The rule is not to be shy, speak-up in time, it is much more embarrassing to say one year after the arrival that the donated instrument is not operating because its maintenance contract is so expensive that the hospital is unable to support it. Similar situations can develop from bad estimation on operating costs. Say the colour hard-copy printer of the camera needs an ink set twice monthly for a cost of US $189.99, but the hospital has the budget of only US $500 per year for all consumables.

One should be rather careful with the ordering of the equipment. The delivery should be requested so that when it arrives everything should be ready for its reception. Sometimes the building is not ready, or the air-conditioners will arrive much later than the expensive instrument. It could happen that the electrical network in the hospital is unable to give the required amount of kiloWatts and severe voltage fluctuations due to this may inhibit

operations. Nothing is more frustrating than to observe a huge instrument case rotting unopened in the monsoon rains in the backyard of the hospital of a developing country.

Sometimes the buyers do not know that not only the instrument deteriorates out in the rain or under the sun, but all legal claims on damages can be lost if they are reported after the expiry date of the factory warranty. It is a general rule that all efforts should be made to install the new instruments as soon as possible.

Sometimes special accessory options are offered by the vendors for important investigations. Often they are unable to provide complete information on the special talents, if any, of the new instrument. One should analyze all offers carefully before the buying decision.For example one should not spend money on evaluating software for lung ventilation investigations done with $^{99}Tc^{m}$ labelled aerosols if the aerosol generator is unlikely to be available in the Unit.

Much care is necessary in negotiations for instrument purchase. Before one makes a decision the full technical specification should be obtained from all bidders. Such specifications should cover all components of the system and all options as well. Information must be available on power requirements. For example in SPECT systems rather powerful motors rotate the detectors. When they are turned on, the high current transients might trip fuses which were designed on the static power uptake data only. It is proper to seek advice from the manufacturer on capacity, and load distribution parameters of the line stabilizers. One can receive good answers if proper information is provided on local conditions like line voltage and frequency fluctuations and precise well formulated questions are asked.

Information should be at hand on temperature and relative humidity. Manufacturers can give information on requirements for expendable, their prices and availability in users area.

Quotations should indicate the price and terms, the date, mode and cost of delivery options (rail, ship, air), the nature and duration of warranty, and the cost and specific coverage of service contracts. Bear in mind that a factory engineer's service trip is rather expensive, when you have to pay for the travel expenses, hotel and daily allowance, service charges plus the price of the replaced components after the warranty period. One should be rather careful to make full use of the warranty given support.

The quotation should give information on the arrangements for installation, training on use, the accessories, spare parts, users and repair manuals, test devices, extension boards without which no repair or even board testings could be performed and expendable for the first year.

If you have an engineer or a technician with the above mentioned basic skills (Part III Certificate) a factory training could be negotiated. Some factories offer the training on the system which will be delivered, so your engineer might take part in the final quality control procedures on the same unit he or she will take care in the future. Such in-plant training could be very beneficial, because often the manuals leave out items considered "trivial" for

the factory staff, but which could be essential for the understanding of the system to a new man who can ask for explanations on the spot. Certainly the efficiency of the training depends on the language, technical and learning skills of the delegated. It is good to send someone who will be able to train personnel at home on subjects learnt abroad.

A few words on selection policy. An instrument with average performance but with good service support may well be preferred, to one with outstanding performance characteristics but inadequate servicing facilities. Maintenance of an instrument including the supply of spare parts has to be foreseen for its expected lifetime. Purchase price is an unreliable guide to what may be the total cost of an instrument. It is essential to allocate a reasonable sum, between 7% to 14 % of the capital cost annually, in order to keep instrument downtime under reasonable limits. Spare stocking is not a good investment. According to various surveys, an average of six percent was utilized in repairs only, the others lost value and were scrapped within ten years.

Site preparation

The placement of an instrument is largely determined by its expected use in relation to work patterns within the nuclear medicine unit. The availability of sufficient space, floor loading capacity in the case of heavy instruments like a SPECT, electrical power line and grounding, temperature and humidity control, protection against sand and air carried pollutants, good ventilation and lighting, easily washable floor and walls meeting isotope laboratory standards are all needed and should be taken into account appropriately.

One can have information from the manufacturers on required space, floor and electrical line ratings. They can provide drawings on recommended physical layouts, electrical connections and power requirements.

Less information is usually available for the proper design of the temperature and humidity control of these areas. The solution could be simple if the unit has been connected to the central air-conditioning system of the building. In such case one should follow up whether the services are on all day or only during daytime. If it is only a half-day type of service one should install an out-door type chilled water cooler to maintain stabilized temperature in the laboratory. According to the recommendations of the manufacturers the instruments should run day and night. In their stand-by mode they are dissipating several hundred Watts of heat which has to be removed.

If central facilities are not available an out-door type cooler should be installed. The window type units need more maintenance. Forced ventilation should be provided in compliance with the regulations for radiological laboratories and hospitals.

It could happen that due to energy saving regulations you encounter an opposition when you want to keep up the 24 hours cooling of the laboratory. Then fight. Temporarily, even for a week, a properly sized de-humidifier might save the condition of the instruments if they are switched-on. Under the worst conditions, you should try to turn on the instruments

minimum twice weekly for two hours to dry them out. Under such conditions the instruments should be covered while idling.

A few words on the temperature of the instrument area. One should try to maintain a stable temperature between 24°C and 27°C in the laboratory. Much cooler,say 18°C, might cause problems in two ways. The first is related to the maximum temperature gradient which may occur in case of a lasting power break during daytime. Scintillators in the camera detectors are guaranteed for only less than ± 5°C/hour temperature changes, if this is greater then this, the crystal may break due to internal stresses. The greater the temperature difference between the inside and the outside temperature, the greater is the likelihood that the dangerous differential may crack a five to seven thousand dollars worth component.

The second problem with the cold laboratories is the moisture condensation on the equipment during power failures. If the instruments are switched-off they cool down to room temperature, say 18°C. If outside humid air enters the laboratory, moisture formation will take place on the coldest surface in the room that is on the instruments. The moisture might affect the high-voltage system first, then the corrosion of the mechanical parts will follow with increasing failure rate and latter with general degradation.

Power supply

Mains operated electronic instruments are designed to receive energy in form of alternating current within a specified frequency and voltage range. Instrument performance could not be maintained if the mains voltage or the frequency drops below the limits given in the specifications.

Voltage fluctuations may result from many reasons, some types need emergency interventions by electricians, others could be solved only by investments.

Symptoms of power failures and the remedies

Lights abruptly flare-up and diminish in intensity. In such cases all instruments, refrigerators, air conditioners should be disconnected as soon as possible to prevent burn-downs. Electrician should be called to check the ground cable connection at the three phase distribution box. This failure is caused by a loose electrical wire connection which has a high resistance. This resistance might heat-up, if high current flows through and it may cause fire. One can test the condition of the junctions by touching them, certainly in switched-off condition. This can give a rough but quick guidance what should be done. The exact approach is to measure the voltage drop on it with a sensitive voltmeter under full load.

Slow voltage variations with regular daily patterns is the typical case of an overloaded electrical distributing network. In a hospital, one should know whether the same variation appears at the main step-down transformer too. If not, then the problem could be overcome by means of a "dedicated power-line" serving only the nuclear medicine unit directly from transformer. If the same level of fluctuation could be measured, then a constant voltage

transformer or a voltage stabilizer should be introduced between the instrument and the power line.

In some locations, the power failures are frequent during the working hours, resulting in a loss of measured data. The cheapest solution is to install a small motor generator. For five hundred dollars, one can buy a 250 VA unit which can overcome the problems of a Type C Unit. Some laboratories raise money for a so-called Un-interruptible Power Supply or UPS. This units contain batteries which are continuously charged from the mains. The stored energy of the battery is converted into AC and this feeds the instruments. The capacity of the batteries and the load determines how long can the UPS keep up the services during a power break. For example a 1 kVAh unit can deliver 2 kVA's for a half hour or 3 kVA's for 20 minutes. A local strategy should be developed for the power failures. One should decide on the priorities on the basis of available kVAh capacity. The running cost of a UPS is rather high, mainly due to the periodical battery replacement requirements.

In some areas severe problems might develop after the power brake. For a few seconds, the line voltage can be 50% higher then the nominal value. To overcome this problem drop-out-relays (DOR) are connected before the instruments. The DOR provides protection by a delayed action switch which gives energy to the instruments only after a minute when the switching-on transient has safely ringed down. Many air-conditioners and refrigerators are still not protected against this effect. In such cases the use of the DOR can be beneficial.

The IAEA has developed a DOR with some additional protective features against line voltage surges often encountered during tropical thunderstorms. If lightning strikes the overhead cables, short but very high voltage pulses might reach the instruments. The IAEA DOR can provide a high level of protection against such occurrences.

There are companies manufacturing voltage stabilizers or constant voltage transformers with built in DOR and surge arresters. These units are available in a wide range of power ratings meeting all kinds of needs.

A few words on instrument stabilization after a power brake. One should rely on the data provided by the manufacturer. This is the basic rule. Counters, sample changers and scanners are ready for use after 15 minutes in most cases. Some cameras without automatic uniformity and field correction might need two to twelve hours for stabilization. The best approach is to perform the IAEA recommended quality control tests after all fresh start-ups.

Air-conditioning

The operational temperature range of most nuclear medicine instruments is between 10° and 27°C. If the outside temperature is higher then this, the instrument area should be provided with proper cooling, sufficient to remove the heat generated by persons, instruments, lights etc.

One should take it into consideration, that in all nuclear laboratories, it is necessary to change the air several times every hour according to the Nuclear Safety Regulations. A scintillation camera and computer system plus patients, operators and medical staff together might represent 5 kWh or 12.400 BTU/hour maximum heat dissipation during working hours. This amount is less at night when the instruments are in their stand-by mode.

Without cooling, say, due to the breakdown of the central air-conditioning system of the hospital, the instrument area will be soon dangerously warm for the electronic system. In such cases, the instruments should be switched-off. Some instruments have thermal cut-off relays providing protection against overheating. However this is not available in all instruments and one should ask from the manufacturer on its availability in their product. If it is not available, one should be bought and installed.

If central air-conditioning is not available, then the use of out-door units are preferred against the window type air-conditioners. The periodical maintenance of these systems are essential for the safe operation of the electronics instruments. Filters should be cleaned regularly otherwise ice formation will take place and this increases the electricity bill.

Dust-free environment

As in all radioisotope laboratories the dust-free environment is a sine quo non condition. Floors and walls should be washable according to radiation safety regulations. It helps if street footwear and clothes are not brought into the laboratory. Frequent floor washing with clean water after vacuum-cleaning helps to keep up the necessary standards. Sweeping should be avoided. In some laboratories, the windows and the doors are not closely fitting. In such cases, first the necessary repairs should be carried out before the installation of the equipment. An un-clean radioisotope laboratory is a potential health hazard to the patients and the staff as well. The instruments are badly affected by the dirt in the laboratory environment. If dust collects on the surface of the electronics components, their cooling suffers and the elevated operational temperature leads to an earlier failure. Bad effect of sand on all mechanical systems is well known. During sand storms it is very important to switch-off and properly cover the instruments. The switching-off is needed to stop sand transfer into the systems by their own fans. After the storms, all air filters should be cleaned before returning to normal work.

Humidity control

Continuously running instruments are warm and since moisture formation takes place on the coldest surface in a closed system they are not affected by high relative humidity. Problems start when the instruments are not operating. Under such conditions, the instruments tend to be the coldest surfaces. If a de-humidifier operates in the room, the cooling coil of this device will be the coldest surface so the moisture condensation will be limited to that place. When a de-humidifier is not available, all instruments should be kept covered, this keeps them warmer by a few tenths of a degree and this can effectively inhibit the condensation.

Obviously de-humidifiers have only a limited capacity and they are efficient if the doors and windows are tightly closed. Rooms with persons and often opened doors could not be controlled properly by such techniques because a typical 200 watts power up-take unit can handle only 400 ml per hour while under tropical conditions more then 50 ml water can be present in a cubic meter of air.

Spares

In present day corrective maintenance, the circuit boards are replaced if they are faulty and the actual board repair is done in the factory with computer assisted diagnostic tools. Keeping in stock all the circuit boards which might be necessary for kind of eventual failures in the instrument, is a rather expensive approach. A typical board costs four to six thousand dollars. If the board is sent back to the factory for repairs, they charge 40% of the board's price even if it is a US $50 component which has to be replaced. The average cost of a service trip from Europe to Africa or from the United States to Latin America starts from US $6000. In an average camera there are eight to twelve boards so the spare kit will tie down a minimum of US $48 000. The interest from this sum if invested can provide for a service engineer's visit. The economics of spare keeping improves if more systems could be serviced by a central repair agency, having factory trained personnel with special tools, extension boards, pulsars and simulators supplied by the manufacturer.

The IAEA-TECDOC-426 [Troubleshooting of Nuclear Instruments] gives a list on components,tools and instruments needed in repair workshops.

Manuals

About ten years ago, it was natural to get not only the operators instructions with the equipment but the circuit diagrams as well. At the present time manufacturers provide circuit diagrams only on special order and the non-factory trained troubleshooters rarely have access to them. What is the problem and why did the manufacturers change their policy during the past years?

The development of complex systems like a SPECT is rather costly, it might be a few million dollars at least. The competition is strong and the unlawful utilization of technical achievements are easy if access to circuit diagrams is freely permitted. However there are other reasons too, for example, the protection of the local suppliers service organization. Only if the drawings are made available to them, there is some chance for a successful repair attempt by other parties.

Even if the drawings are available but you do not have the description of the alignment procedure, it takes quite a lot of sweat and luck to reinvent an efficient approach.

Sometimes the electronics boards are of the multi-layer type with surface-mounted parts, just like in computers. Without proper tools and parts-removal technology generally the repair attempts end up in the complete destruction of the board. A faulty, destructed board

might cause more harm, so the policy of the manufacturer is not to provide any information in order to deter tampering.

The form of the operating manual has also changed. Earlier, it was a listing of the control elements only, latter some applications were included, now some contain a self-paced educational package with multiple choice tests as well. Some companies offer the manual both in paper and diskette form. The latter has advantages, one can make as many copies as it is needed and the loss of the diskette is less likely. Most company service engineers take their "repair manuals" in such form too instead of the heavy fat books.

Even if one might succeed in getting the repair manuals from the factory, one can make real use of it only if a factory-based training was provided.

Role of the international organizations

The IAEA has various programs to co-operate in the training of local specialists for nuclear medicine instrument maintenance. Within the frame work of the Asian and latter in the Latin American Regional Co-operative Agreements, national and multi-national training courses and work-shops were organized with the participation of local and foreign lecturers, on quality control, preventive maintenance and repair of instruments in nuclear medicine. In 1990, a similar program has started for Africa.

For individual training, fellowships are available both from the IAEA and from the WHO. Such programs are really beneficial if the delegated person already has acquired a good repair practice, has no language problems and is willing to transfer the acquired knowledge to the colleagues. The length of such fellow-ships range from one to six months. The in-plant training are rather intensive and more efficient then the others, because they are focused on that special equipment which is likely to be installed in the nuclear medicine unit.

According to surveys, out of four trained persons, one person is lost after the first year and a second leaves after the third year on an average from the delegating nuclear medicine unit. This is an area where the international organizations are unable to help. The proper "fixation" of the trained specialists has to be solved locally by providing adherent incentives.

Since 1979, the IAEA operates an emergency spare part supplying service, to overcome local convertible currency problems inhibiting the repairs. Every year, few hundred institutions are served from this spare part bank in Vienna with hard-to-get components.

Role of the PC in maintenance

In all fields of activities efficiency could be achieved only by good planning and conscious supervision. Personal computers can help these efforts if proper software are available. Organization of maintenance requires information from several data bases. First there should be an inventory on all the items which has to be taken care, second a data base must be available providing data on the estimated repair costs of the items and on their life

expectancy. A data base should cover the available skills, tools, manuals, for the repair of the items. Data should be stored on the instrument locations , environmental and supply conditions. For work load capacity calculations there should be data on the estimated repair times and on the time required for the regular preventive maintenance and quality control inspections. If the number of items one should take care is under thirty the planning work could be done manually if proper training is available in the house to do this job. The advantage of the computer is that one can feed in data requested by the soft-ware and the organizational work, budget and replacement planning, preventive maintenance scheduling will be done automatically. The great advantage of the PC that it efficiently can monitor and supervise the work done. It can evaluate the local efficiency against the international standards and it can pin-point in which areas should be the available skills further developed.

A PC based system is able to analyze minute trend changes in instrument parameters, warning signals can be had much before major breakdowns occur. For example isotope orderings can be adjusted in a planned way to expected repairs or overhauls, such actions can save money and down-time.

In the future more and more educational soft wares will be available on quality control, and the operational and repair manuals will be on diskettes so the need for a PC will increase.

Troubleshooting

Standard electronics skills are not enough for the efficient repair of the nuclear medicine instruments, even nuclear instrument practice is not enough to handle properly a problem in a scintillation camera detector system. The minimal know how was condensed into the IAEA-TECDOC-426. Anybody wishing to know more on the subject should refer to this book as an introductory material.

The reliability of the electronics systems is increasing but the improvements are rather few in respect to cable and connector failures. The first question of the service engineers arriving to a bad instrument are generally whether the instrument was moved recently to a new location, because most of the cable problems are developed during such operations.

Often the board connectors are affected by the transportation and the engineer has to pull them out and push it back only to overcome the tiny misplacement leading to faulty operations.

The next item is to check the back-up battery of the computer memory unit. Sometimes even after a few days of switched off state, a two or more years old rechargeable battery might stop operations of an otherwise completely good system.

The next item on the quick diagnostic check-list is the power supply. To be able to check it certainly one should know where and what should be present for good operation. This test requires practice because a bad setting of a meter for example set for current measurements when the high voltage is attempted to be measured might destroy a H.V.

power-supply and even can cause a severe electrical shock. The rule is do not allow tampering with expensive instruments. Only personnel with proven and certified knowledge should plan the diagnostics and make decisions on the repairs. Generally power supply related problems are easy to identify and repair.

All operators should be informed how to switch-off the instruments in case of emergency:

- when smoke or burning cable smell is present,
- when sound of sparking is heard or displays blink,
- when loud or unusual sounds are heard from the system during operations,
- if collimator could not be securely fixed
- if movements are uncontrollable
- if detectors were contaminated by patients.

If outside help is needed for the repairs it is advantageous if information could be provided on the nature of the problem. It is absolutely essential to write down in the instruments log book the so called reference settings of the instruments. Before any diagnostic procedures, one should put all control elements into this reference setting and only after this one should start with the functional checks. If skills are available one should try to follow the signal in the instrument. Say if a radioactive source is before the detector, do we have the scintillation pulse, does the clock operate in the CPU etc. During the factory training all check-points are explained, even on some drawings, this information is provided so with some skill one could find out where the fault originates.

All information related to the instrument should be entered into a log book. The first page should be dedicated to the reference settings, on the second page should be the names of those operators who passed the test in operation skills. It is a good practice to have it in a written form who is permitted to operate the device.

The log-book should contain short description of the quality control procedure in *local language* and the results of the tests should be entered with date and signature. It is a good practice to mark on wall-charts the most important parameters of the tests. This approach helps to motivate personnel on the continuation of the quality control tests. If the head of the laboratory checks on every occasion this and explains the efforts of the staff to visitors. After the daily work a closing remark should be entered on special occurrences, if there were any.

It is very important that the last operator's name should be available in the records because in case of a breakdown, the service engineer will need information on the last hours of operation.

All repairs, transfers, room modifications, air-conditioner and voltage stabilizer repairs should be entered in the log book. It is a good practice to enter the cost of the repair as well to increase the feeling of the responsibility of the staff for the high value of the instrument.

Cost

Preventive maintenance proved to be a rather efficient approach for the reduction of the repair and major breakdowns related expenditures. Airlines spend more on preventive maintenance testings then on actual repairs because this is the only way to secure the necessary reliability needed for airborne transportation. In space technology, the spending is even higher on the preventive maintenance because the failure of a rocket might cost hundreds of million dollars. The relationship between reliability and cost of repairs and cost of preventive maintenance are given in the **Fig. 9.1.**

The important feature of the diagram is that a minimum exists in the total cost curve as the preventive maintenance is increased. It is a well established fact that bad environmental conditions reduce the mean time between failures compared to laboratories in mild climate The same laws apply for the electronic instruments, in this respect, as for human health or for automobiles.

The total cost curve, however, changes during the life of the instruments, they need more care as they are aging. It is a very important financial aspect of this problem, how long should one keep alive the instruments? The cost analysis gives different solutions in different economical systems. In high GNP category countries, the limit is under five years. In poor countries when they try to extend the life of already old instruments, they more often loose than gain. Old instruments fail more often, this increases the repair costs, down time increases and the cost of the old components is always higher because they are hard to get. In addition, the climatic stress also becomes more troublesome. In the preventive maintenance soft-ware of the IAEA some data is provided on the life expectancy of various instruments under various climatic conditions.

Mechanical safety hazards

More attention is usually paid to the electronics maintenance and repairs but the electrical and the mechanical components of the instrument are equally likely to break down.

To improve the signal-to-noise ratio of the nuclear detectors heavy metal shielding is put around them. They consist of two parts. A fixed part serves to reduce the background and an interchangeable part called collimator determines the field-of-view of the detector. The weight of the shielding of a single probe detector might be between 15 and 30 kg. In a scanner, this can be up to 60 kg, while it could be several hundred kilograms in a camera.

The weight of the collimators range between 7 and 25 kg.

Many accidents have been reported from time to time with these heavy parts from all over the world. While the mechanical designs of the modern systems have improved the safety features, many old units, still in use, are potential hazards. By regular preventive maintenance check-ups and proper administrative measures this problem could be overcome.

About 60 % of the accidents happen during the changing of the collimators. It happens often when the technician tries to change the heavy collimator without the factory supplied tool. It slips out from the hands and after falling can break bones, furniture and floor. In many cases, the part itself is distorted as well. Another 38% happens after the collimator is inserted into its place but the position securing mechanism is not functioning properly due to worn out screws, disabled locking mechanisms or plain carelessness in securing the locking system or screws as much as necessary. In such cases, the component may not drop too far down and with a little bit of luck, no one may be injured seriously but in any case, such accidents are quite unnerving to both the patients and the technicians.

In accident prevention, it is an effective administrative measure to give out orders that collimator changing should be done only with the proper factory supplied tools and after the insertion a second person should check the safe locking of the fixing mechanism. (Sometimes the problem is related to the physical strength of the technician; one should take care of this too).

The preventive maintenance check-up should evaluate the safety of the fixing elements and their operation, no worn-out or missing screws, distorted locking elements should be tolerated. The safety awareness of the operators should be maintained by repeated training on this subject.

Another hazard exists around the supporting and positioning mechanisms of the detector shields. Some of the probe stands are spring or weight balanced to make the positioning of the heavy detectors easy, and few have motors to move the detectors. In many of these stands, a steel rope and a pulley system connects the balancing system to the detector. Neglected maintenance, such as greasing of the rope and the pulley, might lead to accident because of the deterioration of these components. Care should be taken of the emergency brakes as well, because a corroded mechanism could not save the patient from a falling detector.

In the motor-driven-stands an acme screw system positions the detector in vertical direction. Preventive maintenance should be performed on a routine basis around this component. During a visual inspection, one should check for any debris from the bottom of the acme screw. All debris should be carefully removed and checked for metallic parts, because this is the early sign of wear or beginning of misalignment. The screw should be greased as often as possible with proper factory recommended quality and brand. It is very important that the grease should be rubbed on full acme screw length and the trolley must be run up and down to spread the grease evenly.

During the greasing, all loose bolts should be tightened. Cables near the screw should be checked for fraying, wear or friction effects because they can lead to accidents. The checking should include the operation of all limit switches and safety relays as well.

It is a sound practice to check the correct operation of the patient contour safety switches before each investigation to overcome physical trauma or discomfort to the patient.

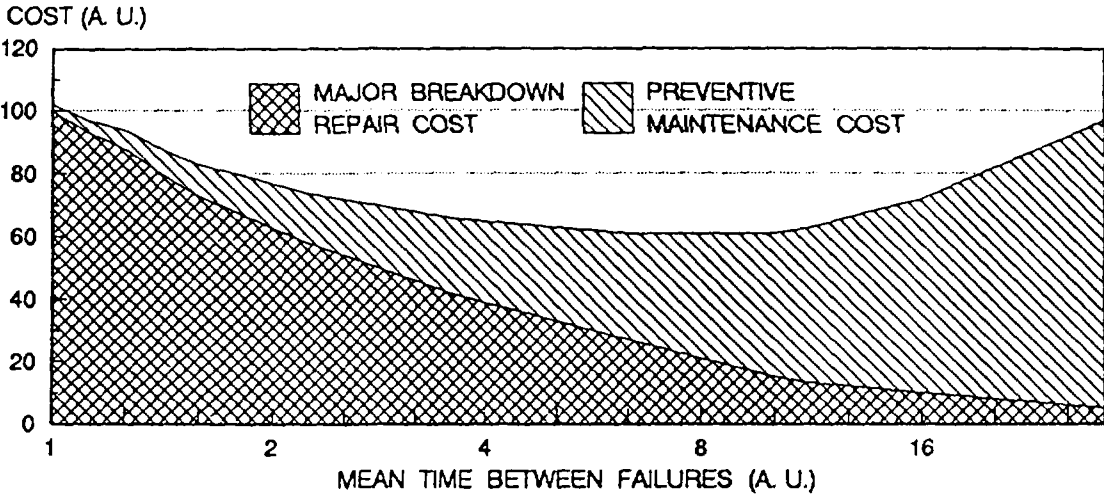

Fig. 9.1 Typical measuring instrument maintenance cost distribution versus mean time between failures.

CHAPTER 9

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Chapter 10

ENVIRONMENT FOR THE INSTRUMENTS

P. Ambro

Power conditioning

A properly conditioned AC power supply is necessary for reliable functioning of instruments. Electric mains power is produced primarily for industry, workshops, lighting and household uses. Its quality is adjusted to these uses. In areas and countries with a fast growing demand for electric power, these requirements are far from being met. Electronic instruments and computers, especially in these countries, need protection against disturbances of the mains supply.

Mains disturbances can be categorized as:

(a) voltage fluctuations,

(b) power failures, sometimes for few seconds and sometimes for several hours,

(c) frequency fluctuations,

(d) harmonics,

(e) transients and

(f) electric noise.

To prevent instrument damage by erratic power supply, an effective and economical power-conditioning system is necessary for the nuclear medicine departments. **Fig. 10.1** shows a scheme for this purpose. It consists of a dedicated power line (i.e., a separate shielded power line from the last step-down transformer), which reduces interference from other power users, a drop-out relay with varistors, which prevents damage by power failures and transients, a ferroresonant constant voltage transformer, which filters out transients and noise and minimizes voltage fluctuations and a dedicated earth line, which prevents interference from currents to earth from other laboratories and workshops **(Fig. 10.1)**.

Air conditioning

A clean and dry environment is needed for reliable functioning and long life of instruments. High humidity, specially at higher temperatures, changes the characteristics of electronic components. Moreover, under these conditions fungal growth causes leakage of currents and corrosion causes poor contacts. The presence of dust enhances these effects. They give rise to malfunction of instruments, particularly of high voltage equipment.

Guidelines for an economical air-conditioning system to be used in tropical areas are drawn up with the aim of the well-being of instruments and for the comfort of staff and patients using the equipment. Economical air-conditioning requires minimizing heat, dust and humidity. Therefore, walls and ceilings must be good heat insulators and double window and door systems must be installed. Windows, doors, and other openings should be kept closed and well sealed as far as possible and direct sunlight should be screened off. To dehumidify most effectively and to cool most economically, two air-conditioners, each of half of the needed capacity, should be installed. One could run continuously and one be controlled by its thermostat. Patients, usually partly undressed, feel uncomfortable when exposed to low temperatures and cold drafts. Detectors can break easily when temperature changes quickly or unevenly. The water condenses readily on cold instruments. Therefore, the temperature in the laboratory should usually be about 8°C lower than the outside temperature. The airflow of air-conditioners should not be directed at patients or instruments. The relative humidity around 50% would be a best compromise between care of instruments, comfort of persons and cost (**Fig. 10.2**).

Figs. 10.1 and 10.2 were prepared by P. Vuister, a former member of the staff of Nuclear Medicine Section, Division of Life Sciences, IAEA.

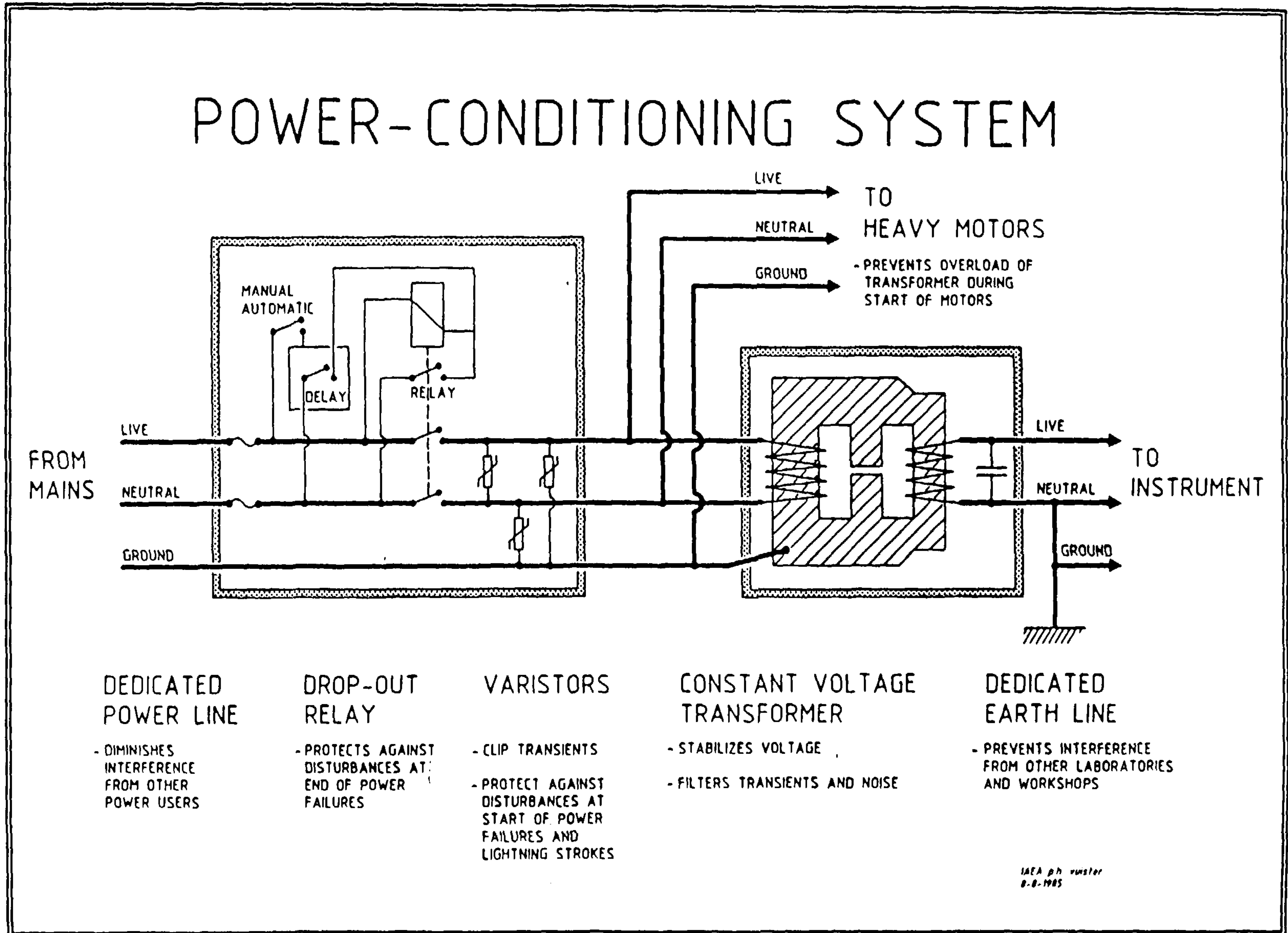

Fig. 10.1 Power-conditioning system

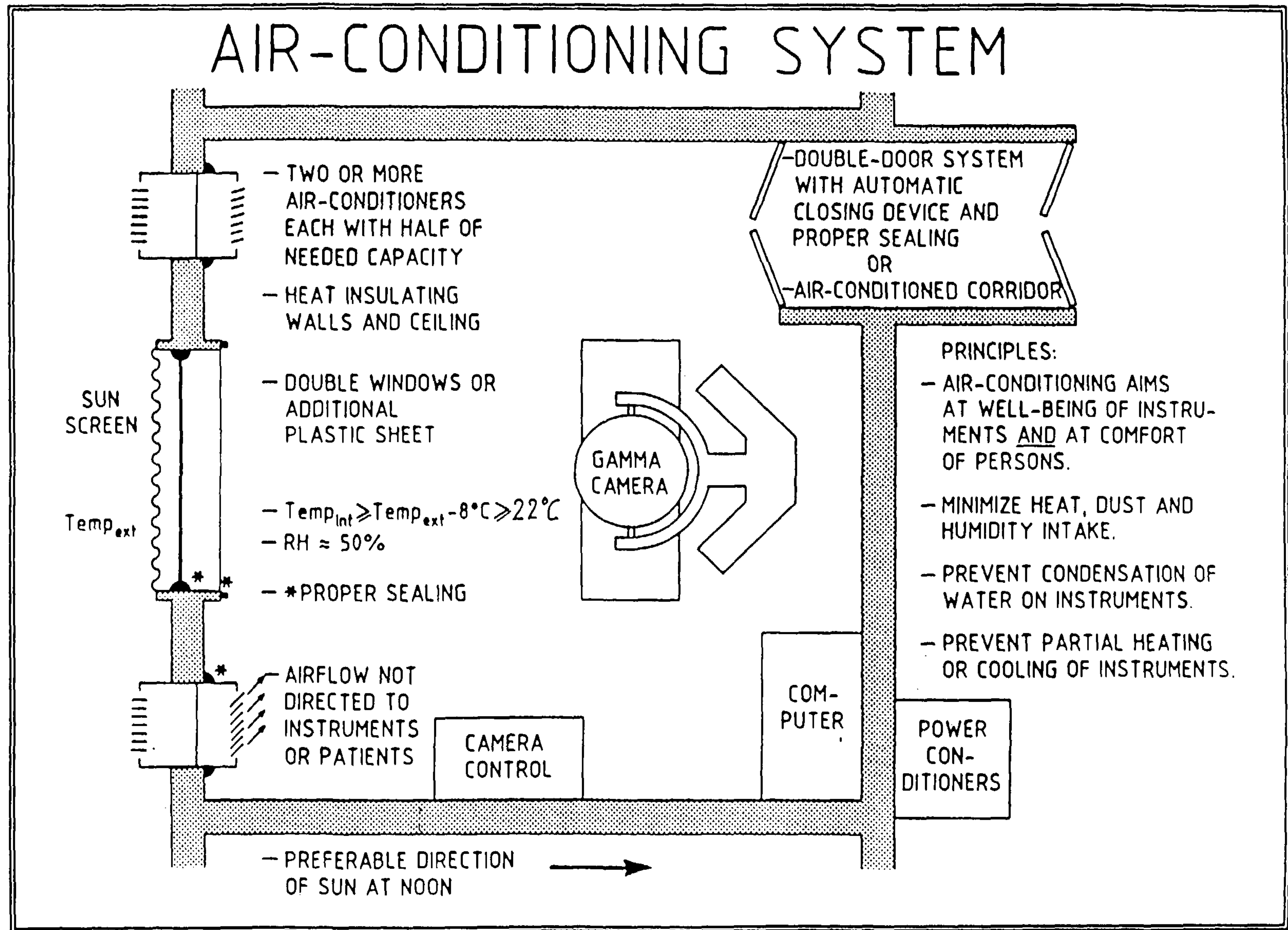

Fig. 10.2 Air-conditioning system

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Chapter 11

RADIOPHARMACEUTICALS

R.D. Ganatra

Introduction

Speaking in April 1989, at the "Fifty Years with Nuclear Fission" conference, Rosalyn Yalow observed that radionuclide procedures for medical diagnosis are received by more than one-third of all patients in U.S. hospitals. Yalow, who received the Nobel Prize in 1977, for her work on radioimmunoassays commented, that this was possible because after the World War II, reactors made available much more abundant, and much less expensive supplies of radionuclides. Today there are an estimated ten million nuclear imaging procedures, performed each year, in just the United States, and the number is still growing. More than 30 000 therapy procedures are performed in the USA each year using radiopharmaceuticals. Moreover, while the numbers continue to grow, so also do the variety of the procedures being employed.

A weakness of nuclear medicine is related also to one of its strengths. Unlike other types of imaging where only an instrument and the patient are required (e.g., with ultrasonics); nuclear medicine requires a radiopharmaceutical. At the same time, the variety of radiopharmaceuticals offers the ability to trace one or more particular functions of the human body. This provides nuclear medicine with great versatility in detecting specific pathologies. Various nuclear medicine studies are possible because of the localization of radiopharmaceuticals in different organs.

Mechanisms of Localization

There are various ways in which a radiopharmaceutical localizes in an organ. In the present day nuclear medicine, the emphasis is on the radiopharmaceuticals, which are function-specific or pathology-specific.

(a) Physiological or metabolic: e.g. radioiodine in the thyroid gland, radioiron in the bone marrow;

(b) Precursor-Product relationship: e.g. Labelled cholesterol for adrenal, Selenomethionine for the pancreas;

(c) Pools: e.g. Blood pool as with labelled RBCs, Placenta;

(d) Particles: e.g. MAA Albumin for lungs, denatured RBCs for spleen, labelled colloids for the reticuloendothelial system;

(e) Passage: e.g. hepatobiliary agents, labelled Hippuran for the kidneys;

(f) Permeability: e.g. altered blood brain barrier for cerebral agents;

(g) pharmacological: e.g. localization of a drug at the site of action, receptor binding of a labelled drug or a biochemical transmitter;

(h) immunological: e.g. labelled monoclonal.

It would be seen from the above list that truly metabolic agents are few. Most of the biologically important substances have C, H, N, O as integral part of their structures, but unfortunately all these radionuclides are produced in the cyclotron and they are all having short half life. Metabolic agents can be prepared by ingenious chemistry, whereby a technetium is introduced in a biologically important molecule. However, in such labelling, it is necessary to ensure that the labelling procedure itself has not altered the functional integrity of the compound. As the technetium has optimum physical characteristics for use with the gamma camera, a large amount of research effort is directed towards labelling suitable chemical moiety with Tc. The chemistry of Tc is quite complex and that has been one of the limiting factors in the radiopharmaceutical research.

Distribution studies

The commonest and the easiest way of testing biological behaviour of a labelled compound is a distribution study in experimental animals. Usually mice or rats are commonly used for such a study. The labelled compound is administered to a set of animals. They are sacrificed at various time intervals and all the organs taken out and counted in an appropriate scintillation counter. The results of distribution study are expressed in a variety of ways, all of which are not very meaningful.

(a) as % of the administered dose in an organ. This is the best way, when evaluating a compound for scintigraphy.

(b) as % per gm of an organ. Useful for evaluation of toxicity.

(c) cpm / gm. For pilot studies, it may be acceptable but for batch to batch comparison, it is not very ideal.

A compound is suitable for scintigraphy studies only if it achieves optimum concentration during the effective life of the compound in the body.

Radioiodine

Iodine is a highly reactive chemical and as radioiodine has been used to label many chemicals. However, the physical characteristics of ^{131}I are far from satisfactory. The energy

is very high for the thin crystal of the gamma camera. Its fairly long half life and the beta radiation can give considerable radiation dose to the patient.

As ^{125}I, it is quite suitable for the in vitro procedures. A large number of hormones, vitamins and proteins have been labelled with this radionuclide for radioimmunoassays. Cyclotron produced ^{123}I has physical characteristics acceptable for the gamma camera and many of the newer radiopharmaceuticals have been labelled with this radioisotope. However, its short half life and its production in cyclotron has made it unavailable in many of the developing countries.

Cows

A radionuclide generator (cow) has a parent radioisotope loaded in a suitable column from which a daughter radionuclide is eluted by a simple chemical procedure **(Fig. 11.1)**. In the most widely used Mo-Tc generator, ^{99}Mo is the parent radionuclide, available on an alumina column, from which the daughter product Tc is eluted by a passage of normal saline. In another commonly used generator radioactive Tin is loaded on a zirconium column from which $^{113}In^{m}$ is eluted by passing dilute hydrochloric acid solution.

The use of short half life radionuclides in nuclear medicine is not an unmixed blessing. Use of these radionuclides puts constraints on the chemical manoeuvring that can be done, because the short half-life of a radionuclide limits the time available for processing, transportation, storage and quality control. The greatest advantage of a radionuclides generator is that it allows the use of short-lived radionuclides at long distances from the site of production.

A radionuclide generator is nothing but a radioactive series in which a long-lived radionuclide parent decays into a short-lived radionuclide daughter. Some examples of commonly used generator systems are given below:

(a)	^{99}Mo >	$^{99}Tc^{m}$ >	^{99}Tc >	^{99}Ru
Half-life:	67hr	6hr	long	stable:
(b)	^{113}Sn >	$^{113}In^{m}$ >	^{113}In	
Half-life:	115d	1.67hr	stable.	

In a generator, the daughter radionuclide is being continuously produced by the decay of the parent radionuclide. The daughter product also undergoes decay at its own decay rate. If the half-life of the parent radionuclide is longer than the half-life of the daughter

radionuclide, in due course, an equilibrium is established between the parent and the daughter radionuclides. In a state of equilibrium, the ratio of the two radionuclides becomes constant. In fact, it appears that the daughter apparently decays with a half-life of the parent.**(Fig.11.2)**

Once equilibrium is established, the two radionuclides can be separated only by chemical means. In a typical Mo - Tc generator, the column is filled with alumina, the ^{99}Mo is loaded on the column as sodium molybdate, and the eluting solution is normal saline. The elute is in the form of sodium pertechnetate. The Molybdenum loaded in these generators is produced either by bombardment of molybdenum with neutrons in a reactor or by fission of ^{235}U in a reactor. Molybdenum produced by fission is carrier free and has a very high specific activity. On the other hand, ^{99}Mo produced by neutron irradiation has low specific activity.

The following characteristics of a generator define its suitability for clinical use.

(a) the elution process should be convenient and quick.

(b) The efficiency of a generator is defined in the following manner:

$$\textbf{Efficiency} = \frac{\textbf{amount of activity eluted}}{\textbf{Total daughter activity on the column}} \times 100$$

(c) Parent breakthrough, as low as possible .

(d) Radiation Shielding: the generator should be properly shielded for the safety of nuclear medicine personnel.

(e) Specific concentration: This is defined as the number of mCi per millilitre of the elution. In dynamic studies, it is important to have a small concentrated bolus.

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^{99}Tc^{m} has a short half life of six hours and its physical characteristics are very suitable for use with gamma camera. The parent ^{99}Mo has a half life of 67 hours, which makes it necessary to obtain it twice a week. For those countries, which depend on their supply of radiopharmaceuticals on foreign countries, this kind of frequent import against foreign exchange, is a big hassle.

In the case of tin - indium generator, the parent has a long half life of 280 days and the daughter product has a short half life of only 100 minutes. Unfortunately, the physical characteristics of ^{113}In^{m} are not very suitable for use with the gamma camera. Its gamma

energy of 394 keV is too high for the thin crystal of the gamma camera. Otherwise, the long half life of the parent requires its import only twice a year. As the demand for this generator is very low, its commercial cost is quite high. The total cost of weekly import of Tc generator is comparable to twice a year import of the Indium generator.

The generators commonly used for clinical work have a longer half life parent and a short half life daughter radionuclide. There can be inverse cows, where daughter has a longer half life than the parent but there is no practical advantage of using this type of a cow. The former type provides a convenient source of a short half life radionuclide in the nuclear medicine department. The use of this type of cow has virtually revolutionized the present day nuclear medicine.

With the use of a generator, it is necessary to obtain the generator and kits containing non-radioactive chemicals. The radioactive elute and the kit are mixed together prior to its use in the nuclear medicine department itself. This puts the responsibility of the quality control of the final preparation on the user. A radiopharmacy thus becomes an integral part of a nuclear medicine unit. The kits are usually very simple to prepare but the attendant quality control guarantee make them quite expensive. There is a tendency on the part of the developing countries to lower the recurring cost of the kits by producing them in the hospital radiopharmacy itself. With a trained staff, it is not a difficult task. More attractive approach in a developing country is that of a central radiopharmacy, where several laboratories in a large city or in a country, pools their resources together to set up a central radiopharmacy as a collective venture. The subsequent chapter deals with this kind of venture extensively.

Ideal properties of a radiopharmaceutical

= the compound should trace some identifiable biological process;

= the radionuclide label should not alter the biological behaviour of the radiopharmaceutical;

= target to non - target ratio should be high to permit good imaging;

= the radionuclide should have physical characteristics, like the energy of the photon, which are acceptable to the imaging device. For use with gamma camera, a monochromatic gamma energy between 100 and 300 keV is preferable.

= effective half life of the radiopharmaceutical should be such that it permits gathering of all possible information. It should not be unduly long so that the radioisotope remains in the body, long after all the requisite information is obtained.

= the carrier amount associated with the radiopharmaceutical should not be such that it produces undesirable side effects.

Nuclear medicine's primary role has shifted over the years; a shift that demands new radiopharmaceuticals for dynamic functional studies. The early imaging was the observation of an essentially static event: the monitoring of radioactive "tracers" after they had distributed throughout the body. Today's techniques emphasize the observation of the tracers, while they are moving through the body; consequently, today's methods are better able to discern whether the body's organs are functioning as they should. Research, too, has benefitted from these changes. The ability to image static and dynamic aspects of physiological functions has opened up a number of new areas of research.

The developing countries have not realized as much of the benefits of nuclear medicine as have the developed nations. The needs of the developing countries are for the simpler nuclear medicine work procedures and for the less complicated equipment. However, radiopharmaceutical research is one area where developing countries can contribute a lot because investment in terms of men and money is not very high for this purpose.

Nuclear medicine now influences, to a lesser or a greater extent, all branches of medicine. It now plays an important role in maintaining and improving the health of millions of people, and that this role is still expanding.

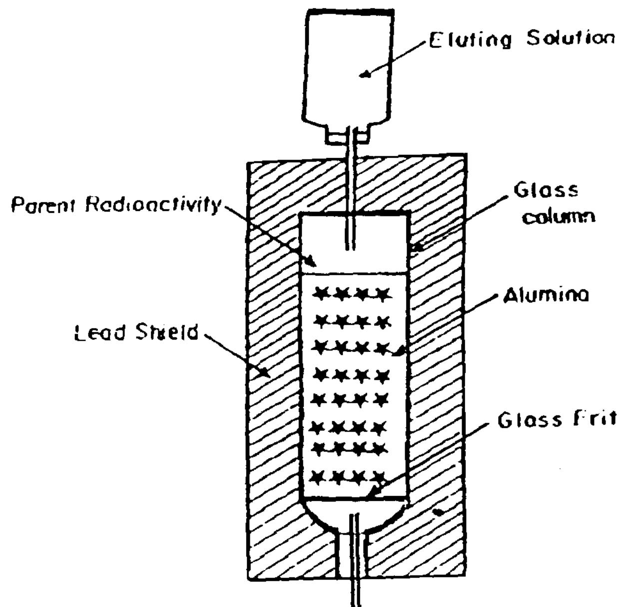

Fig. 11.1 Schematic diagram of a radionuclide generator

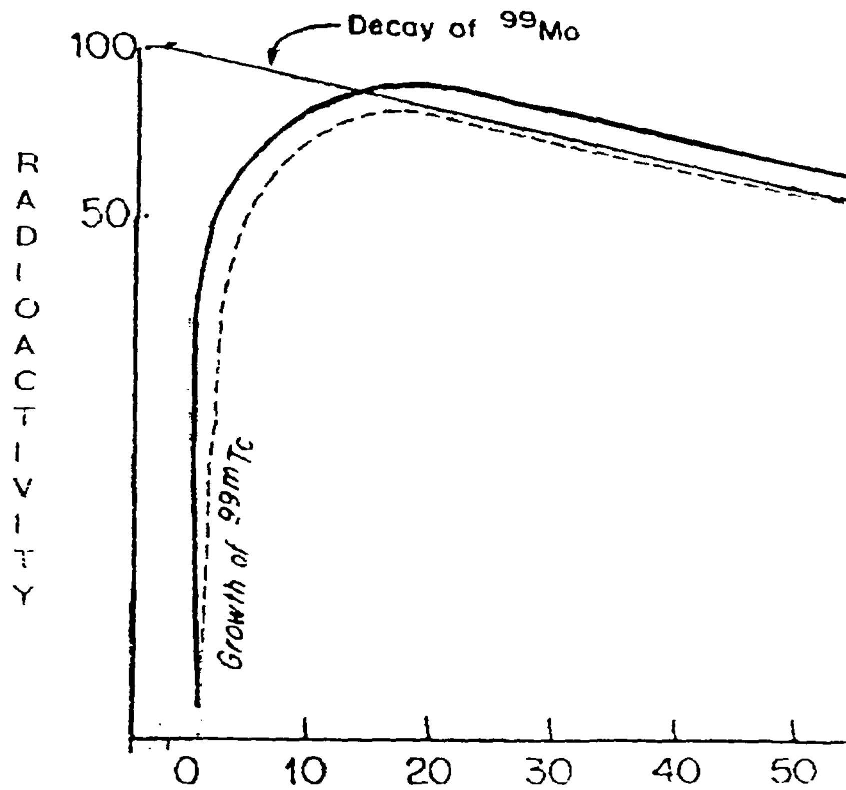

Fig. 11.2 Decay curve of ^{99}Mo and ^{99}Tc^{m} in the generator, showing that after few half lives, an equilibrium is reached between the parent and the daughter

CHAPTER 11

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Chapter 12

RADIOPHARMACY PRACTICES

R.S. Mani

Introduction

Ready availability of a good range of radiopharmaceuticals is an important prerequisite for the organization of nuclear medicine service. Table 1 gives details of the radiopharmaceutical preparations which are currently used in nuclear medicine and their main applications.

With the exception of cyclotron-produced short-lived positron emitting radionuclides and their compounds, which are employed in positron-emission-tomography (PET) in advanced countries, all the radiopharmaceuticals listed in this Table are of interest to developing countries for providing cost-effective and practicable diagnostic and therapeutic nuclear medicine procedures.

The major part of nuclear medicine applications - covering more than 80% of all in-vivo applications - is accounted for by preparations of ^{131}I and $^{99}Tc^{m}$. The favourable nuclear characteristics of ^{131}I and $^{99}Tc^{m}$ and the versatile chemistry of the elements I and Tc, have enabled the development of many labelled compounds, and have given the pride of place for these two radionuclides in radiopharmacy.

Radiopharmaceutical preparations of relatively long-lived radionuclides (with half-lives ranging from few days to few months) are available from many commercial suppliers; in addition, national atomic energy organisations in many countries have undertaken programmes for their production and supply to meet the national needs. $^{99}Tc^{m}$ is available in the form of generators which consist of the parent ^{99}Mo retained on a column of alumina from which the $^{99}Tc^{m}$ is eluted out with normal saline. Other generator systems which enable the separation of the daughter $^{99}Tc^{m}$ from the parent ^{99}Mo by selective solvent extraction or sublimation are also in use in some countries. $^{99}Tc^{m}$ is obtained from the generator in the form of pertechnetate solution; from the pertechnetate the $^{99}Tc^{m}$ is reduced to a lower valency cationic state and is then converted into various labelled pharmaceuticals.

Preparation of short-lived radiopharmaceuticals produced from generators is usually undertaken in the hospital itself in a facility - called hospital radiopharmacy or a centralised radiopharmacy if it caters to the needs of more than one user hospital. Such centralized service may also undertake bulk imports of longer-lived radiopharmaceuticals and radiochemicals, dispensing and dose preparation, preparation of labelled compounds, quality control and training.

This chapter describes the specifications of the commonly used radiopharmaceuticals, preparation of dosage forms of longer-lived products, in-house preparation of "kits" for short-lived generator-produced radiopharmaceuticals, their formulation and quality control. Relevant aspects of good radiopharmacy practice, organisation of a radiopharmacy facilities

are briefly reviewed keeping in view the requirements and conditions prevailing in developing countries.

^{131}I-radiopharmaceuticals

In the initial years of nuclear medicine ^{131}I was the work-horse and radiopharmaceuticals of ^{131}I were more frequently employed than those of any other radionuclide. ^{131}I radiopharmaceuticals are still widely used in many developing countries for imaging with rectilinear scanners which form the main-stay of nuclear medicine in these countries rather than the expensive gamma cameras which are still not available. Sodium iodide ^{131}I in the form of aqueous solution or absorbed on anhydrous sodium phosphate or other suitable absorbent in gelatin capsules is widely used for thyroid investigations and therapy of thyrotoxicosis and thyroid cancer. The specifications and characteristics of these products are given in appendices.

Sodium iodide ^{131}I

Sodium iodide ^{131}I in solution form suitable for oral or intravenous human administration is obtained by chemical processing of reactor irradiated tellurium targets. In addition to ^{131}I, other isotopes of iodine (^{127}I and ^{129}I) are also produced by this irradiation. The formation of these isotopes dilutes the specific activity of the ^{131}I.

Labelled pharmaceuticals of ^{131}I

The ease with which iodine in its oxidised state labels many organic compounds has proved very useful for preparation of many ^{131}I labelled compounds of interest in nuclear medicine. Labelling of proteins is achieved by iodine substitution for hydrogen at the tyrosine unit; the imidazole ring of histidine is also labelled at higher levels of substitution of iodine involving several atoms of iodine per molecule of protein.

Radioisotopes of iodine can also be readily incorporated into many iodo-organic compound (example - Rose Bengal, Hippuran, Diodrast etc.) by exchange of radioiodine for stable iodine. Many compounds whose molecular structure has a double-bond linkage in the carbon chain such as unsaturated fats and fatty acids can be readily iodinated with elemental iodine or iodine monochloride, yielding the diiodo or iodochloro derivatives of these compounds. In the use of these labelled compounds, it is important to ensure that the label remains firmly attached during storage of the product and for the duration of the patient study. The presence of "unbound ^{131}I" is a source of error in tracer investigations using ^{131}I labelled radiopharmaceuticals and it is essential to have quality control analysis to estimate the percentage of free iodine ^{131}I activity in all ^{131}I labelled products.

The specifications and characteristics of the commonly used ^{131}I labelled radiopharmaceuticals are given in Appendices. These products are available in multidose forms from several commercial and other large-scale producers.

$^{99}Tc^{m}$ radiopharmaceuticals

$^{99}Tc^{m}$ is the work-horse of nuclear medicine and is expected to continue to be so in the foreseeable future also.

$^{99}Tc^{m}$ generator

The main source of $^{99}Tc^{m}$ for hospitals is a "generator" or "cow" which consists of a system where the parent ^{99}Mo is retained and the 99mTc is "milked out" at periodic intervals. Two types of generators are commonly used:

(a) The "column generator" which is available from many commercial suppliers and consists of a chromatographic alumina column on which very high specific activity ^{99}Mo (usually produced by the fission of ^{235}U and present in the chemical form of molybdate) - the $^{99}Tc^{m}$ which is produced by the decay of the parent (and present in the form of pertechnetate) is eluted out with normal saline solution.

(b) The "solvent extraction generator" where the parent ^{99}Mo (usually of medium and low specific activity, produced by direct irradiation of natural Mo) in the form of molybdate in alkaline solution is extracted with an organic solvent (usually pure methyl ethyl ketone - MEK) which selectively extracts the pertechnetate. The MEK extract is evaporated to dryness and the pertechnetate is leached out with normal saline solution. Solvent extraction generators for $^{99}Tc^{m}$ are in regular use in a few countries which produce the parent ^{99}Mo using their local irradiation facilities.

The advantageous features of the 'column generator' include ease of operation, rapid recovery of $^{99}Tc^{m}$ with high yield and purity (usually in a sterile, pyrogen-free isotonic saline solution), compact size, attractive presentation and ready availability from many commercial suppliers. The disadvantages include high cost, occasional problems of poor elution yields and impure $^{99}Tc^{m}$ with long-lived radionuclide contamination. In view of the relatively short half-life of the parent ^{99}Mo, regular imports of these generators may pose difficulties of transport delays, in transit decay loss and uncertain availability.

The preparation of column generators involves a very complex technology firstly to produce the parent fission-product ^{99}Mo with high purity and specific activity and, secondly, to fabricate a reliable generator system which will assure a sterile pyrogen free eluate. The production and purification of fission product ^{99}Mo involve the handling of large quantities of long-lived fission product wastes, the storage and disposal of which pose serious problems which may be beyond the infrastructural capability of many developing countries. In view of these disadvantages and problems, many developing countries have focused attention on the development of alternate technologies for $^{99}Tc^{m}$ generators involving the use of low and medium specific activity ^{99}Mo produced by the direct irradiation of natural Mo-containing targets in medium flux research reactors which are available in these countries.

The advantageous features of the solvent extraction technology for $^{99}Tc^m$ include low cost of production, since indigenously produced medium and low specific activity ^{99}Mo can be used for the extraction. The process also involves production of the minimum of radioactive waste (essentially only spent ^{99}Mo solution) which does not pose serious problems of storage and disposal. Further, the process yields $^{99}Tc^m$ of high radioactive concentration and purity. Several designs of apparatus have been employed in various centres for the solvent extraction process and large centralised generators as well as small portable units suitable for operation in hospital radiopharmacies have been in use. The disadvantages of the solvent extraction procedure include the rather lengthy and complicated operations involved which are time-consuming as against the simple direct elution of the column generator. The possible fire hazard associated with the use of an inflammable solvent such as MEK, and the need to have an adequately ventilated fumehood to evaporate off the MEK phase are other handicaps. The operation of the solvent extraction process demands highly trained personnel. Further, problems have been encountered occasionally due to incomplete evaporation of MEK, formation of labelled organic impurities arising from condensation products of MEK, and poor extraction yields.

Two other types of generators which are under development are of interest to developing countries. The first is based on a low temperature sublimation of technetium oxide from irradiated molybdenum containing targets and the second is based on elution of $^{99}Tc^m$ from a matrix containing ^{99}Mo in the form of a gel.

$^{99}Tc^m$ labelled radiopharmaceuticals

General aspects

The starting material for the preparation of $^{99}Tc^m$ radiopharmaceuticals is $^{99}Tc^m$-pertechnetate obtained from a ^{99}Mo- $^{99}Tc^m$ generator. Essentially a $^{99}Tc^m$ labelled radiopharmaceutical preparation is a compound of Tc or a complex formed with a specific ligand by $^{99}Tc^m$ at an oxidation state lower than +7. The exact composition and structure of many of these compounds (complexes) are not known with certainty. Depending on the reaction conditions, in some of the $^{99}Tc^m$ -ligand systems several complexes with different biological behaviour may be formed.

In all $^{99}Tc^m$ labelling procedures, $^{99}Tc^m$ pertechnetate of high purity is reduced to a lower oxidation state using a suitable reducing agent. This is followed by formation of a stable complex(es) with the ligand or by binding to suitable particles etc. The reduction of the $^{99}Tc^m$ pertechnetate is advantageously carried out using stannous ions. The high reduction efficiency of Sn(II), ease of handling and low toxicity have made the $^{99}Tc^m$- (VII)-Sn(II)-ligand systems the most widely used for labelling.

The sequence of steps in the labelling procedure is:

(a) Preparation of the Sn(II) complex of the ligand,

(b) reduction of $^{99}Tc^{m}O_4$ with the Sn(II) complex with simultaneous binding of the reduced $^{99}Tc^{m}$ to the ligand compound.

Having fixed the amount of the ligand, the other conditions and parameters are carefully standardised to get the optimum labelling yield, radiochemical purity and reproducibility. The important parameters to be optimised include amount of Sn(II), pH of the reaction, effect of trace impurities such as Al(III), MoO4=, H2O2 and ^{99}Tc and heating, if required. At present, there is no simple physicochemical method for separating the different components and polymers arising out of the complexation of $^{99}Tc^{m}$ with various ligands. It has been generally observed that the overall biological distribution and behaviour of the ligand-complex and polymer mixtures obtained under meticulously controlled optimum conditions are adequate for the intended diagnostic applications and a rigorous purification of the labelled complex from small amounts of homologues and polymers is not an essential prerequisite for their clinical applications. It is, however, essential that the reaction conditions are carefully standardised to obtain a product with satisfactory biodistribution in experimental animals. The specifications for the acceptable biodistribution pattern for several $^{99}Tc^{m}$ labelled radiopharmaceuticals are given in appendix. Once these optimum experimental conditions are established, they should be followed carefully with appropriate quality controls on all ingredients used (including the ligands and $^{99}Tc^{m}$ pertechnetate) and analysis of the final product by chromatographic and biodistribution studies.

Labelling kits

The labelled radiopharmaceutical formulations of $^{99}Tc^{m}$ are prepared by reacting the corresponding ligand and other ingredients with the $^{99}Tc^{m}$ pertechnetate obtained in a sterile, pyrogen-free form. The ligand and other ingredients are usually prepared, tested and kept in a ready-to-use form in what are known as "kits". The "kits" contain the required amount of the non-radioactive precursor of the $^{99}Tc^{m}$ radiopharmaceutical along with requisite quantities of sterile, pretested essential ingredients. The kit is designed to enable the simple and convenient preparation of the radiopharmaceutical in a closed system, often by a single-step addition of the pertechnetate $^{99}Tc^{m}$ to the "cold" ingredients contained in a vial.

The most popular form of kits is based on stabilising the stannous ions by freeze drying against air oxidation and hydrolysis. Even though other forms of kits like liquid or frozen solutions under inert atmosphere have also been used, the freeze dried kits have an advantage of their long shelf life, the procedural reliability and ease of reconstitution into a clear solution, or suspension (in case of labelled colloids or particles) suitable for parenteral administration. The long shelf life of the kits, (of the order of 6 months) makes it possible to carry out thorough quality control before human use.

Conveniently formulated freeze-dried kits for the most widely used radiopharmaceuticals of $^{99}Tc^{m}$ are available from many commercial sources. However, they are expensive. In most cases the kit costs constitute about 50% of the total cost of a dose of the radiopharmaceutical for a patient. It is practicable and cost-effective to undertake preparation of kits in a centralized radiopharmacy and the equipment and the facilities for such a programme are not too expensive. (see further under "organization of a central radiopharmacy facility").

Procedures for preparing freeze dried kits for a few $^{99}Tc^{m}$ radiopharmaceuticals have been described in this chapter. The composition of the kit preparations for specific agents described in the literature vary somewhat with respect to the amounts of ingredients, pH, and other additives. Each one of them could result in a product acceptable for the intended use. A comparative study of such procedures with a view to standardisation of a single acceptable formulation has not yet been attempted. The procedures given in this chapter have been used over many years for kit preparation with acceptable results. Nevertheless they cannot be claimed as the only procedures or the best procedures. Similarly, the $^{99}Tc^{m}$ radiopharmaceuticals described here have been included primarily with a view to help radiopharmacists in developing countries engaged in kit preparation to start the programme and produce them with the resources available. It is also to be kept in mind that many of these kits are finding newer applications other than the originally intended ones (such as $^{99}Tc^{m}$ pyrophosphate for myocardial infarct imaging, $^{99}Tc^{m}$ glucoheptonate for lung tumour imaging, and $^{99}Tc^{m}$ DTPA for in vivo labelling of red blood cells). The same logic applies to the analytical procedures described here and variations, such as in chromatographic systems and choice of animals etc. have also been reported.

In-house preparation of kits

General:

The procedures described in this section outline the details of the preparation of kits with a batch size of 100 vials. The batch size can be decreased or increased by proportional decrease or increase in the quantities or volumes of reagents. However, while increasing the batch size over 500 or so it has been observed that special precautions are needed, such as cooling the reaction mixture after addition of stannous chloride, purging with inert gas and dispensing into precooled vials. In-process control of stannous ions becomes particularly important in such batches.

Shelf life of the kits and formulations:

The freeze dried kits obtained by the procedures described usually have a shelf life of six months. However, it would be necessary to determine the shelf life of the kits under local conditions. It is recommended that while undertaking the production of kits, in the initial stages a few kits are retained for collecting stability data on kits. It is also advisable to imitate some improper handling of the kits, as it might occur during transportation or storage, by applying stress conditions such as enhanced temperature or exposure to light of a few batch control samples, and then study the behaviour of the kits under these conditions.

Quality assurance programme in kit preparation:

Since the ingredients of the kits for $^{99}Tc^{m}$ formulations are administered intravenously in humans after the formulation processing, a well designed quality assurance programme should be implemented keeping in mind the scale of operations and their intended use in small volumes, as diagnostic agents. Since $^{99}Tc^{m}$ radiopharmaceuticals contain very low

concentration of ingredients of not well defined molecular structure, an unequivocal analysis with conventional chemical methods cannot be performed. Hence, the quality assurance programme should involve clear description of the raw materials, premises, facilities, and equipment, preparation procedures, product control and documentation. The batch size should include sufficient samples for all the control tests, taking into account the possibility of their repetition. Batch control samples should be maintained through the shelf life of the batch for examining the complaints if any received about the quality of the product. Proper record should be maintained of any complaints of improper quality of the product which should be investigated and remedial measures taken. Similarly the shelf life (in vitro stability) of the $^{99}Tc^{m}$ formulations obtained using the kits is recommended to be determined experimentally using the locally available $^{99}Tc^{m}$ pertechnetate and prevailing conditions. The shelf life indicated in the Chapter are not very rigid and are to be taken as guidelines only.

Kits for individual $^{99}Tc^{m}$ Radiopharmaceuticals.

Only few kit preparations are described in detail below just to give an idea to the reader what is involved in making the kits indigenously. A good chemist or a pharmacist is supposed to make them after an appropriate training. An IAEA TECDOC giving details of preparation of kits currently in use in a nuclear medicine laboratory is under preparation.

Kit for $^{99}Tc^{m}$ MDP Injection

Batch size: 100 (100 ml dispensing solution)

CHEMICALS: The main chemicals used are:

(a) Methylene diphosphonic acid (MDP)

(b) Stannous chloride dihydrate

(c) Hydrochloric acid

(d) 1 N Sodium hydroxide solution

(e) water for injection

(f) Ascorbic acid

ACCESSORIES: The main accessories required for kit preparation are:

(a) Sterilised glassware (beakers, pipettes, measuring cylinders, etc.)

(b) Sterilised vials, split-type rubber closures

(c) Sterilised membrane filtration assembly fitted with 0.22 μm (or 0.45 μm) filter

(d) Aluminium caps

(e) Capping and decapping tools

PREPARATION OF STOCK SOLUTION:

This solution should be prepared just prior to kit preparation

Solution of stannous chloride, 8% (w/v):
400 mg of stannous chloride to be dissolved in 0.5 ml of conc. hydrochloric acid and warmed if necessary to get a clear solution. Final volume to be made to 5 mL with water for injection.

(a) 500 mg of MDP and 50 mg ascorbic acid to be dissolved in 80 mL of water for injection. Some drops of 1 N sodium hydroxide solution to be added to get a clear solution.

(b) 0.60 mL of the stannous chloride solution to be added dropwise to the above solutions.

(c) The pH of the solution to be adjusted to 6 to 7 by dropwise addition of 1 N sodium hydroxide solution.

(d) The final volume to be adjusted to 100 mL with water for injection.

(e) The solution to be sterilised by filtration.

(f) The filtered solution to be dispensed in 1 mL quantities into sterilised vials and fitted with dry sterilised rubber closures.

(g) The vials to be transferred to a freeze-dryer and lyophilized for 24 hours.

(h) The vials to be sealed under vacuum or under dry, filtered nitrogen gas.

(i) The vials to be stored at 2-10°C with the following label affixed to each vial:

Kit for 99mTc MDP Injection Code; ... Batch No.: .... Cons. No.: .... 5 mg of MDP 0.5 mg of $SnCl_2.2H_2O$ 0.5 mg of Ascorbic Acid To be stored at 2-10°C

Kits for other $^{99}Tc^m$ radiopharmaceuticals

Kits for other radiopharmaceuticals of $^{99}Tc^m$ are prepared by a similar stepwise procedure: the quantities of ligand, $SnCl_2$, pH of final solution and dilution are shown in Table II. The main accessories required for kit preparation and the procedures for freeze-drying and labelling of the final vials are as indicated for $^{99}Tc^m$-MDP.

Kit for $^{99}Tc^m$ Sulphur Colloid Injection

Batch size: 100 kits with three components each

CHEMICALS: The main chemicals used are:

(a) Sodium dihydrogen orthophosphate dihydrate

(b) Disodium hydrogen orthophosphate dodecahydrate

(c) Sodium thiosulfate pentahydrate

(d) Hydrochloric acid

(e) 1 M sodium hydroxide solution

(f) Water for Injection

(g) Nitric acid

(h) Rhenium metal

(i) 3.5% Infusion solution of gelatine for intravenous administration

ACCESSORIES: As in the case of MDP kits.

PREPARATION OF STOCK SOLUTION:

(a) 0.3 N Hydrochloric Acid solution:
2.8 mL of conc. hydrochloric acid to be diluted to 100 mL with water for injection.

(b) Solution of Sodium thiosulphate, 10% (w/v):
5.0 gm sodium thiosulphate pentahydrate to be dissolved in about 40 mL of water for injection. Final volume to be made to 50 mL with water for injection.

(c) Solution of Rhenium, 2.0% (w/v):
300 mg of rhenium metal to be dissolved in about 0.3 mL of conc. nitric acid. The solution to be warmed for 4-5 minutes and pH adjusted to about 7 using 1 M sodium hydroxide solution. Final volume to be adjusted to 15 mL with water for injection.

(d) Phosphate buffer, pH 7.4:
13.6 g of disodium hydrogen orthophosphate dodecahydrate and 1.2 g of sodium dihydrogen orthophosphate dihydrate to be dissolved in 80 mL of water for injection. Final volume to be adjusted to 100 mL with water for injection.

KIT PREPARATION:

Component A:

(a) The 0.3 N hydrochloric acid solution (Stock Solution a) to be dispensed in 0.5 mL quantities into sterilised vials and capped with sterilised rubber closures and clean aluminium caps. The vials to be sterilised in an autoclave.

(b) The following label to be affixed to each vial:

Kit for 99mTc Sulphur Colloid Injection Code: ... Batch No.: .... Cons. No.: .... Component A (Reaction vial) 0.5 mL solution of dilute hydrochloric acid Expiry date: ....

Component B:

(a) 130 mL of 3.5% (w/v) solution of gelatin for infusion, 15 mL of the solution of sodium thiosulphate, 10% (Stock Solution (b) and 8 mL of the rhenium solution (Stock Solution (c) to be mixed well in a beaker.

(b) The solution to be dispensed in 1 mL quantities into sterilised vials and capped with sterilised rubber closures and clean aluminium caps. The vials to be then sterilised in an autoclave.

(c) The following label to be affixed to each vial:

> Kit for 99mTc Sulphur Colloid Injection
> Code: ... Batch No.: .... Cons. No.: ....
> Component B
>
> 1 mL solution containing gelatine, thiosulphate and perrhenate
> Expiry date: ....

Component C:

(a) The phosphate buffer, pH 7.4 (Stock Solution (d) to be dispensed in 1 mL quantities into sterilised vials and capped with sterilised rubber closures and clean aluminium caps. The vials to be then sterilised in an autoclave.

(b) The following label to be affixed to each vial:

> Kit for 99mTc Sulphur Colloid Injection
> Code: ... Batch No.: .... Cons. No.: ....
>
> Component C
>
> 1 mL phosphate buffer, pH 7.4
> Expiry date: ....

Formulation processing of $^{99}Tc^{m}$ radiopharmaceuticals

Formulation processing of $^{99}Tc^{m}$ labelled radiopharmaceuticals is carried out under aseptic conditions by transferring sterile, pyrogen-free pertechnetate solution into the vial containing the kit ingredients. The contents are mixed well, allowed to stand for a few

minutes in some cases at an elevated temperature. The transfer should be carried out using sterile, preferably disposable syringes and needles. Appropriate radiation shielding should be provided during the operations. The stepwise details of formulation processing for a few $^{99}Tc^{m}$ formulations are given below:

Formulation of $^{99}Tc^{m}$ Sulphur Colloid Injection

(a) Two to three mL of $^{99}Tc^{m}$ sodium pertechnetate injection should be added to Component A containing vial.

(b) To the above vial 0.5 mL of Component B should be added. The contents should be mixed well and heated in a boiling water bath (vent needle without connection to the solution) for about five minutes till a brownish colloidal suspension is formed.

(c) The vial should be cooled and 0.5 mL Component C should be added to the vial and mixed well.

(d) The vial should be fixed with a label with a radioactive symbol and giving the details of the formulation such as:

> 99mTc Sulphur Colloid Injection
> Batch No.: .... Date: .... Vol.: ....
> Activity/mL: .... at .....
> Use before ...... hrs.
> Shake well before use

Formulation of $^{99}Tc^{m}$ DTPA Injection

(a) The kit vial should be allowed to attain ambient temperature after removing from refrigerator.

(b) Two to three mL of $^{99}Tc^{m}$ sodium pertechnetate injection should be added to the kit contents and mixed well. The contents be allowed to stand for ten minutes.

(c) The vial should be fixed with a label with a radioactive symbol and giving the details of the formulation such as:

> 99mTc DTPA Injection
> Batch No.: .... Date: .... Vol.: ....
> Activity/mL: ..... at ......
> Do not use later than ..... hrs.

Other generator systems and radiopharmaceuticals derived from these generator-produced isotopes:

The only other generator system of some interest for developing countries is the ^{113}Sn-$^{113}In^m$ generator. This generator consists essentially of a column of hydrous zirconium oxide on which high specific activity ^{113}Sn (in the form of a chloro complex) is adsorbed. The ^{113}In activity is eluted out with very dilute HCl (pH N 2.0). The ^{113}Sn break through in an acceptable generator is less than 0.01% ^{113}Sn at the time of elution. The high cost of this generator system and the poor nuclear characteristics of ^{113}In (particularly for use with a gamma camera) have limited the use of this generator system.

In view of the long half-life (115.1d) of ^{113}Sn, the ^{113}Sn-$^{113}In^m$ has a useful shelf-life of at least six months. It is necessary to ensure that the generator is protected against microbial contamination during its long shelf-life and the hundreds of elutions which can be performed during its useful life period. It is desirable to house the generator in a laminar air flow (LAF) unit. It is a good practice to elute the generator at least once a week (irrespective of its use) and it is essential that the operating and maintenance instructions for the generator recommended by the supplier are rigorously adhered to. Some suppliers recommend the replacement of the elution tube at intervals (two to three months) because of the possible corrosive effect of dilute HCl on the needle.

Quality control of the ^{113}Sn-^{113}In generator consists of:

(a) Evaluation of ^{113}Sn break through. The permissible limit of ^{113}Sn activity in $^{113}In^m$ is not more than 0.3 kBq ^{113}Sn per MBq of $^{113}In^m$ at the time of administration. This is estimated by allowing the $^{113}In^m$ eluate to decay for 2 days and then evaluating the ^{113}Sn activity by measuring the daughter activity of $^{113}In^m$ using a ^{133}Ba reference source. A gamma spectrometer with a single-channel analyzer or a simple gamma scintillation counter may be used for the assay.

(b) Test for zirconium break-through: Soluble zirconium may be eluted out of the generator and the acceptable upper limit is 10 μg per patient dose.

(c) Tests for sterility and apyrogenicity: The $^{113}In^m$ eluate should be tested to confirm sterility and apyrogenicity by the standard procedures.

Quality assurance and quality control

General aspects

Quality assurance procedures and quality control measures have to be built into a programme of indigenous production of radiopharmaceuticals. The quality control system should be comprehensive, yet simple, inexpensive and practicable at the level of production

envisaged. The sequence of operations covered under quality assurance and quality control encompass the following:

(a) Control of raw materials (documented specifications, analysis and certification of pooled stocks, periodic analysis of bulk stocks and stability studies while stored under optimum conditions).

(b) In process control and supervision of various production parameters (pH, temperature of reaction, concentration of reactants, role of impurities, etc.).

(c) Control of additives, preservatives, diluents, carriers etc.

(d) Quality control analysis of the final products.

These operations are an integral and essential part of Good Pharmaceutical Practices which are briefly outlined in a subsequent Section.

Quality control of raw materials

The raw materials required for the preparation of medical radioisotopes and radiopharmaceuticals in a hospital radiopharmacy consist mainly of the following:

(a) Miscellaneous chemicals and reagents such as methyl ethyl ketone (MEK) for extraction of $^{99}Tc^{m}$ from molybdate solutions, buffers, ingredients of buffer systems, vehicles, additives, etc.;

(b) Sodium iodide ^{131}I solution, reducing agent free and of high radioactive concentration for the preparation of ^{131}I-labelled radiopharmaceuticals;

(c) Generators of $^{99}Tc^{m}$, $^{113}In^{m}$;

(d) Ligands/ingredients for kit preparation.

Quality Control of Generators

The generators for $^{99}Tc^{m}$ and ^{113}In are generally procured from reputed suppliers who provide their detailed specifications and operating instructions. Typical details of a commercially available generator are given in Appendix X.

Quality controls of such generators consist of the following:

(a) Examination of the label affixed on the package and the package insert (leaflets) giving details of the generator and its operation. The activity of $^{99}Tc^{m}$ expected from the elution of the generator and the characteristics of the eluate should be recorded.

(b) Trial elutions of the generator should be carried out soon after its receipt and also at periodic intervals during its useful storage life to assay or confirm the following:

(i) Clarity of the eluate solution

(ii) Total $^{99}Tc^{m}$ activity and radioactive concentration of the eluate

(iii) Sterility and apyrogenicity

(iv) Suitability of the eluted $^{99}Tc^{m}$ for preparation of labelled radiopharmaceuticals

(v) Adequacy of shielding and integrity of packaging of the generator

When generators based on solvent extraction separation or sublimation of $^{99}Tc^{m}$ are employed, the performance characteristics of these systems and the purity of the separated $^{99}Tc^{m}$ should be ascertained in the same manner.

Similar quality control tests are required to be carried out on $^{113}In^{m}$ generators. In view of the long shelf-life of these generators (six to eight months) it is necessary to carry out analysis of the eluates at carefully scheduled periodic intervals to confirm the purity of the eluted ^{113}In and good performance of the generator.

Quality control of ligands and ingredients used for kit preparation.

The ligands and chemicals used in the kit preparation should be of high purity. However, for many of these ligands it may be difficult to lay down standards specifically for use in kit preparation, since, sufficient data on the effect of trace chemical impurities present in them on the behaviour of $^{99}Tc^{m}$ kit preparations is not available. The general approach adopted here is to use the specifications available in recognised pharmacopia wherever available or the specifications prescribed for AR grade reagents are adopted. In case no specifications are readily available, as in case of a few ligands to be synthesised, the user should lay down their specifications. It may be difficult to carry out confirmatory analysis for such specifications in a small set up for radiopharmaceutical preparation. In such a case the tests may be done in a reputed outside laboratory or alternately materials certified for the purity requirements may be obtained from reputed sources.

The following protocols suggested for the analysis of methylene diphosphonic acid (MDP) could serve as an example of the quality control analysis of ligands used in kit preparation:

Methylene diphosphonic acid (MDP)

(a) white crystalline powder

(b) MW 176.000

(c) melting point: 197-199°C

(d) For kit preparation, a product certified more than 97% purity should be used.

Keeping in mind the final intended use, it is recommended to evaluate the product for its radiochemical purity and biodistribution after having prepared a kit and the corresponding $^{99}Tc^{m}$ radiopharmaceutical.

Quality control of kits.

Quality control of kits includes test of appropriate physical appearance, pH of reconstituted solution, determination of stannous ions, test of sterility and apyrogenicity, as well as in-vitro stability of the $^{99}Tc^{m}$ radiopharmaceuticals. The best proof of their appropriateness with the intended use will be provided by biodistribution studies of the kit-prepared $^{99}Tc^{m}$ radiopharmaceuticals. Specifications for the reconstituted $^{99}Tc^{m}$ radiopharmaceuticals obtained by adding pertechnetate solution to a kit vial, have been published in monographs in pharmacopoeias, e.g. British Pharmacopoeia 1988, European Pharmacopoeia, and the United States Pharmacopoeia (USP XX).

Typical specifications and quality assurance analysis for a kit for $^{99}Tc^{m}$ Sulphur Colloid are outlined below:

Description:

Kit for $^{99}Tc^{m}$ Sulphur Colloid is a set of reagent vials as specified on the labels of the vials. When used with $^{99}Tc^{m}$ sodium pertechnetate for Injection according to the instruction of the kit, these would give a sterile and pyrogen free suspension suitable for imaging the reticuloendothelial system after intravenous injection.

Components of the kit:

Component A:
A vial containing a clear, colourless solution of 0.5 mL of 0.3 N hydrochloric acid

Component B:
A vial containing a clear, pale yellow solution of 29.9 mg gelatin, 10 mg sodium thiosulphate pentahydrate and 1 mg rhenium as sodium perrhenate in 1 mL

Component C:
A vial containing a clear colourless solution of 136 mg Na2HPO4.2H2O and 12 mg $NaH_2PO_4.2H_2O$ in 1 mL

Assay for main ingredients in the vials:

The amount of main ingredients in the components A, B and C will be confirmed by monitoring and double-checking the amount of starting materials used in the kit preparation, and the volumes.

Identification of the kit:

(a) A nonradioactive formulation of the product should be carried out by the procedure described under "formulation" using 0.9% NaCl solution instead of sodium $^{99}Tc^{m}$ pertechnetate. The resulting product will be a brownish colloidal suspension.

(b) The results of radiochemical purity and biodistribution tests taken together for a $^{99}Tc^{m}$ sulphur colloid injection prepared with the kit should be taken as an identification test for the kit.

pH: The pH of the nonradioactive preparation should be between five and eight as measured by a pH meter.

Sterility:

Sterility tests should be preformed using a nonradioactive preparation as described above, using sterile pyrogen free 0.9% NaCl solution. The product should be confirmed to be sterile before release for human use.

Pyrogen test:

Pyrogen tests should be carried out on rabbits using an inactive colloid preparation using sterile pyrogen free 0.9% NaCl solution. The product should be confirmed to be pyrogen free before human use.

Formulation of $^{99}Tc^{m}$ Sulphur Colloid Injection

The formulation should be carried out without opening the kit components and using aseptic practices. The transfer should be carried out using sterile (preferably disposable) syringes and needles. Appropriate radiation shielding should be provided during these operations.

(a) Two to three mL of $^{99}Tc^{m}$ sodium pertechnetate injection will be added to Component A contained in the vial.

(b) To the above vial 0.5 mL of Component B will be added. The contents will be mixed well and heated in a boiling water bath (with a vent needle without connection to the solution) for about five minutes till a brownish colloidal suspension is formed.

(c) The vial will be cooled and 0.5 mL Component C will be added to the vial and mixed well.

(d) The vial will be fixed with a label with a radioactive symbol and giving the details of the formulation such as:

99mTc Sulphur Colloid Injection Batch No.: .... Date: .... Vol.: .... Activity/mL: .... at ..... Use before ..... hrs. Shake well before use

Description:

$^{99}Tc^{m}$ sulphur colloid injection is a sterile, pyrogen free colloidal suspension, brownish in colour, stabilised by gelatin, suitable for intravenous administration.

pH: will be between five and eight as checked by pH paper.

Radiochemical purity:

This will be determined shortly after preparation of the radiopharmaceutical, and for the evaluation of its in-vitro stability every hour up to 4 hours, by ascending paper chromatography using 85% v/v aqueous methanol on Whatman No. 1 paper. $^{99}Tc^{m}$ sulphur colloid will remain at spotting point and $^{99}Tc^{m}$ pertechnetate will move with an Rf value of about 0.6. The radioactivity corresponding to $^{99}Tc^{m}$ sulphur colloid will be not less than 92% of the total activity.

Biological Distribution:

This should be done as described in the next chapter.

The acceptable biodistribution characteristics for the sulphur colloid injection and for several other $^{99}Tc^{m}$ radiopharmaceuticals are given in the next chapter.

Storage:

(a) The nonradioactive kit for $^{99}Tc^{m}$ sulphur colloid injection should be stored at room temperature (approx. 23°C). Shake well before use.

(b) The radioactive formulation $^{99}Tc^{m}$ sulphur colloid injection should be stored at room temperature with adequate shielding.

Expiry:

(a) The kit should be used not later than 3 months after satisfactory completion of al! the quality control tests or 4 months after kit production.

(b) The radioactive formulations $^{99}Tc^{m}$ sulphur colloid injection is recommended to be used as early as possible but not later than 4 hours after preparation.

Good Pharmaceutical Practices (GPP)

General

GPP is broadly defined as a comprehensive system, designed, documented and implemented such that the finished products will be of a quality appropriate for their intended use. GPP guidelines are well known and accepted in the pharmaceutical industry. Preparation of radiopharmaceuticals has to be carried out according to these general guidelines which concern the premises, equipment, hygiene, starting materials, preparations, labelling, packaging, storage, the quality control system, and documentation. Besides implementing GPP, the preparation and use of radiopharmaceuticals are subject to legal regulations by national authorities.

Personnel

The personnel deployed for radiopharmaceutical preparation and testing should be well qualified and trained. They should be graduates in either chemistry or pharmacy with additional training in radiochemistry and preparation and testing of radiopharmaceuticals. In many places formal, regular training in these areas is not available. It is often necessary to provide on-the-job training in centres producing radiopharmaceuticals. A new recruit to the facility should be given adequate on-the-job training before deploying for these activities. It is desirable to divide the responsibilities for of product preparation and their quality control between two independent persons.

Premises

Radiopharmaceutical preparation must be performed in premises of defined cleanliness. According to GPP, for the manufacturing of drugs that are intended to be sterile but cannot

be sterilised in their final containers, separate enclosed areas, specifically designed for the purpose, should be provided. This area should be provided with air supply which is filtered through a microbes retaining filter. Entry to this area should be through an air-lock. Provision of Laminar Air-Flow (LAF) benches, which provide a sterile atmosphere, is essential for preparing and dispensing of injectable solutions, kits, etc. In addition, the area in which the LAF benches are installed should be designed to facilitate easy maintenance.

The typical premises for preparation, dispensing and quality control of radiopharmaceuticals may consist of three rooms of approximate size 6 x 6 m each, and additional areas of packaging, storage and service. It is suggested that all preliminary work such as cleaning and sterilisation of glassware, containers, closures, filters and preparation of bulk solutions for the kits are performed in the room adjacent to the preparation and dispensing room. The two areas may be interconnected by hatches for material transfer. All surfaces in the area should be designed to facilitate cleaning and disinfection. To keep the contamination in the dispensing room to a minimum, equipment should be reduced to the minimum necessary. It is advisable to house equipment such as LAF benches, freeze-drying unit etc. in such a way that only the working areas are accessible to the dispensing room.

A periodic sanitation programme, for the premises indicating cleaning procedures and cleaning schedules, should be designed and implemented. The contamination level in the room as well as dispensing areas should be monitored at regular intervals.

Equipment

In addition to standard laboratory equipment, such as work benches and storage cabinets, essential equipment for radiopharmaceutical dispensing, kit preparation and quality control work includes LAF bench, freeze-dryer, deep-freezer, membrane filtration device, semi-micro balance, autoclave for sterilisation, and dry-air steriliser.

Laminar Air Flow (LAF) Bench

Standard LAF benches, either of the vertical or horizontal air flow type can be used as far as they meet the requirements as outlined in BRITISH STANDARD 5295 Class I. After the installation of a LAF bench the conformation to the standard must be verified prior to use. The LAF bench should be disinfected before use by swabbing with an appropriate disinfectant. The performance of the LAF bench should be regularly checked by measurement of air velocity and periodic exposure of nutrient agar culture plate in the working area to detect microbial contamination in air.

Membrane filtration device

Pre-sterilised disposable membrane filtration devices or reusable filtration devices made of either plastic or glass or stainless steel could be used. The membrane filter should not be used more than once. For filtration of nitrogen gas for purging solutions or for sealing the vials under nitrogen atmosphere, membrane filters meant for gas filtration should be used.

Lyophilizer

A shelf-type freeze drying unit capable of accommodating about 200 vials of 10 mL capacity each can be typically used. The unit should have facilities for stoppering the vials under vacuum or nitrogen gas by mechanical means. The other operational features are:

(a) mechanical condenser capable of reaching temperatures of less than - 40°C,

(b) in ice-removing capacity of more than 2 kg in 24 hrs operation,

(c) capacity for retaining a vacuum of less than 50 mtorr without load,

(d) facility for cooling and heating the shelf from -40°C to +40°C by a circulating fluid,

(e) facility for monitoring the above parameters as well as the product temperature. On installation of the equipment and every time prior to loading of the samples the freeze dryer should be operated without load and the performance of the machine with respect to the above parameters checked. Freeze drying is an intricate process and careful standardisation of the various parameters is essential to get a good product.

Containers and Closures

Container

For dispensing the radiopharmaceutical formulations, kits and reagents USP type-I flint neutral glass vials of 10 mL capacity are commonly used. The vials should be obtained from reputed sources certified for conformity to USP specification. Cleaning and sterilisation protocols for the vials should be established and followed. A typical protocol is given in Appendix XI to serve as a guideline. Other glassware such as beakers, measuring cylinders, graduated pipettes, glass rods, filters, flasks, etc. made of good quality boro-silicate glass should be used. They should also be cleaned and sterilised as per established protocol such as given in Appendix XII for glass vials.

Rubber stoppers.

Rubber stoppers with split end for use in freeze drying operations should be purchased from reputed sources certified for conformity to the physical and the chemical tests described in pharmacopoeia. Cleaning and sterilising protocols for rubber stoppers should be established and followed. A typical such procedure is given in Appendix XIII for guidance.

Process

$^{99}Tc^{m}$ labelling kits

The general procedure for the preparation of $^{99}Tc^{m}$ labelling kits essentially consists of the following simple steps:

(a) Weighing and dissolution of stannous chloride in dilute hydrochloric acid.

(b) Weighing and dissolution of the ligand in water or dilute alkali.

(c) Addition of the required amount of stannous chloride solution to the ligand solution with constant stirring.

(d) Adjustment of the pH to the required value using a pH meter.

(e) Sterilisation of the above solution by membrane filtration.

(f) Dispensing of this solution aseptically into sterile vials.

(g) Lyophilisation.

The steps in the lyophilisation process are:

(i) freezing the dispensing solution - this can be done either in the freeze dryer itself or outside in a deep freezer.

(ii) cooling the condenser to less than -40°C.

(iii) evacuating the system to less than 0.1 torr.

(iv) providing controlled heat input to the product during the freeze drying cycle.

(v) Sealing the vials under vacuum or under nitrogen gas in the freeze dryer after completion of the drying cycle.

To obtain a product of reliable and reproducible quality, the process parameters should be continuously monitored throughout the cycle, and properly recorded for every batch.

The normal lyophilisation cycle is for 24 hours. However, when the salt concentration is high and when the volume taken in the vial is more than 1 mL the cycle may have to be extended to 48 hours. The physical appearance of the final product very much depends on the solid content in the starting solution. If it is too less, during the drying cycle the dry powder may crumble and light particles may escape from the vials. Normally this problem

can be taken care of by ensuring sufficient solid content in the dispensing solution. Some workers have found addition of inert substances like inositol, dextrose etc. helpful in the freeze drying process.

Normally the product will be dry following the freeze-drying cycle given under the individual procedures. A qualitative test should be carried out approximately one week after freeze drying, by observing the kit contents. No change in the physical appearance of the product such as shrinkage or presence of water droplets should be seen.

Quality Control.

Good Quality Control Practices form an integral part of GPP and cover quality controls of all raw materials, their certification, control of process parameters including environmental microbial levels, and comprehensive quality controls of the finished products including physicochemical, radiochemical, biological, microbiological and immunological evaluation and stability studies. The quality controls of radiopharmaceuticals, generators and kits have been described in detail in an earlier Section.

Records and Record Keeping

Documentation and record keeping of the various stages of the preparation, dispensing of radiopharmaceuticals and kit preparation including maintenance of premises, processing of formulations, processing of the kits, results of test procedures, and the supply of the kits is an essential aspect of Good Pharmaceutical Practices. A master copy of the compilation of all preparation procedures and testing methods for various products as well as their specifications should be prepared and made available for reference to the personnel involved in the preparation and analysis. These procedures may be periodically reviewed in the light of the in-house experience and developments reported in the literature. Any modifications in the existing procedures should be accepted as routine practice only after its satisfactory validations and should be authorised by the Head of the unit. These should be effectively communicated to all persons involved in the production and quality control work as well as the user.

Log books, preferably printed, giving details of all processing and analysis should be maintained. Typically this should include details of raw materials, containers, glassware, processing details, batch number, identification numbers, history of use, test results and any other relevant information.

The raw materials, chemicals and ligands for use in preparation should preferably be given in-house batch numbers after acceptance by due analysis. Complete record of their procurement, use, analysis and disposal should be maintained in a log book. In addition, the original label on the containers giving the manufacturers batch number, date of analysis, etc. should be affixed on the container to clearly indicate that the reagent is for use in radiopharmaceutical production. Since the quantities of reagents and ligands used in kit preparation are small and to avoid contaminating the bulk reagents and ligands by frequent handling, it may be advantageous to redistribute them in smaller vials, close them air-tight

and store them safely. Once identified for use in production it is preferable to store them in safe custody thereby avoiding mix up with other chemicals.

Batch Control Samples

A sufficient number of samples from each batch should be kept aside under safe custody during the lifetime of the product. In case of receipt of complaints of unsatisfactory performance of the product, the batch control samples can be used to check the product quality and identify reasons for the poor performance.

Organization of a central radiopharmacy facility

A centralised radiopharmacy could undertake the following services:

(a) Bulk imports of ready to use radiopharmaceuticals such as ^{131}I sodium iodide, preparations of ^{51}Cr, ^{59}Fe, ^{32}P etc. and their dispensing and distribution to user hospitals.

(b) Bulk imports of $^{99}Tc^{m}$ generators and their distribution.

(c) Preparation of kits for $^{99}Tc^{m}$ radiopharmaceuticals.

(e) Preparation of "instant $^{99}Tc^{m}$ formulations" and their supply to user hospitals.

(f) Preparation and distribution of $^{99}Tc^{m}$ generators from imported ^{99}Mo.

An economically designed centralised radiopharmacy, handling and processing the various products listed above and the activity levels shown in Table III (Appendix III) should have a total constructed area of about 400 m^2 and a staff of three scientific and three technical personnel. At the optimum level of operation this centralised radiopharmacy would be able to prepare and supply 4000 to 5000 consignments of various radiopharmaceutical preparations per annum which would be adequate for 15 000 to 20 000 patient procedures. The operating costs of such a centralised radiopharmacy (inclusive of staff salaries, running costs and costs of raw materials) would be about US $60 000 per year, while the import costs of the radiopharmaceuticals processed and supplied from the facility per year would exceed US $150 000. With marginal increases in space and staff, the range of products and levels of activity handled in this facility could be substantially scaled up. The experience of several developing countries has shown that local production and/or distribution of medical radiopharmaceuticals results in substantial savings in foreign exchange and ensures the provision of a more streamlined nuclear medicine service. The raw material costs of such a programme ranges from 10-15% of the overall production costs, the staff salaries account for about 15%, while the operating costs of laboratory and equipment and the depreciation provision account for about 20-25%. Indigenous kit production costs have been found to be less than 5% of the import costs.

Legislative Aspects

A wide range of radiopharmaceuticals is now regularly used in nuclear medicine and many of these products have been included in the pharmacopoeia of advanced countries. The specifications and testing procedures for these products which have been documented in the Pharmacopoeia have been finalised and accepted after extensive studies had been carried out concerning their physicochemical, radiochemical and biological aspects of purity, and clinical utility and efficacy.

The special characteristics and properties of radiopharmaceuticals (as against conventional pharmaceutical products) such as their radioactivity, short shelf-life and the fact that the large majority of these products are diagnostic agents with no pharmacological action have been duly recognised. A special provision has been made in the pharmacopoeia that injectable radiopharmaceuticals may be used on patients by the intending nuclear medicine practitioner before the tests for their sterility are completed as such tests require 14 days for completion which is too long compared to the half-life of most of the medically important radionuclides.

Many developing countries have been importing radiopharmaceutical preparations meeting the specifications and purity criteria outlined in the USP/BP/EP and produced by suppliers in UK, USA, France, Germany, Australia, etc. There are a few radiopharmaceutical products which are not yet documented in the Pharmacopoeia, however, these are in regular use in many advanced countries and are processed and supplied by reputed commercial suppliers. Many developing countries have also been using these products with beneficial results in their nuclear medicine services.

While considering the legislative practices for radiopharmaceutical products in developing countries, the following special characteristics and features of these products and their human applications may have to be kept in view:

(a) Most radiopharmaceutical products contain extremely small quantities of chemical ingredients (a few micrograms to a few milligrams of mostly well known chemicals).

(b) The large majority of radiopharmaceutical products are used essentially as diagnostic agents. These have no pronounced pharmacological action. The radionuclides involved in these preparations are of low and medium toxicity.

(c) Most radiopharmaceutical preparations are administered in small quantities and often only once or on a small number of occasions on the same patient. Repeated administrations are not involved in most cases.

(d) Radiopharmaceutical preparations are supplied only to highly qualified experts who are aware of their special characteristics and end applications. They are not available for sale from pharmacies as is the case with many conventional pharmaceutical products.

(e) The dosage, route of administration and the clinical indications for their use are well established and documented for most radiopharmaceuticals. The contraindications and side effects of their use are also well documented.

(f) Over the years, radiopharmaceuticals have been administered to millions of patients the world over and only a very small number of adverse reactions have been reported which could be attributed to their administration. The adverse reactions have been mostly confined to allergies and a small number of other idiosyncratic reactions of a mild nature.

While the legislation practices regarding medical and pharmaceutical products are a matter of national policy, it would be a logical and practical approach to accord legislative clearance and acceptance to all radiopharmaceutical products which are listed in the well known pharmacopoeia (USP, BP, EP, IP). A general clearance for human use may also be accorded for other products which are in regular use in advanced countries though these products are not yet officially recognised in the Pharmacopoeia.

The overall objective of the legislative process should be to ensure the safe, efficacious and cost effective use of well recognised radiopharmaceutical products for patient care and to promote their indigenous production and widespread applications.

Appendix I

TABLE I. COMMONLY USED RADIOPHARMACEUTICALS AND THEIR MAIN APPLICATIONS.

Serial No.	Radiopharmaceutical	Main medical application/s
1.	Chromic (^{51}Cr) Chloride Injection	Determination of loss of serum protein into the GI tract (by direct i.v. injection)
2.	Sodium chromate (^{51}Cr) Solution	In-vitro labelling of red blood cells for red cell volume, and red cell survival
3.	Cyano cobalamin (^{57}Co) Solution	GI absorption tests
4.	Cyano cobalamin (^{57}Co) Capsules	GI absorption tests
5.	Cyano cobalamin (^{58}Co) Solution	GI absorption tests
*6.	Indium (^{111}In) Bleomycin Injection	Localisation of tumours in a variety of neoplastic conditions
*7.	Indium (^{111}In) chloride	For preparation of ^{111}In labelled proteins and cells, and monoclonal antibodies for immuno-scintigraphy
*8.	Indium (^{111}In) calcium DTPA Injection	CSF studies, cisternography and ventriculography
*9.	Indium (^{111}In) oxine solution	For labelling blood cells
*10.	Sodium iodide (^{123}I) capsules	Investigation of thyroid function and thyroid scintigraphy
*11.	Sodium orthoiodo (^{123}I) hippurate injection	Scintigraphic investigation of kidney function
*12.	Metaiodobenzyl guanidine (^{123}I) injection	Diagnosis of increased localised catecholamine metabolism. Also for localization and diagnosis of active catecholamine metabolic tumours and their metastases (ex. pheochromocytoma and neuroblastoma)

TABLE I. (cont.)

Serial No.	Radiopharmaceutical	Main medical application/s
13.	Sodium orthoiodo (^{125}I) hippurate injection	Determination of effective renal plasma flow (EPRF)
14.	Sodium iodo thalamate (^{125}I) injection	Determination of glomerular filtration rate (GFR)
15.	Iodinated (^{125}I) human serum albumin injection	Blood volume determination
16.	Iodinated (^{125}I) human fibrinogen injection	Detection of deep vein thrombosis of the legs
17.	Sodium orthoiodo (^{131}I) hippurate injection	renography
18.	Sodium orthoiodo (^{131}I) hippurate injection containing less than 0.5% non-bound ^{131}I	Determination of EPRF
19.	Sodium iodide ^{131}I solution	thyroid uptake, and localisation of thyroid cancer metastases, therapy of thyrotoxicosis and thyroid cancer
20.	Sodium iodide ^{131}I injection	thyroid uptake, and localisation of thyroid cancer metastases, therapy of thyrotoxicosis and thyroid cancer
21.	Sodium iodide ^{131}I capsules (diagnostic)	thyroid uptake
22.	Sodium iodide ^{131}I capsules (therapeutic)	Therapy of thyrotoxicosis and thyroid cancer
23.	Meta-iodo benzylguanidine (^{131}I) (m-1BG) solution (diagnostic)	Adrenal medulla scintigraphy in investigations of pheo-chromocytoma, neurobastoma and for the assessment of adrenal medullary hyperplasia
24.	Meta-iodo benzylguanidine (^{131}I) (m-1BG)	Treatment of malignant pheochromocytoma and neuroblastoma
25.	Ferric Citrate (^{59}Fe) injection	Investigation of iron metabolism
26.	Sodium phosphate (^{32}P) injection	Treatment of polycythemia vera and related disorders

TABLE I. (cont.)

Serial No.	Radiopharmaceutical	Main medical application/s
27.	Strontium (^{89}Sr) chloride injection	Palliative treatment of bone metastases
28.	Indium (^{113m}In) DTPA injection	Brain scintigraphy
29.	Indium (^{113m}In) colloid-mannitol stabilized or gelatin stabilized	Liver scintigraphy
30.	Technetium-99m EHIDA	Scintigraphic imaging of the hepatobiliary system
31.	^{99m}Tc gluconate	Diagnostic scintigraphy of the kidney or brain
32.	^{99m}Tc R.B.C.	Diagnostic imaging of blood pools
33.	^{99m}Tc DMSA	Diagnostic imaging of kidney
34.	^{99m}Tc DTPA	Measurement of GFR, functional study of kidney and/or brain
35.	^{99m}Tc dl-HMPAO	Diagnosis of abnormalities of regional cerebral blood perfusion; also for leucocyte labelling
36.	^{99m}Tc MAA	Lung imaging
37.	^{99m}Tc-MDP	Bone scintigraphy
38.	Xenon (^{133}Xe) gas	Lung ventilation studies
39.	Xenon (^{133}Xe) injection	Lung perfusion studies, cerebral blood flow studies
40.	Yttrium (^{90}Y) silicate injection	Intrapleural or intraperitoneal injection treatment of malignant disease

* These radionuclides are produced by target irradiation in a cyclotron or similar accelerator.

TABLE II. PREPARATION OF ^{99m}Tc LABELLING KITS

Name of kit	Quantity of Ligand	Volume of solution in step 1	Quantity of $SnCl_2$ used	pH Step 3	Final volume step 4	Storage Temperature of kits.
^{99}Tc^{m} -MDP	500 mg MDP, 50 mg ascorbic acid	80 mL	0.6 mL	6-7	100 mL	2-10° C
^{99}Tc^{m} -EHDP	1 g EHDP	80 mL	1.25 mL	6-7	100 mL	2-10° C
^{99}Tc^{m} - Pyro-phosphate (PYP)	1.5 g Tetra-Na-pyrophos. deca- hydrate	80 mL; warm if necessary	1.25mL	6-7	100 mL	2-10° C
^{99}Tc^{m} DTPA	3.5 g DTPA mix with 10 mL 1N NaOH.	80 mL; warm if necessary	2.5 mL	6-7	100 mL	2-10° C
^{99}Tc^{m} - gluconate	20 g sodium gluconate	160 mL	1.25 mL	6-7	200 mL	2-10° C
^{99}Tc^{m} - gluco-heptonate	20 g sodium glucoheptonate	160 mL	1.25 mL	6-7	200 mL	2-10° C
^{99}Tc^{m} DMSA	100 mg DMSA; 5 mL 1N NaOH	80 mL	0.5 mL	2.5 (with 1N HCl)	100 mL	2-10° C
^{99}Tc^{m} - EHIDA	1 g EHIDA 40 mL 0.1N NaOH	40 mL	0.5 mL	5.5-6 (pH water)	100 mL	2-10° C
^{99}Tc^{m} - Bromo trimethyl IDA	1 g 10 mL 1N NaOH	10 mL	0.25 mL	6-6.5	100 mL	2-10° C
^{99}Tc^{m} - phytate	1 g Sodium phytate	80 mL	1.25 mL	6-7	100 mL	2-10° C

Appendix III

TABLE III. OPTIMUM QUANTITIES OF MEDICAL RADIONUCLIDES WHICH MAY BE PROCESSED/HANDLED IN A MEDIUM LEVEL CENTRALISED RADIOPHARMACY

Serial No.	Radionuclide and pharmaceutical form	Frequency of processing or handling	Activity per batch
1.	^{99}Mo-^{99}Tc^{m} generators	Twice in a week	500 G Bq
2.	^{99}Tc^{m} pertechnetate	Once or Twice a day	1 G Bq of each product
3.	^{131}I as sodium iodide solution and capsules	Once a week	5 G Bq of each product
4.	^{131}I labelled compounds (Rose Bengal, Hippuran, MIBG, etc.)	As required	500 G Bq
5.	^{125}I as sodium iodide for protein iodination,	Once in 2 weeks	100 G Bq
6.	^{125}I labelled compounds (Rose Bengal, Hippuran, MIBG, etc.)	Once in 2 weeks	5 G Bq or as solution
7.	^{57}Co/^{58}C labelled cyanocobalamin	As required	100 M Bq
8.	Miscellaneous medical products (labelled forms of ^{51}Cr, ^{59}Fe, ^{75}Se, ^{32}Pek)	As required	1 G Bq of each product
9.	^{113}Sn-^{113}In^{m} generator	Once in 2 months	5 G Bq

CHAPTER 12

Appendix IV

CHARACTERISTICS AND SPECIFICATIONS OF SODIUM IODIDE ^{131}I SOLUTION

1. Sodium iodide ^{131}I in aqueous solution containing a suitable buffer (sodium carbonate-bicarbonate)

2. Multidose packing in penicillin-type glass vials

3. Clear colourless solution, may have a light brown colour occasionally (due to effect of radiation)

4. Contains sodium thiosulphate added as a preservative (reducing agent)

5. Radioactive concentration 40-400 MBq/mL

6. Radiochemical purity $I^- > 97\%$

7. Radionuclidic purity $^{131}I > 99.9\%$

8. pH: 7 - 9.5

9. Content of $Na_2S_2O_3$ 0.001-0.02 mg/MBq ^{131}I

10. Content of stable iodine ($<$ 0.02 mg/MBq of ^{131}I at the time of (for therapeutic purposes)) patient administration

11. Storage: at room temperature with adequate shielding

12. Useful shelf-life: 4 weeks

Appendix V

CHARACTERISTICS AND SPECIFICATIONS OF DIAGNOSTIC ^{131}I-SODIUM IODIDE CAPSULES

1. Sodium iodide ^{131}I adsorbed on a solid adsorbent such as anhydrous sodium phosphate and contained in a gelatin capsule
2. 1-6 capsules may be packed together in penicillin-type vials which may contain a dessicant in a separate sachet
3. Single dose capsule
4. Activity per capsule 0.5-5 MBq/capsule
5. Radiochemical purity of ^{131}I I^- > 97%
6. Radionuclidic purity ^{131}I > 99.9%
7. Maximum variation in activity of individual capsules in a batch $\pm$ 5%
8. Sodium phosphate content per capsule 50-200 mg
9. Storage: at room temperature with adequate shielding
10. Useful shelf-life: 2 weeks

Appendix VI

CHARACTERISTICS AND SPECIFICATIONS OF THERAPEUTIC SODIUM IODIDE ^{131}I CAPSULES

1. Sodium iodide ^{131}I adsorbed on a solid adsorbent such as anhydrous lactose or sodium phosphate and contained in a gelatin capsule

2. The capsules may be individually packed in penicillin-type vials which may contain a dessicant in a separate sachet

3. Single dose capsules - for therapy of thyrotoxicosis or thyroid cancer

4. Activity per capsule: 40-4000 MBq/capsule

5. Radiochemical purity: I^- > 97 %

6. Radionuclidic purity: ^{131}I > 99.9%

7. Storage: at room temperature with adequate shielding

8. Useful shelf-life: 2 weeks

Appendix VII

CHARACTERISTICS AND SPECIFICATIONS OF ^{131}I-HIPPURAN INJECTION

1. Hippuran (sodium ortho iodo hippurate) ^{131}I in isotonic solution suitable for intravenous human administration

2. May contain a bacteriostatic agent (0.9% Benzyl alcohol solution)

3. Multidose packing in penicillin-type (amber-coloured) glass vials

4. Clear colourless solution, may have a light brown colour occasionally (due to effect of radiation)

5. Radioactive concentration: 4-20 MBq/mL

6. Content of Hippuran: 1-5 mg/mL

7. pH: 6-8

8. Radiochemical purity: $I^- < 2$ %
(less than 1% for some applications)
^{131}I as ortho iodo benzoic acid < 2 %

9. Storage: at 2-10°C in amber coloured glass vials with appropriate shielding

10. Useful shelf-life: 3 weeks

Appendix VIII

CHARACTERISTICS AND SPECIFICATIONS OF RADIOIODINATED (^{131}I) HUMAN SERUM ALBUMIN INJECTION

1. Radioiodinated (^{131}I) human serum albumin in isotonic solution suitable for intravenous human administration

2. May contain a bacteriostatic agent (0.9% Benzyl alcohol solution)

3. Multidose packing in penicillin-type (amber-coloured) glass vials

4. Clear colourless solution, may have a light brown colour occasionally (due to effect of radiation)

5. Radioactive concentration: 4-20 MBq/mL

6. Content of human serum albumin: 1-50 mg/mL

7. pH: 6-8

8. Radiochemical purity: Inorganic iodine ^{131}I } < 2%

9. Storage: At 2-10°C in amber coloured glass vials with appropriate shielding

10. Useful shelf-life: 3 weeks

Appendix IX

CHARACTERISTICS AND SPECIFICATIONS OF META-IODOBENZYLGUANIDINE (^{131}I) (m-IBG) SOLUTION-DIAGNOSTIC

1. Sterile, aqueous solution, containing 9 mg/mL of sodium chloride and 10 mg/mL of benzyl alcohol
2. Multidose packing in penicillin-type vials
3. pH 4.0-7.0
4. Radioactive concentration: 9-20 MBq/mL
5. Specific activity: 30-200 MBq/mg, m-IBG
6. Storage: at 2-8°C
7. Useful shelf-life: 3 days

Appendix X

CHARACTERISTICS AND SPECIFICATIONS OF INDIUM (^{113m}In) STERILE GENERATOR

Tin-113 absorbed on an ion-exchange material contained in a robust plastic column, with puncturable rubber end seals. The sealed unit is sterilized by heating with bactericide. Sterile indium-113m in aqueous solution is obtained by elution with the sterile eluent provided. The shielded generator is despatched with full instructions and accessories for 60 elutions.

1.	Activities available:	Generators containing 200 MBq to 5 GBq of ^{113}Sn.
2.	Purity:	Eluate contains less than 3.7 kBq, as tin-113 and less than 0.74 Bq, as other impurities per 37 MBq, ^{113}In at the time of calibration. Total heavy metal impurities present in the eluate do not exceed 5 μg/ml.
3.	pH:	pH of eluate is 1.4 $\pm$ 0.1.
4.	Storage:	Store at 15-25°C.
5.	Expiry:	The generator should not be used later than 6 months after the reference date.
6.	Description:	The kit provides pre-dispensed sterile reagents which, when used with the eluate from Indium [^{113m}In] Sterile Generator in the recommended manner, produces a sterile solution containing carrier-free indium-113m in the form of a strong DTPA chelate complex. The solution is ready for immediate intravenous injection as a diagnostic brain scanning agent. The kit contains reagents for five individual preparation units. Each prepared unit will provide several patient doses, the number being dependent on the total activity added.

7. Content: Each preparation unit contains two components:

   Component A - Sterile complexant 1 ml of sterile aqueous solution containing 1-60 mg of diethylenetriamine penta-acetic acid and 1.02 mg acetic acid. The solution is contained in a 10 ml neutron glass vial with puncturable rubber insert seal.

   Component B - Sterile buffer 1.3 ml of sterile aqueous solution.

8. Storage: Store at 15-25°C

9. Expiry: Normally at least 6 weeks from day of despatch as stated on the label.

10. Indication: The indium-113m brain scanning kit is indicated for brain scintigraphic studies following preparation by the method described below.

11. Preparation Procedure: Carrier-free indium-113m eluted from an indium [113mIn] sterile generator is complexed with diethylenetriamine penta-acetic acid (DTPA). The pH of the solution is then adjusted to neutrality by addition of a buffer.

CHAPTER 12

Appendix XI

CLEANING AND DISINFECTION OF PREMISES

1. The floors of the laboratory should be cleaned daily by mopping with a disinfectant solution such as Dettol (or other disinfectants as specified in the authorized disinfectant list).

2. The walls, the bench tops and doors should be cleaned similarly once a week.

3. The rooms should be exposed to formaldehyde vapour once a week by keeping formaldehyde/potassium permanganate mixture in Petri dishes in the room during the weekend.

4. The equipment such as freeze drying unit should be cleaned by wiping with aqueous alcohol regularly once a week and just prior to use.

Monitoring:

1. Nutrient agar plates should be exposed for one hour at a few places in the room and the dispensing area.

2. Sterile fluid thioglycolate medium or tryptocase soya broth should be filled and sealed in sterile vials under the same conditions as in the production of radiopharmaceuticals. They should then be incubated and examined for any microbial growth.

This should be carried out prior to preparation of each batch of products and the record of the results should be maintained.

Appendix XII

CLEANING OF GLASS VIALS

1. _____ No. of ______ capacity flint vials should be carefully selected and cleaned with 0.1% Teepol by brushing individually or by ultrasonic cleaning for 30 minutes.

2. These should then be cleaned in running tap water to remove Teepol.

3. After this, these should be all immersed in 15% v/v nitric acid (C.P.) and kept overnight.

4. Next day these should be removed from acid and washed to remove acid completely.

5. At this stage, these should be checked with indicator paper to confirm no acid contamination in them.

6. Then these vials should be individually rinsed twice with double distilled water and kept inverted in a clean container and sterilized by dry heat at 180^{0}C for 2 hrs or 160^{0}C for 6 hrs.

CHAPTER 12

Appendix XIII

CLEANING OF RUBBER CLOSURES

1. _____ Nos. of rubber closures should be carefully selected and cleaned with tap water to remove visible dust and extraneous particles.

2. Then they should be immersed in 1% Teepol solution and scrubbed thoroughly.

3. They should then be rinsed with tap water till they are completely free from Teepol.

4. They should be soaked in 10% w/v sodium hydroxide solution and allowed to remain overnight.

5. Next day they should be removed from alkali and washed with running tap water till they are free from alkali.

6. Further these closures should be soaked in 20% v/v hydrochloric acid and allowed to remain for 24 hours.

7. Next day the acid should be drained off completely and these rubber closures should be washed with double distilled water till acid is completely removed (rinsing should be checked for absence of any acidity).

8. They should be autoclaved in double distilled water for 30 minutes under 15 p.s.i. pressure.

9. They should then be rinsed with double distilled water and then put in cleaned beakers and kept in hot air oven at 70°C for drying.

10. They should then be stored in a clean atmosphere.

11. For use in production they should be sterilized by one of the following methods:

    Sterilization:

    (a) Radiation sterilization: Seal in polythene bags and sterilize by gamma radiation (2.5 Mrad dose).

    (b) Autoclaving: Rinse with double distilled water, seal in polypropylene bags and autoclave at 15 p.s.i. (121°C) for 30 minutes. Dry in hot air oven at 70°C. To be used within one week after autoclaving.

Appendix XIV

SPECIAL EQUIPMENT AND MATERIALS REQUIRED FOR SETTING UP A RADIOPHARMACY

1.	Laminar flow work bench	1
2.	Pyrogen-free water unit	1
3.	Electronic analytical balance	1
4.	pH Meter	1
5.	Medium-capacity freeze drying unit	1
6	Steam sterilizer	1
7.	Dry air sterilizer	1
8.	Incubator	1
9.	Centrifuge	1
10.	Well-type scintillation counter with detector and spectrometer	1
11.	Portable survey meter (beta gamma)	1
12.	Portable beta-gamma contamination monitor	1
13.	Radioisotope dose calibrator	1
14.	Shielding materials (lead bricks, viewing glasses)	As required
15.	Millepore filters	
16.	Automatic pipettes for small volumes	
17.	Special chemicals	
18.	Small tongs, handling tools	
19.	Radioactive standards and long-lived reference sources	
20.	Chromatography paper	
21.	Glassware (vials, chromatography jars, pipettes, etc.)	
22.	Fume hood/beta gamma box	1

CHAPTER 12

Appendix XV

CHARACTERISTICS AND SPECIFICATIONS OF INDIUM [^{113m}In] COLLOID LIVER SCANNING KIT

1. Description — The kit provides pre-dispensed sterile reagents which, when used with the eluate from Indium [^{113m}In] Sterile Generator in the manner described, produces a sterile solution containing carrier-free indium-113m labelled colloid. The solution is ready for immediate intravenous injection as a diagnostic liver scanning agent. The kit contains reagents for five individual preparation units. Each prepared unit will provide several patient doses, the number being dependent upon the total activity added.

2. Contents — Each preparation unit contains two components:

   Component A - Sterile stabilized carrier. 1 ml of sterile aqueous solution containing 4.8 μg of ferric chloride (FeCl3.6H2O) and 50 mg of mannitol. The solution is contained in a 10 ml neutral glass vial with a puncturable rubber insert seal.

   Component B - Sterile buffer 1.3 ml of sterile aqueous solution containing 43.3 mg of sodium dihydrogen phosphate and 68.5 mg of disodium hydrogen phosphate. The solution is contained in a neutral glass ampoule.

3. Storage — Store at 15-25°C.

4. Expiry — Normally at least 6 weeks from date of despatch as stated on the label.

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Chapter 13

BIODISTRIBUTION STUDIES - GENERAL ASPECTS

R.S. Mani

In accordance with the general guidelines for animal experiments, consideration should be given to the appropriateness of experimental procedures, species of animal used, and number of animals required.

Biodistribution studies should be performed in a specially reserved area outside the radiopharmaceutical preparation area by staff well trained in the technique. In handling animals for experiments the guidelines issued by national and international agencies should be followed. Even though desirable, it may not be always practical for a small scale radiopharmacy to maintain an animal house. In such a case, cooperation with another institution or agency having appropriate facilities would be required.

Rats and mice weighing 140-200 g and 18-25 g respectively and in healthy condition should be used for these experiments. The set of animals used in each experiment should be of the same sex, strain, flock and of about the same weight. The radiopharmaceutical formulations in a volume not exceeding 0.3 ml in case of mice and 0.5 ml in case of rats containing the appropriate amounts of radioactivity depending on the sensitivity of the measuring device used, is injected in the tail vein of the animals. Care should be taken to keep the amount of chemicals injected to the same level on a per kg basis as is used in human beings since one may encounter abnormal biodistribution if too much of the chemical is injected. After the time intervals stipulated for each product the animals should be sacrificed and, in addition to collected urine, the organs liver, lungs, spleen, kidneys, stomach, intestine, femur, bladder, and thyroid gland should be removed and placed in containers for counting. They may be weighed if the data required is to be expressed on a per gram basis. In case of blood, a measured aliquot should be weighed and counted. Total activity in blood should be calculated assuming the blood to be 7% (7.8%) of the body weight. The total activity injected should be calculated either from the net activity of the syringe (minus the activity at the injection site in the tail) or by adding the count rate in the individual organs plus the carcass.

For scanning, healthy adult rabbits should be injected through the ear vein, a dose of the formulation containing activity and chemicals proportional to their body weight to the expected human dose in a volume not exceeding 3/ml. After the appropriate time interval, the rabbits should be anaesthetized and a whole body gamma camera or scanner image of the rabbit should be taken in the posterior and anterior views using a low-energy multi purpose collimator. For serial imaging the injection should be made under the camera.

CHAPTER 13

ACCEPTABLE BIODISTRIBUTION CHARACTERISTICS OF ^{99m}Tc RADIOPHARMACEUTICALS

1. 99mTc-MDP Injection

Biodistribution studies are carried out using rats. At 2 hours post injection, the percentage of radioactivity in the femur will not be less than 2% of the injected activity, that in the liver not more than 0.6% per gram and in the kidney not more than 1% per gram.

If the facilities permit, it is advisable to carry out supplementary distribution studies on rabbits using a gamma camera. 99mTc MDP will localize in the skeleton. Significant activity will be seen in the kidneys and bladder with no significant activity seen in the liver, stomach thyroid gland or in other areas.

2. 99mTc-EHDP Injection

Same as in 1 above.

3. 99mTc-Pyrophosphate injection

Same as in 1 above.

4. 99mTc-DTPA Injection

Biodistribution studies are carried out using rats. At 2 hours post injection the combined percentage of radioactivity in urine and bladder will not be less than 85% of the injected activity. Less than 1% of the injected activity will be seen in the liver.

If the facilities permit, it is advisable to carry out supplementary distribution studies in rabbits using a gamma camera. Serial images obtained immediately after injection will show uptake of 99mTc DTPA by the kidneys and clearance into the bladder with no significant activity remaining in the kidney or seen concentrating in the liver, stomach, thyroid gland or in other areas.

5. 99mTc-Gluconate Injection

Biodistribution studies are carried out using rats. At 1 hour post injection, the percentage radioactivity in the kidneys will be no less than 15% of the injected activity, that in the liver and GI tract not more than 5% each. The sum of the activities in the kidneys, urine and bladder must be greater than 75% of the injected activity.

If the facilities permit it is desirable to carry out supplementary biodistribution studies in rabbits using a gamma camera. The kidneys and bladder will be visualized clearly 1 hour post injection. No significant activity will be seen in the liver, stomach, thyroid gland or in other areas.

6. 99mTc-glucoheptonate Injection

Same as in 5 above.

7. 99mTc-DMSA Injection

Biodistribution studies are carried out using rats. At 1 hour post injection the combined % of radioactivity in urine and bladder will not be less than 40% of the injected activity. The ratio of uptake in kidney to that of liver and spleen taken together is more than 6:1.

If the facilities permit it is desirable to carry out supplementary biodistribution studies in rabbits using a gamma camera. Both kidneys will be clearly visualized 1 hour p.i and significant activity may be seen in the bladder with no significant activity seen concentrating in the liver, stomach, thyroid gland or in other areas.

8. 99mTc-EHIDA Injection

Biodistribution studies are carried out using rats. At 30 minutes post injection the percentage of radioactivity in the intestine will be not less than 70% of the injected activity, that in the bladder and urine not more than 15%, in the kidney not more than 2.5% per gram and in the liver not more than 1% per gram.

If the facilities permit it is desirable to carry out supplementary biodistribution studies in rabbits using a gamma camera. Serial images starting immediately after injection will show rapid clearance from the blood, liver uptake and excretion into the intestine.

9. 99mTc-trimethyl Bromo-IDA Injection

Same as in 8 above.

10. 99mTc-Phytate Injection

Biodistribution studies are carried out using mice. At 30 minutes post injection the percentage of radioactivity in the liver and spleen will be not less than 80% of the injected activity. Less than 5% of the injected activity will be seen in the lungs.

If the facilities permit it is desirable to carry out supplementary biodistribution studies in rabbits using a gamma camera. Images obtained 30 minutes after injection will show clear visualization of the liver with no significant uptake in the kidneys, stomach, thyroid gland or in other areas.

11. 99mTc-sulphur colloid injection

Same as in 10 above.

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Chapter 14

DOCTOR'S DILEMMA
(Medical Decision making)

R.D. Ganatra

Einstein is supposed to have said that "God does not play dice". A Doctor has to play God at times and unlike God he has to play dice also. A patient does not go to the doctor for a shopping list of drugs or for a list of investigations to find out what is wrong with him. He goes to him, really speaking, for a decision. A doctor is unable to offer, most of the times, a definite decision because the evidence that he has at hand is more of a statistical nature and not conclusive enough, but he still has to decide about the management of the patient by playing dice, because he cannot wait indefinitely for the evidence to build up so that his diagnosis is irreproachable. How should he play the dice that he has better than an even chance of coming out right?

It should be recognized first of all that there are no medical decisions which are infallible. A doctor takes them in such a way that the statistical probability of their being right is in his favour.

The application of any test will split the population in four ways.

1. **True positive (TP)** - those individuals who indeed have the disease which the test is supposed to detect **and** in whom the test is positive;

2. **True negative (TN)** - those individuals who do not have the disease, the test is intended to detect **and** in whom the test is negative;

3. **False negative (FN)** - those individuals who do have the disease for which the test was used **but** in whom the test is negative;

4. **False positive (FP)** - those individuals who do not have the disease **but** in whom the test is positive.

The above outcomes of a test can be expressed in a matrix format in the following way:

		DISEASE	
T	->	Present	Absent
E	Positive	TP	FP
S			
T	Negative	FN	TN

The specificity (Sp) of a test is its ability to detect those individuals who do **not** have the disease.

$$\text{Specificity (Sp)} = \frac{TN}{TN + FP}$$

It is obvious, that fewer the FP cases found by a test, the higher is its specificity. The highest possible specificity can be 100%, which means that among 100 individuals without the disease the test will find 100 TN and no FP cases. Specificity decreases with the number of the FP cases detected.

Sensitivity (Sv) of a test is its ability to detect the disease among those who indeed have the disease.

$$\text{Sensitivity (Sv)} = \frac{TP}{TP + FN}$$

As in the case of specificity, the highest degree of sensitivity is expressed as 100 %. Higher the FN, lower is the sensitivity of the test.

Accuracy (Ac) is mathematically expressed as:

$$\text{Accuracy (Ac)} = \frac{TP + TN}{TP+TN+FP+FN}$$

Accuracy decreases with increase in the FP and / or FN. When there are no FP or FN, FP+FN is equal to zero and from the above formula, it will be obvious that the Ac will be ONE, which is the highest possible.

A very sensitive test is likely to be less specific. Let us take an example from Liver scintigraphy. There is a total of 344 patients in whom a confirmed diagnosis is available. The decision matrix looks like this:

	D+	D-
T+	231(TP)	32 (FP)
T-	27 (FN)	54 (TN)

If the above numbers are plugged into the formulas given earlier, the following values are obtained:

$$\text{Sensitivity (Sv)} = \frac{231}{231 + 27} = 0.9$$

$$\text{Specificity (Sp)} = \frac{54}{54 + 32} = 0.63$$

$$\text{False Negative} = \frac{27}{258} = 0.1$$

$$\text{False Positive} = \frac{32}{86} = 0.37$$

$$\text{Accuracy (Ac)} = \frac{231+54}{344} = 0.83$$

Whatever one does, certain inaccuracy is inevitable. In what clinical situations, we can tolerate more FP? Not in detecting metastases in the liver because with a positive scintigram, either TP or FP, the chemotherapy will be imperative. These are relative value judgements and compromises are involved in this kind of decisions.

The following figure shows the same concepts graphically for a system of continuous variables such as T4 levels of RIA in a patient suspected of having thyrotoxicosis **(Fig. 14.1)**.

The graph shows two kinds of populations: normal and patients suspected of having toxicity. The central vertical line is the so-called normal value which divides the population between normal and abnormal. If you slide this line of demarkation to left, you end up with more FP and less FN (**Fig. 14.2**). On the other hand, a slide to the right will result in small FP and a large FN. If you apply the above mentioned formulas, you will realize that there is always a compromise between the sensitivity and the specificity. You can never be unimpeachably accurate but you are allowed to temper with your scales of justice in favour of sensitivity or the specificity.

There are few other factors which influence your decision. Prevalence and incidence of a disease influences the predictive value of the test and these terms are defined below.

Prevalence (P) represents the total number of cases of a disease at a certain moment in a given population. Prevalence rate (Pr) is the number of cases of the disease per 100 000 population.

Incidence(I) is the number of new cases of a disease which develop during a defined period of time. It is usually expressed as the incidence rate (Ir), i.e. the number of new cases per year per 100 000 population.

The **Predictive value (PV)** of a test is an important notion in diagnostic testing. Predictive value positive (PV+) is defined as the % of the positive results that are true positive when the test is applied to a population comprising of both the healthy and the diseased subjects. In the same way, the PV- is the % of the negative results in a similar population. The Predictive value depends on the following factors: Prevalence (P) of the disease, sensitivity (Sv) and the specificity (Sp) of the test used, as shown in the following equation for PV+:

$$PV+ = \frac{Sv \times P}{(Sv \times P) + (1 - Sp)(1 - P)}$$

It is clear from the equation that P strongly influences the PV+. In a simple way, PV+ provides probability of a disease, if the test gives a positive result. PV- signifies probability of **no** disease, in the presence of a normal result. There are ready-made Tables of Predictive value at different Prevalence. Table 1, is given as one example:

TABLE I. EFFECT OF PREVALENCE ON PREDICTIVE VALUE WHEN SENSITIVITY AND SPECIFICITY EQUAL 95%.

Prevalence of disease(%)	Predictive value of a positive test(%)
0.1	1.9
1.0	16.1
2.0	27.9
5.0	50.0
50.0	95.0

A simplified approach to the Predictive values of the test can be presented as follows:

$$PV+ = \frac{TP}{TP + FP} \times 100 \qquad PV- = \frac{TN}{TN + FN} \times 100$$

PV+ depends on the number of the FP and PV- is dependent on the number of FN-. The smaller the number of FP or FN, greater is the value of PV+ or PV-.

Here also, an example will make the concept of prediction easier to grasp. As we saw in the previous example, the sensitivity and the specificity of liver scintigraphy is 90 % and 63 % respectively.

In an area, where the prevalence of Amoebic abscess is just 1 %, as is the case in most of the developed world, a PV+ resulting from a positive test, will increase this probability to 3 %, not a very significant gain by any chance. If a negative result was obtained in liver scintigraphy, our confidence in the prediction that the patient does not have amoebic abscess will rise from the original 90 % to 100 %. In some parts of the third world, the prevalence of liver abscess is as high as 50 %. A positive scan will increase this probability to 75 % and a negative result will raise this to 87 % (**Fig. 14.3**). In both cases, the gains in our predictive confidence are not substantial. Medical decisions are not made solely on the basis of the test results but they are also dependent on the geographical location of the patient.

There are other ways also of improving the predictive confidence. Selection of the patient for an investigation will change the prevalence values. High degree of clinical suspicion will put the patient in a high prevalence group. It will at least establish a rationale for the investigation. Is it being done for diagnosis because the clinical examination is strongly in favour of a specific diagnostic probability, or is it being done merely as a screening test to exclude a diagnostic possibility in cases where the clinical examinations were normal?

A similar enhancement of the prevalence in a population group can be obtained by sequential testing. If a previous screening test had indicated a presence of the disease, the second test will be done in the high prevalence group. Doing a variety of tests, all at the same time, does not help very much because all tests are being done in a low prevalence group. Moreover, too many tests have a high probability of one test giving an abnormal result as shown in the graph below. If 15 tests are done simultaneously, there is a 50 % chance of one of the tests showing abnormal results (**Fig. 14.4**).

Receiver Operating characteristics (ROC), was originally devised as a means of displaying the accuracy of an observer to discriminate between signal and noise when using electronic systems such as RADAR. The curve primarily was meant for electronic signal detection. ROC curve can be plotted as the true positive rate (TPR) or sensitivity as the ordinate and the false positive rate (FPR) or (1 - specificity) as an abscissa (**Fig. 14.5**) In a sense, the curve represents possibilities of compromise between (or a sort of trade-off) the sensitivity and the specificity. The cut-off line can be moved to right or left, increasing or decreasing on one side TP and FP and thereby the sensitivity of the test or on the other side the TN and FN and thereby the specificity of the test. An optimal operating point should be determined for each test. The optimal operating point defines the aim with which the test has been applied. If the aim is to exclude the disease, higher specificity is required at the expense of the sensitivity, e.g. testing pilots for their fitness. On the other hand, if it is

desired to detect the disease, (say, T.B. among the recruits) , higher sensitivity is required at the cost of specificity. The following figure shows a typical ROC curve between TP and FP. It will be obvious that you can not increase TP without some increase in the FP. Where the curve levels off at the top, there is no substantial gain by any costly refinement of the technique.

Fig. 14.6 shows ROC curve between CT and ultrasound for detecting focal diseases in the liver. Obviously, CT has more TP with less FP. ROC curves not only vary from patient to patient or from instrument to instrument but can also vary from doctor to doctor.

Do doctors disagree?

Disagreement between experts is presumed to be uncommon in medical diagnosis. Radiology is considered to be a particularly objective means of diagnosis and expert radiographic interpretation is expected to be infallible.

Five military radiologists were made to review independently chest radiographs of 1256 patients recorded in four image formats and interpret each as positive or negative for tuberculosis. The results were unexpected. Ability to detect tuberculosis varied little between various image formats but the extent of disagreement between doctors was remarkable. The number of cases judged positive varied from 56 to 100 among the five readers. Of cases judged positive at least once, the mean rate of disagreement between pairs of readers was 19%.

The validity of these findings have been confirmed repeatedly in several subsequent trials. Other diagnostic modalities show equally surprising rates of diagnostic dissonance. Extensive observer disagreement was found to be a universal problem in medical diagnosis, giving credence to the proverbial adage that "no two doctors agree".

The magnitude of disagreement between experts is the principal theoretic problem of diagnosis. Even a stochastic theory of diagnosis is devised which accounts for the disagreement between experts, where the disagreement approaches a theoretic maximum even for ideal diagnosticians.

In another similar trial, three radiologists and three chest physicians, read three sets of chest X-rays of 150 patients with proven tuberculosis. The rate of disagreement between pairs of readers ranged from 26 to 38%. Readers made a second interpretations of the radiographs several weeks later. The rate of disagreement with oneself ranged from 19 to 29%!

The receiver operating characteristic (ROC) curve, as explained above, is a graph of sensitivity versus specificity. A particular point on an ROC curve represents a **diagnostic strategy,** i.e, "under-read" or "over-read" the test. There are always sins of 'omission' and 'commission' and whichever way you lean you either increase the sensitivity and decrease the

specificity or vice versa. A physician defines his strategy depending upon the prevalence of the disease, treatment possibilities available and the management outcome of a false positive or a false negative diagnosis. Experts can and do disagree systematically as a result of diagnostic strategy. Disagreement does not indicate a disparity in diagnostic skill.That is why the Agency's insistence that a clinical training program should be in the candidate's region only, so that his ultimate clinical decisions are not based on the diagnostic predictions prevalent in the developed world.

If I am in a country where there is no facility for cardiac surgery and where I am doing nuclear cardiology to decide which patient should go abroad for cardiac surgery, I would prefer to be sensitive, rather than specific. If I am in a hospital where I have to give opinion to a cardiac surgeon on the next floor who is waiting for my results to decide whether to operate on the patient or not, I shall try to be specific and not unduly sensitive.

Coordinated Research Program (CRP)

IAEA had conducted a CRP on "Evaluation of Liver Imaging Procedures" from 1984 to 1988. It started with an analysis of the images of a liver phantom with the aim of evaluating the performance characteristics of the imaging instruments available in the developing countries. During the course of this initial analysis, we found that a same image of the phantom can be interpreted differently by different observers. There was an observer variation with a wide divergence. A way of statistically analyzing this observer variance was developed on the basis of the Receiver Operating Characteristics (ROC) curves. The project then entered into its second clinical phase at this stage. A set of 293 liver images of patients with confirmed diagnosis were provided to each of the participating countries. Several nuclear medicine specialists from each country interpreted these images and entered their findings on a specially prepared proforma. Obviously there was a great deal of variation in the diagnostic interpretation from doctor to doctor.

The variation in the interpretation of clinical liver images does not come as a surprise to a practising nuclear medicine specialist. A two dimensional image of a large solid organ like liver without any specific geometric shape poses a serious challenge for interpretation. Faced with such a challenge, each nuclear medicine specialist forms his own diagnostic strategy. This would differ from country to country.

Cost of a test

The cost of a test is difficult to determine because the cost is to be ascertained not only in terms of money but also in terms of cost of the medical consequences of FP or FN results of an investigation. The average cost resulting from the use of a diagnostic test C can be expressed by the following complicated formula:

$$C = Co + C_{TP} \cdot P_{TP} + C_{TN} \cdot P_{TN} + C_{FP} \cdot P_{FP} + C_{FN} \cdot P_{FN}$$

where:
Co = overheads of performing the test;
C_{TP} = the average cost of the medical consequences of a True positive decision;
P_{TP} = the probability of a True positive outcome; and so on for the True negative, false positive and negative decisions. The above formula looks formidable but very easy to understand conceptually, if it is written in words as given below:

cost of error = cost of test + P of false +ve x cost of false +ve + P of false -ve x cost of false -ve

The following table shows the results of Renogram and Angiogram in a group of patients with renal artery stenosis.

	RENOGRAM		ANGIOGRAM	
	Abn.	Norm.	Abn.	Norm.
Patients who do not need surgery	11	85	1	95
Patients who needed surgery	4	0	4	0

Obviously the renogram did miss quite a few abnormalities but it did not miss any where the disease was advanced enough to need surgery. Renogram is non-traumatic and if the aim is to detect patients who might require surgery, it is far less costlier than the angiogram.

It is now commonplace to build decision tree, where you go from non-invasive to invasive and from less costly to more costly tests. The tree progresses sequentially and all the tests are not done at the same time and at any stage on the tree, if a doctor got enough confidence in his diagnosis, further investigations need not be done.

Efficiency, and efficacy

Efficiency is a simple definition derived from the above:

$$\textbf{Efficiency} = \frac{\textbf{TP + TN}}{\textbf{100}} \textbf{ x Total}$$

A more realistic approach will be to define efficiency as the ability of the test to provide diagnostic information of the highest relevance with the lowest investment both in terms of work and money.

Efficacy is the ability of a diagnostic test to influence a doctor's decision regarding the patient's management. Which diagnostic test is more efficacious in a particular clinical situation depends on a variety of factors. A test acceptable or preferable in one part of the world may not be considered that beneficial in another part of the world.

Diagnostic utility of investigations

How a doctor arrives at a decision is of interest to both the developed and the developing countries. The developed and the developing want to walk on the same road but from different directions: one wants to develop a little more and the other wants to develop a little less for cost containment.

Advanced countries want to reduce unnecessary diagnostic tests in an attempt to bring down the sky-high cost of the medical care. The developing countries want to set up only the essential tests because their resources are limited. Both of them are approaching the same target but from different directions. These economic considerations have led us to new ways of accessing the utility of diagnostic tests. They are increasingly evaluated in terms of "how they affect the outcome of the patient's management". Apart from establishing the diagnosis, does the test provide any prognostic information, does it help in evaluating the results of the treatment that the patient is receiving?

To justify nuclear medicine in a developing country we have to see nuclear medicine in a new role. It is not for putting the diagnostic labels, not for differential diagnosis as we have been conditioned to think so far. In a developing country it should be for differential management. How does it alter the management decision in respect to a particular patient ? If management outcomes are restricted, there is no need for an investigation which does not help in any way the management of the patient. If there is no bypass surgery, what use is the thallium perfusion? Although primarily a diagnostic discipline for its justification and survival in the developing country it should lead to a sensible differential management.

May be a rule of thumb for deciding whether a test should be done or not, could depend on the following dialogue between a patient and a doctor:

"What will you do if the test is negative?"

"Nothing is indicated then."

"What will you do if the test is positive?"

"Nothing again, because nothing is possible."

"Why do you want to investigate, then?"

CHAPTER 14

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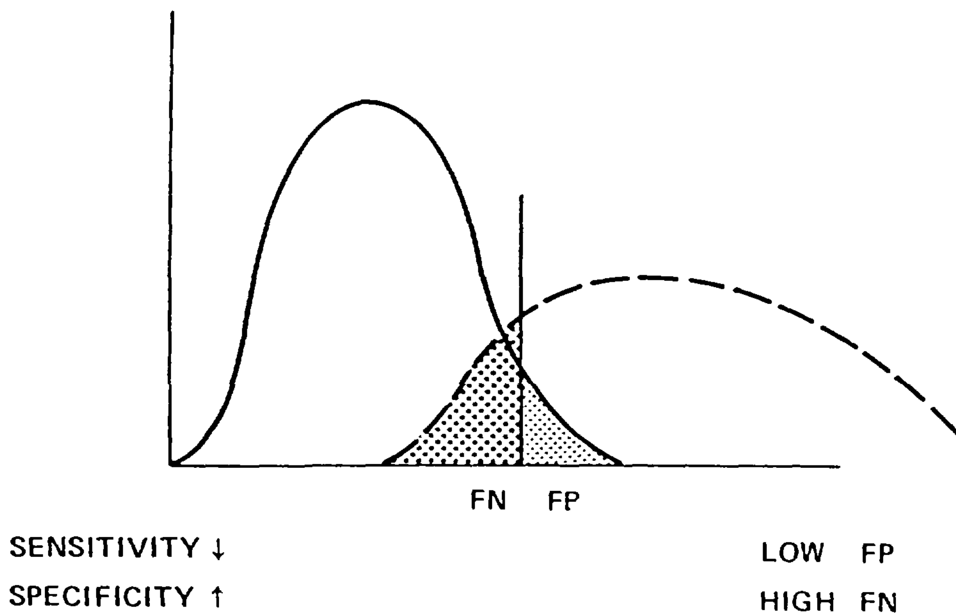

Fig. 14.1 The continuous line shows normal population. The interrupted line shows thyrotoxic population. The straight line in the middle shows the cut-off value between the normal and the abnormal. The cut-off point shows a low FP, indicating that the in vitro test is more specific and less sensitive.

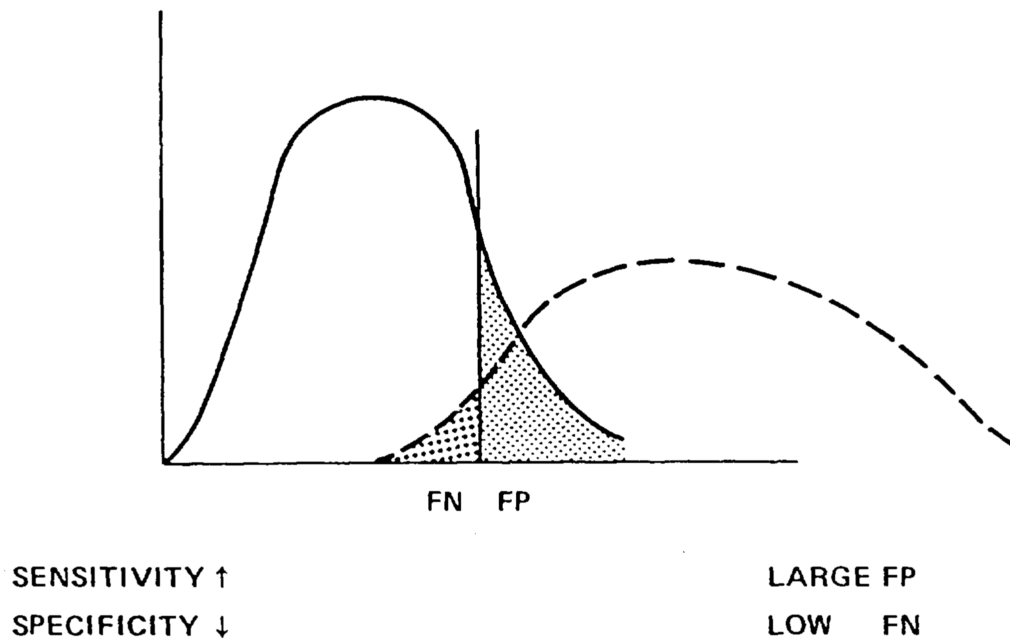

Fig. 14.2 Sliding the cut-off point to the left increases the number of FP, thereby increasing the test's sensitivity but lowering its specificity.

LIVER IMAGING SN 90

SP 63

Am. Abscess

DP	1%	
P +	3%	P – 100%
DP	50%	
P +	75%	P – 87%

Fig. 14.3 Sensitivity and the specificity of the liver imaging for Amoebic abscess, in populations with markedly different prevalence rates.

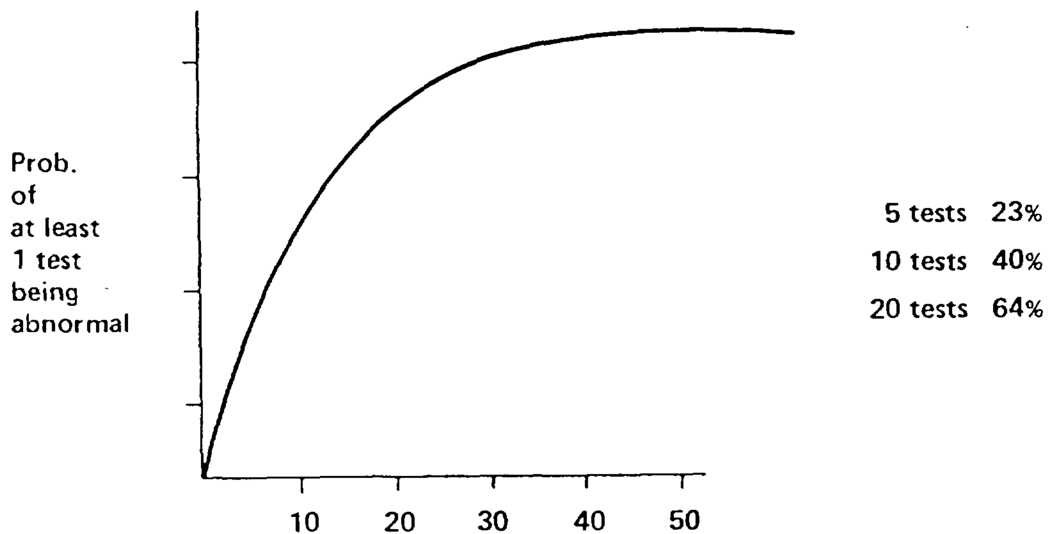

Fig. 14.4 The probability of at least one test showing abnormal results when a series of investigations are done at the same time. With 15 tests, there is a 50% chance that one test shows abnormal results.

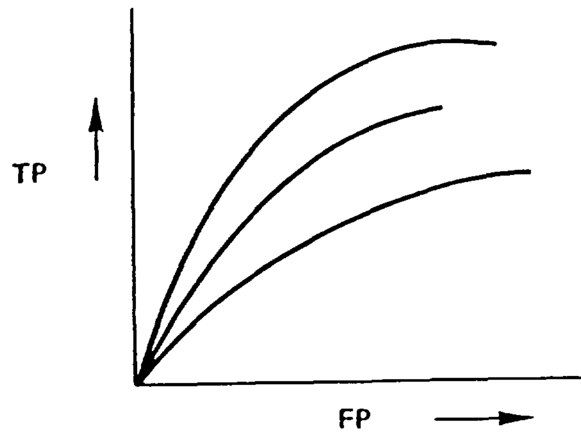

Fig. 14.5 ROC curves. X-axis shows false positives and the Y-axis shows false negatives.

CT
US
LIVER

CT
US
PANCREAS

2.4 cms
2.1
LESION SIZE
1.4
1
P OF DETECTION
INF. DENSITY

Fig. 14.6 ROC curves of CT and US. The CT has less FP, while having less FP.

CHAPTER 14

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Chapter 15

EFFECTIVE CHOICES FOR DIAGNOSTIC IMAGING IN CLINICAL PRACTICE

(Excerpts from a Report of a WHO Scientific Group on Clinical Diagnostic Imaging)

Introduction

There are so many different methods of diagnostic imaging that medical practitioners may need guidance to choose the best through the maze of options for each clinical problem. Advice may be required for more than just the first choice, because the first imaging procedure does not always give the desired answer and, depending on the results, further imaging may have to undertaken. The alternative is to submit the patient to a barrage of imaging and hope that one type, at least, provides the diagnosis. This is a quite unacceptable way to practice medicine because of the cost and the risk of radiation damage from unnecessary examinations.

If the WHO's goal of health for all by the year 2000 is to be achieved, the money available for health care must be efficiently used in every country. In an ideal world, the cost of medical care would not be a limiting factor affecting the quality or quantity of the health services provided, but unfortunately this situation does not exist in any country. Even the wealthiest nation must be aware that final support is not unlimited. As the wealth of a country grows, so do cost and complexity of medical care, often at rates which exceed other aspects of the economy. Such an imbalance can have an effect throughout the community which no society can ignore.

Cost and complexity in health care are always reflected in diagnostic imaging services that can be provided. The WHO established the principle that radiological services are of great importance at the first level of patient care, and has provided specifications for X-ray equipment and general-purpose ultrasound scanners. WHO manuals guiding radiographic and darkroom techniques and the interpretation of X-ray films are available in several different languages and are deservedly popular, and a manual on ultrasound interpretation is in preparation. In addition, several WHO published documents are concerned with the efficacy and efficiency of radiological imaging, and with the technical specifications of the equipment to be chosen. However, none of these reports has addressed the vital question of which approach is the most effective for the imaging of any specific clinical problem. What order of imaging should be followed to make the diagnosis accurately, quickly and economically? Equally important, is diagnostic imaging needed at all in some cases? It must be emphasized that imaging can never replace clinical history-taking and examination as the first steps in helping each patient.

The choice of the most effective imaging is often difficult and frequently controversial. The sequence to be followed varies with many factors: the equipment available, the skills of the practitioner, the expected quality of the results, the quality of interpretation, and conclusion which can be drawn. Local circumstances will alter need; for example, the exact diagnosis of the type of cerebral tumour is not an urgent matter if there is no neurosurgeon available. The quality of the local clinical service, and the simple question, "What can be

done with this patient after the diagnosis is made?", must also be taken into account. Priorities must therefore be established and the way in which a patient undergoes imaging will have many local variations. Unfortunately, not everyone can have the benefit of all methods of diagnostic imaging; indeed, the majority of the world's people will never even see an X-ray unit, let alone have radiographic examination. Ultrasound equipment is similar scarce. Both types of facilities are unevenly distributed and predominate in large cities. Nuclear medicine withers when departments are unable to obtain radionuclides promptly. The use of magnetic resonance imaging (MRI) is expanding at an astonishing rate in some countries, while remaining only a hope for the future in many others. Any recommendations on the use of imaging must allow for all these variants, so that in each case the best choice can be made from what is available. Despite all the difficulties, some attempt needs to be made to establish the best approach to the most common clinical problems.

Although the economic situation, which cannot easily be changed, may be the ultimate factor limiting the choice of diagnostic imaging techniques, the risk of ionizing radiation, which is controllable, must also be a consideration. At the primary care level, radiography is still the most common method of imaging, because the majority of patients can benefit from basic radiological diagnosis. This applies equally at secondary and tertiary levels of care, because throughout the world 80% of all diagnostic images are of the chest and skeleton. Ultrasonography, which is less expensive, cannot satisfy either of these needs. Computerized tomography (CT), using ionizing radiation, is not a survey tool suitable for most initial examinations. MRI is a remarkable technology, particularly for imaging the central nervous system and spine, but even its most ardent advocates have yet to recommend it as a first step when the patient has a cough or a suspected limb fracture. Scintigraphy is seldom of initial importance, except in a few cases such as osteomyelitis or suspected stress fractures. The principle that no patient should be exposed to unnecessary (meaning ineffective, clinically useless) radiation is a very good reason why the sequence of imaging must be carefully chosen by a radiologist or medical practitioner who has a clear idea of what should be done first after the clinical examination, and what subsequent imaging may be needed when the first results are available.

The growth of ultrasonography in the last decade has changed the pattern of imaging: in many clinical situations ultrasonography is now the first choice. Few clinicians would disagree that all pregnant women who need imaging should have an ultrasound scan first, and it is seldom that any other method of obstetric imaging will be contemplated throughout pregnancy. Equally, for the liver, pancreas and spleen, ultrasonography should be used first, even where CT and MRI facilities are available. CT should be deferred because it uses ionizing radiation, costs more and does not always give more information. MRI should be delayed because of even higher costs, and because ultrasonography will provide equally reliable guidance in a high proportion of cases. Similarly, ultrasound is considered the primary imaging modality for most gynaecological problems, the scrotal contents and the prostate. In some countries, ultrasonography is used as a screening procedure for detection of *echinococcal* infection, and has demonstrated a significant number of new cases. However, its predictive value is strongly influenced by the prevalence of the disease. The thyroid may be examined using scintigraphy, and for bone scanning there is no substitute for

radionuclide techniques. With the present state of knowledge mammography should be done with X-rays, despite exposure of patient to radiation, because no other method has the same accuracy, ease of access and availability.

None of the images obtained by these techniques, be they radiographic, ultrasonic or from magnetic resonance, can be interpreted without knowledge of the clinical examination of the patient, and in many cases of the results of laboratory tests also. Information should be thoroughly and carefully assessed, and the laboratory investigations as carefully chosen as the image sequence. All steps in assessing the patient are interrelated and very few images, however spectacular, are valid in the absence of all other information. There will also be circumstances where local skill and experience in one modality may override other considerations, because the images are only as good as the physician who interprets them.

This report, then, is concerned with the many different indications which will guide the physician in the diagnostic imaging of each patient. There are, today, so many different choices and such wealth of information that it is often too much for any individual to master, and consultation with colleagues has become essential for good care of patients. While this report may provide useful guidance, it will not displace, wherever available, personal discussions between patient's physician and the specialist in diagnostic imaging. Such consultations should precede and guide the choice of imaging, rather than being merely a review of images after they are taken. Early consultation will improve the results, and the twin goals of high quality of patient care and cost restriction may be reached. If, at the same time, ionizing radiation is used more sparingly, the process will be further justified.

The Scientific Group was guided by three previous WHO reports, which outline the criteria for diagnostic imaging. The first considers the indications for and the limitations of the most common diagnostic X-ray investigations, and provides recommendations for limiting their use when unlikely to provide any clinically significant information. The second considers the use of ultrasonography and CT in developing countries, as well as the specifications of equipment required, and outlines the major clinical indication for these imaging methods. The third considers the rational use of diagnostic imaging in paediatrics, and provides recommendations for improving the use of the various imaging techniques which are valid for children up to the age of 14- years. It includes criteria, not only for those who have to decide which imaging technique is best for their patients, but also for those who are performing examinations, in the hope of limiting the use of diagnostic imaging to cases where it will really benefit the individual.

The Scientific Group was well aware of the wide variety of imaging techniques available, and yet at the same time, the many parts of the world where the choices are very limited. The report attempts to reflect this practical situation and gives as many alternatives as possible. Throughout it has also been made clear when imaging is not really helpful, or when there are significant limitations on the benefits that will result.

The report is arranged on an anatomical basis, and within anatomical section the clinical indications for imaging are discussed. While anatomy and illness are much the same

everywhere, available equipment is not so uniform. The Group has therefore described the sequences to be followed, envisaging three different levels of imaging equipment, while being aware that there is to be overlap and discrepancy. It is hoped that within the useful life of this report most facilities will approach the levels of imaging described. Unfortunately, Level I represents the most likely situation for most of the world and the Scientific Group was unanimous in recommending that this represented the minimum which should be accepted for good patient care.

 # Level I

Standard radiography, as with the WHO Basic Radiological System;

General-purpose ultrasonography;
Where possible within the health care facility or available within a reasonable distance:

- conventional linear tomography
- fluoroscopy with image intensification.

 # Level II

All Level I techniques and:
Sophisticated radiography
Sophisticated ultrasonography, including Doppler
Mammography
Angiography
Digital subtraction angiography
Computerized tomography
Radionuclide scintigraphy, including SPECT
Thermography (of limited use).

 # Level III

All Level I and Level II techniques and:
Magnetic resonance imaging (MRI)
Positron emission tomography
Advanced radionuclide scanning: labelling by means of monoclonal antibodies (immunoscintigraphy).

It is assumed that any Level II department will have all the imaging facilities that are available at Level I, and similarly for Level III, which must be fully equipped to perform any type of imaging. This does not imply, however, that diagnostic tests performed at Level I, for example, necessarily have to be applied at Levels II to III; the choice of imaging procedures will strongly depend on the particular clinical situation.

More important than the equipment is the availability of skills. An error in diagnosis because of inadequate education and experience is as dangerous as being without the equipment, and the success of any interventional procedure (e,g. angiography) is very dependent on the skill and the experience of the responsible physician. Qualified radiologists are not available in many parts of the world, and it should never be suggested that an adequate standard can be reached in any type of imaging on the basis of self learning and reading. In particular, the effective use of an ultrasound scanner, although less expensive than other imaging equipment, is very dependent on the physician. The minimum required training for ultrasonography and CT, which should be completed in a large centre, has been described elsewhere. The recommendations made by this Scientific Group are based on the assumption that at least an equivalent level of expertise will be available to interpret the images obtained in every facility. If a choice of imaging can be made, the decision should always be in favour of that for which there is the most local experience. Thus, many of the Group's recommendations can only be, at best, broad guidelines to be adapted according to local conditions and disease patterns.

Yet, the principles remain the same, and the message is very clear. An orderly and logical approach to the diagnostic imaging of patients will result in more accurate diagnosis, less harmful radiation and less expense. All three are goals well worth achieving in any country.

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Chapter 16

RADIOIMMUNOASSAY IN DEVELOPING COUNTRIES [1]
(General principles)

R.D. Piyasena

Radioimmunoassay (RIA) is probably the most commonly performed nuclear medicine technique. It is an in vitro procedure, where no radioactivity is administered to the patient. But this alone is not the reason for its widespread use. It provides the basis for extremely sensitive and specific diagnostic tests, and its use in present day medicine has brought a virtual information explosion in terms of understanding the pathophysiology of many diseases.

The fact that the technology involved is within the technical and economic capabilities of the developing world is evident from the increasing demand for its introduction or expansion of existing services. RIA facilities need not be restricted to urban hospitals, as in the case of in vivo nuclear medicine techniques, but may be extended to smaller district hospitals and other laboratories in peripheral areas. It is also possible to send blood samples to a central laboratory so that a single centre can serve a wide geographical area. There are many laboratories in the industrialized world that receive a major proportion of samples for assay by mail. In recent years, substantial RIA services have been established in many of the developing countries in Asia and Latin America. The International Atomic Energy Agency (IAEA) and World Health Organisations (WHO) have made vital contributions to these activities and have played a catalytic role in assisting member states to achieve realistic goals. In the past five years, more than 250 individual RIA laboratories in developing member states have been beneficiaries of IAEA projects.

RIA is a microanalytical technique that employs radionuclides to detect the presence (e.g. bacterial and parasitic antigens and antibodies, hepatitis B markers) or measure the concentration (e.g. hormones, drugs, vitamins, enzymes) of substances of interest in biological fluids, most commonly blood serum or plasma. The discovery of immunoassay as such may be traced back to the historical publications about 30 years ago from Soloman Berson and Rosalyn Yalow (USA) and, independently, Roger Ekins (U.K.). Ms. Yalow from the former group, who subsequently received the Nobel Prize in 1977, developed an assay for insulin that employed antiinsulin antibodies. The first assay described by Ekins was for serum thyroxine. It employed a naturally occurring binding protein (TBG) rather than an antibody, but the basic principle is the same.

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[1]This chapter has two layers. Text in normal type is considered essential for every one working in nuclear medicine. The parts shown in small letters need not be read by those who are not interested in RIA technology but reading of these portions, perhaps later, may apprise the clinician of techniques that might have to be set up if bulk reagents or production of indigenous reagents are substituted for the imported ready-to-use kits. Portions in the Annexes are definitely for those who do the techniques themselves.

Requirements of a RIA System

These are essentially a binding agent - hereafter referred to as the REAGENT - having the capacity to combine with or bind to the substance whose detection or measurement is required, the ANALYTE, (ligand, substance of interest). Reagents most commonly used in practice are antibodies. Ekins (3) describes the process in a broader context as one of progressive "saturation" (occupancy of binding sites) of a saturable reagent by the analyte and RIA thus becomes one example, albeit the commonest one, of "Saturation Analysis" where an antibody serves as the reagent. This broad frame can cover assay systems such as protein binding assay, radioenzymatic assay, and radioreceptor assay (where naturally occurring proteins, enzymes, and receptors serve as reagents).

The quantitation of analyte in RIA depends on determining the proportion bound to the antibody and the proportion remaining free after it has been allowed to react with the antibody under optimum conditions. The measurement of the bound and the unbound fraction is enabled by a radioactive label (tracer) being attached either to the analyte, in case of a RIA, or the reagent, in case of immunoradiometric assay (IRMA). In addition, most assays in practice require a method to physically separate the free from the bound fractions so that the radioactivity in them may be individually measured by means of a suitable counting system.

From all of the above, therefore, it would become clear that the essential components of a RIA or IRMA system would be,

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(a) The analyte, as standard or in the "unknown" or test sample;

(b) The reagent (antibody or other);

(c) Radiolabel, (attached to either analyte or antibody);

(d) Separation system;

(e) Device for the measurement of radioactivity.

Basic Principles

The basic principles of RIA and IRMA have been extensively described in the literature. Immunological recognition in Immunoassays is "structurally specific" with analyte and reagent recognizing molecular structure, in contrast to "functionally specific assays" such as bioassays

where the end point is some biological response obtained in a whole animal or an animal preparation, (e.g. uptake of glucose in the rat hemidiaphragm under the influence of insulin). The activity or potency of a biological substance as assessed by bioassay and immunoassay methods may therefore not be equivalent (e.g. in the case of hGH or TSH).

Essential to an understanding of the difference between RIA and IRMA is the fact that, in the former case, the reagent (antibody) is present in small amounts relative to the analyte whereas in the latter it is present in excess. In a RIA, therefore, all available reagent binding sites are occupied by analyte. When the analyte is present in both labelled and unlabelled forms they would occupy identical reagent binding sites. In that case, the amount of label that is bound will depend on the amount of unlabelled or "cold" analyte also present in the sample. The more "cold" analyte there is, the less would be the probability of tracer being bound. In an IRMA, on the other hand, the (labelled) reagent is in excess and all analyte present can find binding sites available. The fractional occupancy of tracer, as determined after the separation step, is thus between free and bound labelled analyte in the case of a RIA and free and bound labelled reagent in that of an IRMA.

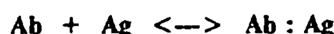
These basic principles are illustrated in **Figs. 16.1 and 16.2**. The figures show that, when the radioactivity in the bound fraction (e.g. the bound counts or the normalized counting ratio, percentage bound counts/total counts) or the degree of occupancy of binding sites are plotted against increasing amounts of unlabelled analyte, the curve obtained, in a RIA, would be of a descending type and, in an IRMA, of an ascending kind. **Figs. 16.3 and 16.4** provide typical examples where the concentration of analyte is plotted on a logarithmic scale.

These are "standard curves" or calibration curves which are model dose response curves relating responses (on the y axis) to calibrators (standards) of known concentration. For each unknown sample, the dose can be read or "backfitted" from the standard curve at the relevant response point, provided that the assay has been done under such conditions as would make the comparison valid. To ensure this, concentrations of all assay components, (reagent, tracer, buffer, matrix protein etc.) are kept constant except for the one factor of analyte concentration (dose) and all assay tubes, standards and unknowns, are processed in exactly the same way and under the same conditions (incubation time and volume, pH, temperature, centrifugation conditions, etc.).

Reaction between analyte and reagent is governed by the Law of Mass Action. Initially, the analyte (antigen, Ag) reacts with the reagent (antibody, Ab) to form what is referred to as the complex.

$$\mathbf{Ab + Ag \longrightarrow Ab : Ag}$$

As this reaction is reversible, the complex, as it builds up, will also begin to dissociate.



$$\mathbf{Ab + Ag \longleftrightarrow Ab : Ag}$$

If k_1 refers to the forward (association) rate constant, and k_2 to the backward (dissociation) rate constant, then, at equilibrium,

$$\frac{k_1}{k_2} = K = \frac{[Ab:Ag]}{[Ab][Ag]}$$

where K is the equilibrium constant and the square brackets [] denote molecular concentration. The value of K is a measure of the affinity of binding between analyte and reagent under the conditions employed.

Not only the concentration of analyte but also that of the reagent or binding agent can be determined in a RIA system. For example, the presence and concentration of hormone sensitive receptors in breast tissue may be determined.

Quantitation, in this case, is by means of a Scatchard Plot (8), and the equation is,

$$B/F = -KB + Kq$$

where B and F refer to the concentration of bound and free analyte respectively, K to the equilibrium constant, and q the molar concentration of the total number of binding sites, i.e. the binding capacity.

As the above equations are of a straight line, the slope of the curve would yield the value for K. When $B/F = 0$, then $Kq = KB$ and therefore $q = B$. The intercept of the curve on the x-axis would thus yield the value for q. This principle is illustrated in **Fig. 16.5.**

A homogenous one analyte, one reagent system reacting and reaching equilibrium under the Mass Action Law is assumed. Scatchard Plots will not be straight lines if there is reagent heterogeneity (curve concave upwards), analyte heterogeneity (curve concave downwards and assuming an extreme form if K for the tracer is much reduced compared to that of the unlabelled analyte) or misclassification error. In the last instance, if B is misclassified as F, the curve will only be displaced downwards so that estimation of K (from slope) will be unaffected whereas that of q will be reduced by the same factor as the misclassification error, provided this is the same in every tube. If on the other hand F is misclassified as B, the effect is more drastic with the curve concave upwards and K over-, and q underestimated. Other less well understood causes of non-linearity of Scatchard plots are disequilibrium, allosteric effects and reagent polymerisation or cross linking.

RIA Reagents

Some essential characteristics of RIA reagents need now to be dealt with particularly as they would apply to local production of them which is to be encouraged, where technically and economically feasible, in developing countries.

A standard is the material against which the substance being assayed (the analyte) in test samples is being compared and is most commonly the pure analyte itself in known quantity. Primary standard reference preparations are of known composition and high purity. Low molecular weight substances of simple chemical structure are obtainable in pure form and reference preparations are usually available, e.g. steroids, iodothyronines. The situation is much less certain, however, when it comes to several other analytes which are heterogenous, and may exist in several forms of varying biological potency. In these cases, international agencies such as WHO or major centres such as the National Institute of Health in the U.S.A., or the National Institute for Biological Standardisation and Control in the U.K.

produce, calibrate, and distribute primary standards which are meant to serve as reference materials to which secondary or working standards may be related. Common examples are hGH, TSH, PRL, LH, hCG, Insulin, Pro-insulin, PTH, etc.

Most immunoassays are now performed in "neat" or unextracted serum or plasma and it thus becomes necessary to have supplies of this material "analyte free" in which the standard may be made up, so as to avoid matrix effects.

Analyte free serum may be prepared by:

(a) Immunoadsorption with a specific antibody on a solid phase. This method has the disadvantage that any antibody that may break free and spill over from the column into the serum will cause interference.

(b) By physiological manipulation of normal subjects, e.g. cortisol free plasma following administration of dexamethasone.

(c) Physical adsorption. Charcoal is most commonly used but the method is technically demanding: charcoal is difficult to remove from serum, and may also remove other naturally occurring substances, besides the analyte in question, including potential cross reactants that may be present in the test samples. Nevertheless, physical adsorption remains a convenient method for obtaining analyte free serum, especially in developing countries, and recent developments have resulted in evolution of a method which, although still using charcoal as adsorbent, is much less drastic and also less wasteful of primary material. The protocol for this procedure, which has come into increasing usage recently, is given in detail in Annex I. Devised at the North East Thomas Regional Immunoassay Service (NETRIA) in the U.K., the method has been taught at almost all IAEA organized training courses over the past 4 to 5 years. A further protocol for preparation of the analyte free serum using an anion exchange resin is provided in Annex IA.

Working standards may be prepared in analyte free serum obtained as described above. They need to be calibrated over as wide a range of working conditions as possible, and where practical, by different operators in many laboratories. Working standards may not be as stable as primary ones (e.g. AFP, TSH, LH, FSH). After calibration, the working standards are diluted to appropriate concentration in analyte free serum and stored in lyophilized form or at subzero temperatures; (-70°C is better than -40°C and this in turn is better than -20°C). A typical protocol for preparation of thyroxine and triiodothyronine standards is given in Annex II.

Antibodies are immunoglobulins (mostly IgG) capable of combining with a defined antigenic determinant. An antigen is a substance that can produce an immune response (i.e. it is immunogenic). Haptens are small molecules not immunogenic in themselves but capable of being made so by combination with carriers such as bovine or human serum albumin (e.g. Cortisol, Thyroxine). They react with their corresponding antibodies.

Antibodies are conventionally produced by injection of immunogen into an experimental animal (rabbit, sheep, guinea pig). It is preferable that the animal has not had any previous immunisation. The immune response depends, amongst other factors, on the species of animal selected, the nature and amount of immunogen, and the route of immunisation.

Antibody production is considered as much an art as a science. Immunisation schedules are variable and depend on experience and personal preference. A typical and convenient schedule is given in Annex III.

Antibodies need to be characterized and evaluated prior to use in a bulk reagent based RIA system. The practical features that need to be looked into in this regard are:

(a) Titre

(b) Avidity

(c) Specificity.

Titre is the apparent dilution of antiserum to be used, determined by the construction of antiserum dilution curves. Generally, a single curve may be constructed relating percentage bound tracer to antiserum dilution (on a log scale) and that dilution yielding 50% displacement of tracer selected. A better approach, however, would be to construct two curves, one with tracer alone and the other containing some amount (say, the first or mid concentration of the proposed standard curve) and select that dilution of antibody at which the maximum displacement between the two curves is seen. The principle is illustrated in **Fig. 16.6.** The mass of tracer used should always be stated in tracer studies. Titre is a function of both the avidity of the antiserum and its binding capacity.

Avidity, determined by construction of a Scatchard Plot as described above, is a measure of the strength of binding between antibody and antigen. It is one of the two main determinants of the precision and sensitivity of an assay, the other being experimental error.

Specificity indicates the ability of an antibody to distinguish one particular analyte from several closely related substances including metabolites, subunits, and analogues. The specificity of an RIA is improved by:

(a) The use of pure immunogens or conjugates;

(b) Pure labelled compounds;

(c) Pure analyte for assay, pre-extracted and purified if needed.

(d) Immunisation with specific subunits, e.g. b-hCG;

(e) Saturation with cross reactants, e.g. TSH antibody with hCG;

(f) Use of monoclonal antibodies of high specificity.

Cross reactivity curves are a series of standard curves, one with the specific analyte and others with increasing amounts of possible cross reactants. The amounts of antibody and tracer are kept constant. Cross reactants usually have lower K values towards the reagent than the analyte itself and an observed cross reactivity may also be reduced when the specific analyte is present, which is usually the case. The bias caused by a cross reactant may be assessed

and sometimes corrected by construction of a "Cross Reactivity Profile" which is a plot relating the absolute or fractional bias caused by the cross reactant to analyte concentration at several levels and not only at the ED_{50} response point, at which this is most usually assessed. Typical cross reactivity curves are illustrated in **Fig. 16.7**.

As second antibody is one of the commonest separation methods used for RIA in developing countries, a brief discussion on its character and mode of production will now follow. Polyethylene glycol is usually added with the second antibody to facilitate separation. Second antibody is made by immunising a different animal species with pure IgG from the species in which the first antibody was raised. The antiserum precipitates the first antibody complex in insoluble form. Non-immune serum from the first antibody species is added to increase the bulk of the precipitate.

Second antibody is used at low titre, generally less than 1:50, so larger animals are used for production. The immunogen is purified IgG (caprylic acid fractionation, DEAE ion exchange, immunoelectrophoresis) from the first animal species and the immunisation schedule is essentially similar to that used for first antibody production although booster doses may be higher.

Second antibody (as in the case of first antibody) should be tested against a reference serum. Dilutions of 1:5 to 1:25 may be used with a 1:400 dilution of normal serum. The point of maximum precipitation and the width of the precipitation plateau are assessed. The latter is less secure with decreased dilution of normal serum. Choose the best combination of concentrations of second antibody (maximum precipitation) and carrier serum (widest plateau) and test under different conditions of pH, temperature, etc. The principle is illustrated in **Fig. 16.8**. Accelerated second antibody precipitation is achieved by inclusion of Polyethylene glycol (PEG), M.W. 6000, 2-4%.

In a RIA for low molecular weight analytes, first and second antibody may be added together. Better sensitivity may be obtained by delaying the addition of tracer until the primary reaction is complete in the case of higher molecular weight analytes.

Haptens need to be initially activated and then linked to carrier protein by peptide linkage. The commonest such carrier used, Bovine serum albumin (BSA) which contains 59 lysine units, is antigenic, and readily available in pure form. A typical protocol for producing an immunogen from a steroid hapten, using the mixed anhydride reaction is given in Annex IV.

Peptide haptens may be directly coupled on to carrier protein via amino or carboxyl groups. A typical protocol for the conjugation of thyroxine (T_4) to BSA using the carbodiimide reaction is given in Annex V. In either case, direct or following activation, the carrier protein should be attached to the hapten at a site remote from the characteristic functional group of the latter substance.

Antibodies, particularly when they are intended for coating on to solid phases or labelling for IRMAs, need to be purified. Crude IgG fractions may be obtained by precipitation using

either ammonium sulphate or caprylic acid. Such crude fractions, containing IgA and ceruloplasmin besides IgG, may be used for labelling but not for coating.

Further purification can then be achieved using DEAE, Protein A or affinity chromatography. Of these, the last is probably the best but is not applicable in all cases as supplies of pure antigen for preparation of the column are required and this can be very expensive. DEAE cellulose ion exchange chromatography will yield very pure IgG but has the disadvantage that large sample volumes (at least 5 ml) are required. Where sample sizes are small, Protein A purification is more suitable and is therefore commonly applied to the purification of monoclonal antibodies. Recommended practical protocols for the purification of antibodies using ammonium sulphate, caprylic acid, and DEAE cellulose are given in Annex VI.

Antibodies coated on to solid phases are increasingly used in developing countries where solid phase separation methods, particularly those avoiding centrifugation, are sometimes the most convenient technique. The methods for immobilization of antibodies are described below, under separation systems.

The final RIA reagent that needs to be considered is the tracer. In RIA, the label is attached to pure analyte which is then made to participate in the reaction in the same way as the unknown. Labelled and cold analyte need to be identical only if recovery studies are needed, not necessarily for estimation of distribution between free and bound moieties.

^{125}I is being increasingly used for preparation of label even for compounds with no tyrosine or similar radicles (steroids, drugs, prostaglandins). It has several advantages over other radionuclides that may be incorporated into antigen or antibody molecules. It is also readily available with virtually 100% radiochemical purity, and is readily introduced into many proteins and other organic molecules that contain a suitable aromatic ring by a mild oxidation reaction. Direct substitution or replacement of naturally occurring ^{127}I is also possible as in the common examples of T_3 and T_4. It is a gamma emitter and is therefore detectable without the need for special sample preparation. Incorporation of radioactive iodine generally does not make significant alteration to the shape of proteins on the recognition of which immunoreactivity depends. However, it should not be lost sight of that iodine is rarely found naturally in proteins and that incorporation of an iodine atom (which is as large as a benzene ring) can change physiological and immunological properties, particularly in regard to stability.

Iodination methods are of two general types, direct and indirect. In either case, an oxidising agent is used to convert Sodium ^{125}Iodide to reactive $^{125}I^{+}$iodinium ion. At mildly alkaline pH (7.5) this reacts with tyrosine and tyrosyl residues on phenolic benzene rings. It is essential that oxidising conditions should be as mild as possible to avoid oxidation damage and cleavage of the polypeptide chain.

Direct iodination of soluble proteins is inexpensive, simple, and can yield tracers of high specific activity. The commonest oxidising agents employed are chloramine T, lactoperoxidose, and iodogen.

Chloramine T is the sodium salt of n-monochlorotoluene sulphonamide which yields hypochlorous acid in aqueous solution. Sodium metabisulphite is used to terminate the reaction. A typical protocol for iodination by this method is given in Annex VII. Lactoperoxidose [10] is an enzyme that catalyses the oxidation of Na ^{125}I in the presence of the oxidising agents hydrogen peroxide or glucose oxidase. Iodination by this method is considered to be milder and the tracers are more stable than those prepared using chloramine T. It has a distinct practical advantage in that lactoperoxidose can be attached to a solid phase (cellulose) and the reaction can be terminated by centrifugation. The reaction may also be terminated by 0.1% sodium azide, 100 μl, but in this case it is important to ensure that no sodium azide, which is a strong enzyme inhibitor, is added until the iodination reaction is complete. It must be noted, therefore, that this substance should not be present in buffers used for the iodination reaction. A protocol for iodination using lactoperoxidase on a solid phase is given in the Annex VIII.

A third method of direct iodination is by Iodogen, which is 1,3,4,6, Tetrachloro-3a, 6a-Diphenylglycouril. It is also a mild oxidising agent useful for the iodination of labile proteins. No reducing agent is required. The oxidation and iodination reactions take place slowly and maximum iodine incorporation with minimum chemical damage is thus achieved. The reagent is slightly more expensive than chloramine T, the technique is simpler, and is therefore gaining in popularity. However, it has the disadvantage that it is not suitable for iodination of peptides less than 20 000 Daltons, a notable example being Prolactin. Note also, that iodogen coated on iodination tubes is not stable. A typical protocol for iodination by the Iodogen method is given in Annex IX.

The three methods of direct iodination mentioned above are only applicable where the radioiodine can be incorporated into a part of the molecule far removed from the characteristic binding site so that immunoreactivity is unimpaired, where direct substitution for a naturally occurring ^{127}I atom is possible, and when the substance to be iodinated contains tyrosine, histidine, tryptophan, or sulphydryl groups. Where these conditions are not present indirect or conjugation iodination provides a suitable alternative for tracer preparation.

Here, a phenyl group on a small molecule such as tyrosine is iodinated and this is then coupled onto the antigen, after activation, via a free amino group. The antigen itself is therefore spared from exposure to an oxidising agent. One of the commonest reagents used for conjugation labelling is the Bolton and Hunter reagent, named after the workers who devised the method, which is commercially available in a pre-iodinated and purified form. Another example would be diazo or isothiocyanate derivates of iodinated sulphanilic acid or aniline. A typical protocol is provided in Annex X.

When labelled hapten conjugates are prepared, the conjugation reactions should not be the same as those used in the preparation of hapten-carrier protein conjugates for antibody production. The antibody may recognize the bridge as well as the hapten and, if the bridge structure on the tracer is identical, the tracer may have a different affinity for the antibody than the analyte. This problem of "bridge binding" can be avoided by attaching the conjugates to different sites on the hapten for preparation of immunogen conjugates and tracer conjugates respectively.

A good method for the iodination of antibodies is to use N-bromosuccinamide which is a mild oxidising agent. The method is essentially similar to that used with Chloramine T except that the reaction is terminated by dilution of the reaction mixture with buffer or addition of excess tyrosine or potassium iodide. A protocol is provided in Annex XI

Ideally, a radiolabelled tracer for use in a RIA should be a homogenous preparation of labelled antigen exhibiting the same affinity for antibody as the unlabelled analyte. The quality of tracer affects specificity and sensitivity of the assay, which will be reduced if the K value of the tracer is less than that of the analyte. The iodination reaction is reversible and "free" radioiodine will be released from the iodinated protein. In addition to free radioiodine, other impurities present in the original material may also be labelled and these labelled impurities can interfere.

Of several alternative methods available for obtaining the required labelled material in pure form (e.g. ion exchange, adsorption, electrophoresis), the commonest is gel filtration. Practical details of a typical method, using Sephadex G25, are included in the protocols describing the preparation of ^{125}I tracers for T_3 and T_4. This method of separation is generally adequate when the material being iodinated is pure but where this is heterogenous or may have deteriorated on storage, purification is better achieved by thin layer chromatography (TLC) or high performance liquid chromatography (HPLC). The latter method is becoming more widely available in developing countries due to decreasing equipment cost and a typical protocol is given in Annex XII. Note that heterogeneity of an antigen may only be revealed after labelling by the presence of peaks of radioactivity distinct from those due to the labelled moiety and free iodine.

In theory, two ^{125}Iodine atoms can be incorporated into each tyrosyl group and, where several such groups are available, the potential specific activity of tracer can be very high. Use of such high specific activity tracers may serve to improve sensitivity but there is the danger that excess ^{125}I labelling may alter immunoreactivity. Also, high specific activity tracers tend to be less stable. Therefore, as a general rule, the inclusion of just one atom of iodine per molecule of protein is preferred. Preparations of $Na^{125}I$ for protein iodination from commercial sources usually have a specific activity of about 1mCi/0.5 nmol of iodine. Thus, reaction of one mCi of ^{125}I with 0.5 nmol of the substance to be iodinated will yield tracer with the desired incorporation of one iodine atom per molecule.

The calculation of specific activity of tracers is fairly simple where there is one primary iodinated product. As an example, if 10 μg of protein is reacted with 1 mCi of ^{125}I and if 80% of the iodine is uniformly incorporated into the protein, the specific activity of the tracer will be 80 mCi/ug. However, when two tracers are obtained from a single starting material, as in the example in Annex VII where both ^{125}I T_4 and ^{125}I T_3 are obtained by iodination of T_3, the calculation of specific activity is more difficult as it depends on the quantity of radioiodine incorporated into each tracer which has to be determined independently.

When the mass of tracer is known, an experiment should be performed to check that its immunoreactivity is the same as that of the "cold" native antigen. This is done by construction of two standard curves, one containing the native antigen (the same as was used for labelling) and the other the same amounts of tracer only with no native antigen. If the two curves, plotted as % bound vs mass of antigen are superimposable, then immunoreactivity is unimpaired [15]. If the curves are parallel, though not superimposable, excess free ^{125}I is present. Non-parallelism, however, indicates alteration of immunoreactivity and an alternative iodination procedure should be considered.

^{125}Iodinated peptide tracers may be stored lyophilised, if necessary, with addition of carrier protein. Stock solutions of ^{125}I T_3 and T_4 tracers may be stored in 75% ethanol, at low temperature. When stored at low concentrations, apparent losses may be due to adsorption on the walls of the container.

Special safety precautions become necessary as iodinations are carried out using 1 mCi (37 MBq) or more of radioactive iodine. For the handling of this amount of radioactivity, a special "hot" laboratory, well ventilated, and equipped with a fume cupboard is essential.

^{125}I in the free state is volatile and is easily ingested. Protective clothing (laboratory coat and gloves) must be worn. The iodination should be performed in a fume cupboard and the equipment needed for purification of the tracer should also be located in the hot laboratory. Chromatography columns, fraction collectors, etc. used for tracer purification are very likely to be contaminated with residual radioactivity and will not be suitable for other uses. A radiation monitor for checking of all work surfaces and floors should be available and all such surfaces should be continuous and of material that is easily wiped clean.

Phase Separation

The major problem in a RIA does not concern the availability of reagents or the hazards of using radioactivity but the requirement that a physical separation of reactants to free and bound moieties is necessary before the final measurement of radioactivity can be made. This is in fact one of the main reasons why RIAs are difficult to automate while certain non-isotopic methods, which do not need a separation step, can be automated. Although often regarded as the weakest link in a RIA, the basic requirements of a separation step are, at least in theory, fairly straight forward. It needs to be efficient, practical, and not too expensive. In practice, however, this ideal is difficult to realise. Systems commonly used are generally divided into four types.

(a) Physical (solid adsorbents, protein A, sucrose)

(b) Chemical precipitation (PEG, Et.OH, ammonium sulphate)

(c) Immunological precipitation (2nd antibody)

(d) Solid phase systems, with antibodies on solid support or matrix.

Physical separation: Common reagents for physical separation are:

(a) Charcoal (Norit A), usually coated with dextran or albumin. Acts as a molecular sieve that removes free analyte and excludes the bound.

(b) Protein A, prepared from killed Staph. aureus, binds to IgG and thus removes the bound moiety.

(c) Sucrose. Antibody on a dense solid phase such as Sephacryl is made to gravitate down a 10% solution of sucrose.

Chemical precipitation: This is most commonly done by PEG 6000. Used by itself, the method is sensitive to protein concentration in the incubation mixture, and is prone to be erratic. Many of these problems are overcome by use of PEG in association with second antibody.

Other chemical precipitants of the bound fraction such as Ethanol and Ammonium sulphate are now very rarely used.

Immunological separation: As the molecular weight of the first antigen-antibody complex is small, a larger micelle is needed for precipitation. Precipitation depends on concentration which is why the first complex does not precipitate. The first antibody (with bound analyte) acts as antigen for a second reaction with an antibody from a different species. The method is less time consuming when used with PEG, is not affected by protein concentrations and is suitable for use with antibody of relatively low K value. Incubation time is reduced by inclusion of PEG, and addition of carrier serum increases the bulk of the precipitate. Excess of 2nd antibody may redissolve the first antibody/antigen complex (Prozone effect), and therefore the minimum concentration of second antibody corresponding to the precipitation plateau is chosen. Interference from complement is avoided by addition of a chelating agent such as EDTA, if required, to the 2nd antibody buffer. Lastly, the price of second antibody which was relatively high, has been decreasing in recent years.

Solid phase separation: Antibody is adsorbed or chemically bound via glutaraldehyde to a solid matrix. This may be a solid support such as glass or polystyrene in which case centrifugation is not required. Alternatively, insoluble particles such as cellulose, sepharose, or magnetisable cellulose may be used and the separation completed by centrifugation or use of a magnetic separator. Second antibodies are commonly linked to solid phases.

Practical protocols for the immobilisation of antibodies on to tubes or wells, cellulose, and magnetisable cellulose are given in Annexes XIII, XIV and XV.

QUALITY CONTROL IN RIA

Despite the advantages of sensitivity (limit of detection) and specificity (the ability to recognize a particular analyte in the presence of other substances) RIA, as a microanalytical technique, has an inbuilt fragility that derives from the nature of the reagents used, which are biological materials and radiolabelled compounds of limited stability. In addition, the separation step is rather difficult to reproduce or standardize, For these reasons, quality control(QC) becomes very essential in RIA in order that results can be interpreted with confidence.

QC procedures are not ends in themselves but means towards improving the standard of the RIA service being delivered, by identification and quantification of errors that affect assay validity.

The number of Errors that could occur in RIA are, in theory, probably infinite, and some of them, e.g. the so called "blunders", cannot be quantified. For purposes of statistical analysis, however, Errors in RIA are of two broad types. Systematic errors (bias) displace results in one direction only, either up or down. Random errors, on the other hand, cause imprecision, which can displace results in either direction.

Precision is the reproducibility of a measurement as calculated from the "scatter" of replicate estimations. It may be expressed in absolute (Mean and S.D.) or in relative (CV %) units. Bias is the systematic displacement of measurements from the "true" value and can also be expressed in relative terms, (% Bias). No measurement is exact, repeated measurements will yield different results due to random errors.

Random Error or Imprecision:

(a) Counting: from counting statistics,

(b) Non Counting: These are sometimes referred to as manipulation errors and are primarily derived from the pipetting and separation steps.

The counting error (S) is readily calculated from a knowledge of the total number of counts accumulated (n) (NOT the counting rate) using the formula

$$S = \frac{100}{\sqrt{n}} \%$$

Errors add in quadrature (which means that the squares of the individual components add up) and therefore

$$\text{Total error}^2 = S^2 + R^2$$

where S = counting error and R = non-counting error.

Similarly, the accuracy of a result, which is taken to mean the degree to which it corresponds to the "true" value is a function of both bias and precision, and thus

$$\text{Accuracy}^2 = \text{Precision}^2 + \text{Bias}^2$$

Replicate estimations (at least two) of samples are necessary in order to assess within batch reproducibility (precision) and also to recognize outlier samples. An outlier is defined as a sample with measurement error too large to be accounted for by normal scatter among replicates.

Within Batch Errors:

These are either due to imprecision or to bias. Imprecision results in poor agreement between pairs of replicates and is derived primarily from the pipetting and separation steps. Bias, on the other hand, results in poor agreement between sets of replicates.

There are several ways by which within batch imprecision may be estimated. An optimum condition variance may be determined by measurement of aliquots from the same sample many times (say 10 or 20) in a single assay. This may be done at more than one level of analyte concentration and a Mean and S.D. calculated in each case. The value may not be an accurate reflection of reproducibility under routine conditions where a sample is not likely to be measured in as many replicates.

An average batch coefficient of variation (ABCV) may be calculated from the slope of the Response Error Relationship (RER) plot. An RER is a plot of the error in the response vs. the mean response and may be constructed by plotting the standard deviation of replicate responses (counts) vs. the mean to yield a "snowstorm" of points as illustrated in Fig. 16.9. A smooth curve drawn through the points in this figure provides an estimate of the RER, as illustrated in Fig. 16.10. In this case, the CV is seen to be about 3% where the counts are lowest, declining slightly as the counts increase. The general RER equation, which will be used in the discussion on data processing that appears below is,

$$R = A + BP,$$

where R = CV of replicate estimations of response
P = the mean response
A = the intercept on the y axis
B = the slope of the curve.

Different values of A and B may be found for different analytes and procedures. However, once a method has been well standardized, the values of A and B tend to be stable over a long period of time. It is this feature of the RER that makes it very useful.

Manual construction of RER plots from data obtained in one or several assays is a useful practical exercise but is not at present necessary in most laboratories because computer programs that automatically calculate the RER parameters are readily available. These will be further discussed below.

Where special QC samples have been included in an assay the CV derived from these may be compared with the ABCV. The two values should be nearly the same.

The weakness in the above approaches is due to the phenomenon of heteroscedasticity, which means that precision depends on and varies with analyte concentration, being poorer at the lower and upper limits of the standard curve. The construction of an Imprecision Profile (IP) [24] corrects for this and enables the precision associated with any analyte concentration to be more satisfactorily determined, as also to decide on the range of analyte concentrations within which results would be acceptable (the working range) at any desired level of precision, e.g. a CV of 10%.

A typical IP is illustrated in **Fig. 16.11** from which it would be seen that it is a plot of the error (as CV) associated with the estimation of any particular analyte concentration or dose. Like the RER, the IP in a tabular or a graphical form is automatically generated by modern computer data processing programs.

Within Batch Bias:

This is also called within batch Drift. It refers to a systematic (usually unidirectional) change in responses from identical samples repeated at different locations within a single assay batch. Special QC specimens are used to verify drift. However, it is also believed that a more powerful check for within assay drift is provided by the inclusion of two complete standard curves, at the beginning and the end of the batch.

Drift may be present if the variance between sets of replicates is more than twice the variance between individual pairs. It may be quantified as the % change from one group of (QC) specimens to another, within a batch.

The selection of individual specimens from a batch for rejection and reassay can only be done by identification of poor agreement between replicates. Empirical criteria on which this is sometimes done (e.g. CV more than 10%) form an inflexible approach that does not take into consideration heteroscedasticity or that criteria may need to be stricter in the "grey areas" or decision making ranges between normal and abnormal values. The application of statistical criteria would be more satisfactory. Sometimes, a sample is rejected if the CV of its replicates is more than the ABCV by a fixed amount (say 2 or 3 times) but the approach would fail if there is a significant number of samples with poor replicates in a particular batch. A better method would be to consider the error observed in a particular measurement in relation to that expected, from previous experience, under similar conditions. The ratio of observed to expected error is calculated and it is then determined whether any departure from unity is significant or not. This method is used in the IAEA designed RIA/PC data processing programs and will be dealt with in further detail below.

Between Batch Variation:

The concern is to investigate reproducibility of results on equivalent specimens assayed in different batches and to identify batches with bias significantly different to that expected from previous experience.

Within batch imprecision forms one component of the imprecision seen between batches. However, the main source of between batch variation is a change in reagents (standards, tracer, antiserum), or assay conditions (time, temperature, incubation volume). The separation step is often difficult to standardize. Other factors would be a change of technician and of course "blunders" or "catastrophic errors" that may occur.

Batch to batch variation is usually worse in RIA than in other biochemical techniques and its measurement is a vital function of quality control. QC specimens obtained from specially prepared QC serum pools are generally used to assess between batch variation. They may be used to estimate assay precision as well as drift. It is also sometimes recommended that, in addition, randomly selected test samples be assayed in successive batches. They may be more representative of the patient material itself and probably stored for a shorter time than

the QC pools. QC material should resemble test samples in an assay as closely as possible, (e.g. same matrix) and should contain analyte concentrations that cover the working range. They should be prepared in sufficient quantity to suffice for a long period (say, at least 20 assays) and free from any microbiological hazards.

There are several possible sources of QC material:

(a) Residual patient samples. After pooling, these should if possible be filtered or at least centrifuged to remove debris and a suitable preservative such as 0.1% sodium azide added.

(b) Serum from a single or a few individuals who may be specially selected (e.g. post menopausal women) or specially treated (e.g. with T3 or TRH) if required to provide a particular kind of pool.

(c) "Spiked" serum, where known amounts of analyte are added to untreated base material usually of low or normal analyte concentration.

(d) Serum rendered analyte free ("stripped") and then "spiked" to the desired dose levels. Treatment with charcoal itself is too drastic a method of rendering serum analyte free, and may even remove cross reactants that would be present in the other test samples. The milder technique as described in Annex I is preferred. It has also been found that the use of ion exchangers have produced spurious results.

(e) Commercial material. QC sera are cheaper to make than to buy, except in exceptional circumstances, but there are many commercial sources from which they may be obtained.

Stored in suitable aliquots at -20°C or lower, QC sera is stable for at least 2 years. For routine use, three pools of QC material are used. They should contain low, medium, and high analyte concentrations to cover the working range. Each pool should be contained in 3 separate groups in a typical 100 tube assay batch with each pool prepared for assay individually and not together. A number of preliminary assays (at least 5-10) would be needed to establish the target Mean and S.D. for each batch, (outliers excluded). Individual results from subsequent batches may then be analyzed, either manually or by means of a computer program, and the results displayed on typical Shewart (Levy-Jennings) QC charts. A typical example is seen in **Fig. 16.12**.

QC data may be so analyzed that drift, (the % change from one QC group to another) may be evaluated with respect to one QC pool in one batch, all QC pools in one batch, one

QC pool in many batches, or all QC pools in many batches. Modern computer programs allow for analysis of IQC data from as many as 100 batches or more. In addition, other parameters generated by analysis of RIA data may be maintained on control charts. These would include the % Bound/Total at zero dose, the % NSB, the 20, 50 and 80 % displacement (ED) points, the slope of the standard curve, ABCV, the RER parameters and sometimes the mean patient value. These often prove useful in assay troubleshooting as changes in some parameters may indicate tracer or antiserum deterioration or problems in the pipetting or separation steps. For example, a lowered B_o and decreased slope with no change in the ED_{50} may mean that the tracer is damaged. If only the B_o falls but both slope and ED_{50} remain unchanged, this may mean that the tracer is old, with much free ^{125}I.

As in the case where an individual specimen from an assay batch was selected for rejection and reassay on the basis of imprecision or bias, the same criteria may be applied to the selection of entire batches for rejection. On the basis of imprecision, an ABCV which is more than three times the mean of the previous ten batches may qualify a batch for rejection. On the basis of bias, it is often suggested that if all three QC pool results are outside one S.D. of the target mean, or 2/3 are outside two S.Ds, or even if a single pool result is outside 3 S.Ds, the batch should be considered for rejection. There are objections to this kind of stringency and sometimes (as in the RIA/PC program), non-statistical parameters of 10, 15 and 20% of normalized target Mean values are substituted for the respective S.D. points. Whatever criterion is adopted, it is essential that the limits of tolerance or acceptability of the results need to be consistent and well-defined.

In summary, the essence of IQC is the evidence from replicates (which provides data on precision) and the evidence from the IQC samples (which provide data on within assay drift and between assay variability). Whatever may be the contingencies, there is no excuse for avoiding replicates or for excluding QC sera from an assay batch.

External Quality Assessment will not be dealt here at length. A laboratory may consider participation in an EQA scheme when it has firmly established IQC practices. EQA is no substitute for IQC but provides an independent assessment of laboratory performance with samples of unknown analyte concentration. Its main purpose is the recognition of individual laboratory bias against a peer group. It provides little information on within laboratory precision. EQAS may be able to detect method bias if a sufficient number of laboratories in the scheme use the same method or kit and is useful in multicentre research studies where results need to be comparable. However, the real role of EQA may be its educational function, the promotion and monitoring of IQC itself, and in providing a means of contact and communication between different groups of workers.

The Golden Rule in RIA is to find a good method and persist with it. QC is not merely for surveillance but plays an important part in assay design, in choosing reagents and protocols that are optimum but, even in an optimized system, reagents and conditions may change. QC must therefore be a continuous activity always as a priority in a RIA laboratory.

Assay design and validation:

It is desirable that developing countries establish "in house" RIAs using locally produced reagents as far as possible. Following production and characterization of such reagents, as described above, they need to be put together so as to produce an assay that would serve the purpose for which it is intended. Good RIA is not possible without good reagents but "even if good parts are haphazardly assembled, the result will be a ramshackle one" [18].

An assay should be designed to measure the concentration of analyte accurately, conveniently and at an acceptable cost.

The main factors that come into consideration are:

(a) The nature of the analyte

(b) The form in which it is present

(c) Its concentration

(d) The nature of the matrix in which the analyte is present

(e) The presence of cross reacting substances.

The factors that are aimed for in designing an assay are that the RIA should,

(a) be sensitive;

(b) cover the required working range;

(c) yield good precision over the required range of analyte concentrations;

(d) be practical and not too tedious or cumbersome;

(e) not be too expensive.

In sum, reagent (antibody, tracer, separating agent) concentrations and assay conditions (pH, temperature, incubation volume and time) should be so matched as to provide a practical and economical assay with required sensitivity and acceptable precision over the desired working range.

Assay optimization is a process in the course of which reagents and procedures are explored in order to arrive at a satisfactory design. Compromises will be unavoidable, as changes causing improvement in one area may be deleterious in another. For example, reducing tracer concentration would improve sensitivity but counting times may have to be

increased to reduce the counting error. Good assays are not designed on an "ad hoc" basis and some understanding of RIA theory is required.

Ekins has proposed a theory of RIA which is widely appreciated. Optimization of conditions is done in terms of parameters K (equilibrium constant), S (specific activity of tracer in cpm per unit mass), V (incubation volume), T (counting time), and E (experimental error). According to this theory, in an RIA where the bound fraction is counted, the optimal antibody concentration is given by 3/K, the optimal tracer concentration by 4/K, and the theoretical sensitivity, assuming no experimental error by $4 \times \sqrt{2} / \sqrt{STVK}$.

All theoretical models make certain assumptions, the most important being the following:

(a) Identical behaviour of labelled and unlabelled antigen towards antibody;

(b) A univalent reaction to equilibrium;

(c) Complete separation of free from bound fractions;

(d) A constant experimental error, or experimental error minimum when F/B = 1;

(e) the equations depend on which fraction is counted, free, bound, or both.

For a theoretical approach proposed by Ekins, values for K, V, T or parameters derived from a Response Error Relationship (RER) or Imprecision Profile (IP) plot need to be known, but they cannot be known until sufficient data has been generated. On account of this dilemma, many assayists prefer to adopt a pragmatic approach. However, there is no substitute for two things. First, an understanding of theoretical principles, and second, experimental work.

Experts recommend that, for many analytes, it would be a good idea to optimise an assay for sensitivity first and then make amendments as dictated by practical considerations. Sensitivity in RIA means the limit of detection, i.e. the smallest amount that can be distinguished from zero concentration. In theory, this is given for RIA by E/K and for an IRMA by E x NSB / k where E is the experimental error, k the equilibrium constant and NSB is the non-specific binding. NSB can be as low as 0.01 % which is a reason why, theoretically speaking, IRMAs have the advantage of sensitivity over RIAs. In practice, sensitivity is about equal to mass of tracer used, so this quantity can be used for construction of antiserum dilution curves. An idea of the concentration of antibody required may thus be obtained. Alternatively, a series of curves may be constructed with decreasing amounts of tracer. It may be found that decreasing the amount of tracer beyond a certain point may have little effect, thus providing a clue to optimum antibody and tracer concentrations.

Attention may now be directed to incubation time. To begin with, a time of 24-48 hours at 4°C may be used to ensure equilibrium, and other time and temperature conditions then tried out to access the effect on Bo, ED_{50}, slope etc. of standard curves over the working range.

A separation system is then chosen and optimized for maximum precipitation and widest plateau, as illustrated in **Fig. 16.6**.

Delayed addition of tracer may improve sensitivity in assays for proteins of high molecular weight, but not usually for hapten assays.

Finally, using all information available as a working basis, the assay could be optimized using criteria such as the IP. Decisions should be made according to requirements, good precision at low dose or in the areas of most diagnostic interest, widest working range, etc.

The first principle of assay validation, irrespective of whether locally produced reagents or commercial kits are used, is that this should be done in one's own laboratory under the usual conditions of work. Validation procedures are fairly standard and consist of the following experiments:

(a) Cross reactivity studies

(b) Parallelism tests

(c) Estimation of bias at zero dose

(d) Recovery studies with unlabelled analyte

(e) Reproducibility studies within and between batches

(f) Comparison with accepted reference method if possible.

Cross reactivity studies have been dealt with previously and illustrated in **Fig. 16.7.**

Parallelism testing (sometimes called "dilution down a standard curve") is done to establish the identity of standard with unknown analyte in the RIA system. Note that, if parallelism is found, this does not mean that standard and unknown are certainly identical, only that the probability of this being so is high. On the other hand, lack of parallelism almost certainly indicates that standard and unknown are not the same, or a matrix effect, i.e. the diluent does not behave as analyte free serum.

Testing for bias at zero dose is useful because, as previously mentioned, cross reactants may exert a greater effect at zero or low concentrations of the specific analyte. Samples known to contain zero analyte concentrations are more suitable for this purpose than serum artificially rendered hormone free.

Untreated or naturally analyte free serum is also preferable to treated analyte free serum for experiments that estimate the recovery of unlabelled added analyte. Recovery experiments are carried out at low, medium, and high analyte concentrations and the accepted recovery range is 90-110%.

The question of assessment of within and between batch variability has been discussed in the section dealing with Quality Control.

In the case of IRMAs, the theoretical models proposed by various groups are rather more complicated than in the case of RIAs but the effect of critical factors such as NSB, experimental error, and background on assay sensitivity may be predicted. Without dwelling on any of the mathematical approaches it may be stated, from a practical point of view, that sensitivity in an IRMA depends on the "blank" or binding at zero standard, the equilibrium constant of the antibodies used and the specific activity of the tracer. The latter, however, has the least effect in situations where the greatest contribution to the total error is from experimental error.

Data Processing in RIA:

In parallel with methodological developments, advances in data processing (DP) techniques have also made very significant contributions towards realization of the full potential of RIA. One factor above all else that has enabled DP to play this role has been the virtual "explosion" that has occurred in computer technology that has placed programmable calculators and computers of impressive capabilities available at a fraction of the cost of less powerful machines of the past.

One of the obvious advantage of modern computer assisted DP lies in its contribution to time and personnel management in RIA laboratories. Added to this would be the advantages of speed, accuracy, and reliability over manual methods which are much more prone to operator error. Important though all these factors may be, they do not constitute the most significant benefit that a modern computer based DP system provides, which is the possibility of a detailed statistical analysis of assay results, thus enabling the establishment of confidence limits on dose estimations, the identification of "outlier" specimens, and a general assessment of assay quality and performance. It provides the radioimmunoassayist with the ability to store and to retrieve data for computation of composite results on assay performance.

Some initial resistance to the idea of computer based DP was encountered on the grounds that detailed statistical analysis was a prerogative of, and could only be understood by, trained statisticians only, and also that such analysis was not needed by workers with backgrounds in medicine, biochemistry etc. who were using RIA for clinical service or research. The opinion of most experts, however, was that the converse was nearer to the truth. The clinical biochemist "is less well equipped for statistical computations and needs the protection of a sophisticated program".

Objectives of Data Processing:

Analysis of RIA data has three objectives. First, it enables the computation of the concentration of the analyte concerned in test samples. Second, it informs on the degree of validity of the results by providing an estimate of error; and third, the capability to retrieve stored information permits the comparison of current with previous results, thus assessing the consistency of the assay procedure over a period of time. Modern DP techniques are geared

to deal with all of these issues, in such a way that all that is required of the analyst is sufficient understanding of the basic statistical and mathematical concepts to appreciate and use the information that the computer provides. There is no dearth of DP packages from both commercial and non-commercial sources, so that "it makes no more sense to write one's own program than to build one's own computer".

A misconception common to beginners in the field needs to be removed at this stage. No computer program can make up for defects inherent in a badly designed assay or an imperfect technique; no DP package can provide good answers from bad results.

Basic Steps in Data Processing of RIA:

Put in its simplest form, all that is required in order to determine the concentration of the analyte in an unknown specimen is to compare the corresponding response against those obtained from specimens of known concentration (standards), under conditions that would make such a comparison legitimate. What one has at the end of a RIA procedure is not a measurement of analyte concentration but of radioactivity (counts). These results need to be calibrated so that a relationship is established between what is available (the measurement of radioactivity) and what is needed (the measurement of analyte concentration). The first step in this process would be to contruct a standard curve. The responses may be "scaled" (e.g. as B/T, F/T, B/B_o etc.) or "transformed" (e.g. logit, reciprocals, etc.) and plotted as dependent variables (on the y-axis) against independent variables (standard concentrations) on the x-axis. Once the dose response relationship has been plotted in this way, the several data points are then "fitted" to produce the final dose response curve derived from the data from all standards. Data on measured responses from the unknown specimens is then processed and the concentrations of analyte calculated by interpolation on the standard curve. The final result is corrected for any dilution or recovery factors, blanks, isotope spill over, etc., if required.

Construction of Standard Curves:

In RIA as generally practised the measured response is counts in the bound fraction. A dose response curve relating bound counts (B) (or other direct responses such as 1/B, 1/F, B/F etc.) to analyte concentration is not linear, but hyperbolic or sigmoidal depending on whether dose or log dose is plotted on the x-axis. In IRMAs, the curves would be reversed. Design of a computer model to fit the various data points would require an equation, derived from the standard curve data, that would relate responses to doses. It is of vital importance that due consideration be paid to the errors (variance) in the response measurements and the fact that this error is not constant but varies with the concentration of analyte; - the phenomenon known as "heteroscedasticity". Allowance is made for this in curve fitting procedures, (e.g. by the introduction of "weighting" factors).

Calibration curves must be monotonic, uniformly ascending or descending with a positive or a negative slope. Each response must correspond to one and only one dose level. Numerous methods have been developed for the fitting of curves to experimental data. No single one of them is yet accepted as unequivocally superior to all others and applicable in all circumstances. The choice of a particular system may be based on several considerations but it must never be lost sight of that the DP technique should suit the RIA, and not the other way around. The number of standards, for example, and their spacing, should be decided upon clinical grounds and not for satisfying the requirements of a curve fitting program.

Methods of construction of standard curves are basically of 2 types:

(a) Interpolation methods

(b) Regression methods.

In interpolation methods the responses corresponding to the standard points are treated with a confidence that does not extend to the areas in between. Many methods have been used to connect these standard points, but all of them are considered merely to imitate what could be done manually with a ruler or a flexicure. In regression methods, on the other hand, the analysis is based on a statistical model so that a derived function is compared with a measured value and adjusted so that the quotient of distance of the fitted to the experimental point divided by the S.D. of the experimental point becomes a minimum. This is done for each standard point and the final curve drawn when the sum of the squared ratios for all the points is the smallest (least squares fit).

Interpolation DP methods such as linear, polygonal, or spline, are now of only historical interest. They suffer from the general disadvantage of dependence on the number and accuracy of the standards used. In addition, no good mathematical method is available for the rejection of outliers or bad points. These factors make all such methods unsatisfactory for RIA data processing purposes.

Regression methods:

These are of two general types as:

(a) Based on theoretical models and

(b) based on empirical models.

Theoretical models are based on equations derived from the Mass Action Law and use physical parameters obtained by this application; e.g. values for equilibrium content (K), for both labelled and unlabelled analyte, concentration of antibody, concentration of tracer, NSB etc. In practice, these values are not known, and have to be calculated. Although the computation procedures are complex, the non-linear methods are resistant to error because the deviant points would have a minimum effect. The selection of a proper model to suit a particular assay or a given set of data is difficult and the technique does not have the flexibility or universality of application that, for example, a spline technique would allow.

Empirical models have found more favour as regression based methods for DP because of the complexity of the mathematical equations needed for the theoretical ones. Many of these empirical models use transformations (reciprocals, logit-log etc.) in an attempt to linearise the curve, but, as pointed out earlier, such transformations introduce or exaggerate heteroscedasticity to compensate for which weighting factors have also to be included. Furthermore, many of the assumptions made when these models are used, for example, that there is only one species of binding site, full equilibrium is achieved, complete separation of free and bound fraction,etc., do not apply in actual RIA practice, and may result in atypical curves.

Logit log transformation:

This was probably the most popular method of plotting of RIA data until the advent of the four parameter logistic log plot. Statisticians were using it over 50 years ago to linearize sigmoid shaped curves and the technique was extended to linearize RIA curves from equilibrium but not non-equilibrium or "two site" assays.

$$\text{logit } y = \log_e \frac{y}{100 - y} \qquad \text{where } y = B/B_0 \text{, corrected for NSB;}$$

or $$\text{logit } y = a + b \log x$$

x = dose and a and b are calculated by the method of least squares, i.e. where the sum of the squares of the y residuals is a minimum.

Transformation along the y axis scale introduces heteroscedasticity and weighing should therefore be applied, complicating the regression process. Also, the zero point is lost and the standard curve is usable only to the first (lowest) definitive standard concentration although the sensitivity of the assay may in fact be much better.

The four parameter logistic method:

The two parameter logit log method may not always linearize the standard curve at its extremes, i.e. in the low and high concentration regions. The problem may be dealt with by introducing two additional parameters representing binding at zero and at infinite doses. The four parameter logistic log equation is:

$$y = \frac{a - d}{1 + (x/c)^b} + d$$

where y = response
a = binding at zero dose, i.e. the highest normalized counting ratio in a typical RIA where fraction bound is counted
b = slope of the logit-log curve
c = ED_{50} point; (a+d)/2
d = the lowest normalized counting ratio
x = analyte concentration

Although alternative methods exist for computing the values of a, b, c and d, the one generally employed is successive iteration of b and c followed by a and d until the best fit is obtained, (the 2+2 method).

The four parameter logistic log plot is a non-linear empirical regression method of curve fitting suited to most symmetrical dose response curves. Rather than truncate the curve at an useful area, - (at low dose levels) - it enables values for the B_0 and NSB points to be adjusted using other experimental evidence available. As many parameters are used the curve becomes quite resistant to error, and outlier points, which are readily recognized, have a minimum effect on the calculation of final results.

The logistic model has the advantage of almost universal applicability. It does not depend on whether bound or free fractions, or both, are counted and enables a choice of response variable, although counts are usually preferred; (cf. in the logit log plot, y is always B/B_0). Furthermore, the method is applicable to many types of immunoassays irrespective of the label used, whether a radioisotope, enzyme, fluorescent or chemiluminescent marker, etc. It is thus an improvement of the logit log method which is more flexible and adapted to more general use.

The method may be extended by the addition of more parameters in cases where asymmetry of the curve is still encountered. In IRMA assays, for example, the addition of a 5th parameter correct for asymmetry of the curve.

$$y = \frac{a - d}{[1 + (x/c)^b]^e} + d$$

The 5th parameter, e, of this "powered logistic" is calculated on the basis of experimental evidence from several assay batches. It is generally < 1 and its introduction causes the point of inflection of an IRMA curve to be above its mid point.

Sixth and seventh parameters may also be introduced, if required, but those would require quite considerable computing power and involve additional time and expense.

From the review of curve fitting methods given above, it would be clear that there is no really universal, best, or "right" method which may be recommended to the exclusion of all others. A fitting technique that best suits the assay requirements should be selected with regard to such factors as assay kinetics, assay sensitivity and ability to exclude deviant values. The four parameter or five parameter logistic model appears to be the most flexible and versatile presently available.

IAEA Data Processing Programmes (RIA/PC):

The Nuclear Medicine Section of IAEA has recently developed a set of DP programmes that would handle RIA and IQC results, for use on IBM PC or compatible computers. The diskettes and instruction manual (IAEA-TECDOC-509), available free of cost on request, are already in use in over 150 laboratories. The programs use the four parameter logistic model and represent an advance over a previous version (IAEA-TECDOC-346) which, although using the same approach, was for an HP41C programmable calculator. Utilizing the greater computing power of the IBM PC, the programs can provide composite analysis (RER parameters, between batch QC analysis, etc.) for 40 assays, and enable hard copies of standard curves, precision profiles, and Levy-Jennings QC charts to be readily obtained. These interested in obtaining copies of the program and TECDOC should apply to the Head, Nuclear Medicine Section, P.O. Box 100, A-1400 Vienna.

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CHAPTER 16

SETTING UP A RIA LABORATORY.

Equipment requirements for a Radioimmunoassay Laboratory

This list assumes that the laboratory concerned uses bulk reagent based methodology and prepares at least some of its own reagents on a small scale. Ordinary laboratory glassware, test tubes, stationary, etc. are not mentioned.

1. Counters

1.1 A 8-12 well multiwell (multiple manual) gamma counter, preferably with on-board computer and printer and "on line" data processing possibility for RIA and IQC results. Modern machines make it possible to obtain composite results (RER parameters, BBCV etc.) from 100 batches or more. "Automatic" counters with moving platforms are not recommended as the mechanical components are more likely to break down.

1.2 A back-up single well manual counter. This is an essential item for the counting of iodination fractions in laboratories that prepare their own tracers. Most multiwell counters are not suitable for counting of high activity iodination fractions.

1.3 A liquid scintillation counter is only needed if ^{3}H, ^{14}C, ^{32}P, or similar radionuclides are used; e.g. in methods using DNA probes. However, likely future innovations make the use of ^{125}I possible in liquid phase DNA probe systems.

2. Centrifuge

2.1 A RIA centrifuge with swing out head and adapters and carriers to take at least 100 LP3 (75 mm x 12 mm) tubes. Refrigeration is preferable but is becoming less important as most assays are now carried out at ambient temperature. However, centrifugation at 4°C may be needed as in certain protocols for the purification of antibodies. A speed of up to 4 000 rpm is sufficient except for special purposes.

2.2 A clinical centrifugation for the preparation of serum samples.

3. Hand held semiautomatic RIA pipettes

Two to three sets covering the range 25-1,000 ml. Note that the tips used with many RIA pipettes are generally re-usable provided they are washed after treatment with a suitable radioactive decontaminant solution and carefully dried at no more than 50°C.

4. An analytical balance

5. A pH meter, analogue or digital type, with one set of spare electrodes.

6. Rotary mixer capable of taking 120 LP3 tubes

7. Magnetic separators, 2

8. Magnetic stirrers, of which one should be of large capacity (1-2 litres) with hot plate, and 2 others small to mid size.

9. A set of stirring bars

10. Vortex mixers, 2, for mixing small samples in test tubes

11. A water bath, minimum 6 litre capacity, with thermostatic control

12. A distilled water still

13. A portable radiation monitor

14. A general purpose laboratory deep freezer, preferably upright model, about 750 litre capacity

15. One refrigerator, at least 300 litre capacity

16. A desk top computer, minimum 640 KB RAM with hard disk and printer

17. Voltage stabilizers, at least for the counter, deep freezer, and computer.

More advanced laboratories making up their own reagents such as tracers would also need:

18. A fume hood, preferably with laminar flow

19. A fraction collector or HPLC equipment. Moderately priced HPLC equipment is now available and is preferred for separation of iodination fractions.

20 Chromatography coloumns

21. An ultrasonic cleaner

22. A small refrigerator in the "hot" laboratory.

CHAPTER 16

CLINICAL APPLICATIONS OF RIA PROCEDURES

Introduction

In developing countries, RIA procedures find their widest application in the detection and measurement of hormones, vitamins, drugs, tumour markers, and bacterial, parasitic and viral antigens and antibodies. Information about Radioimmunoassay (RIA) in bacterial and parasitic disorders and on viral hepatitis will be found in other chapters of this book. The clinical relevance of measurement of the thyroid related hormones T3, T4 and TSH, probably the commonest procedures in RIA laboratories in developing countries, is also described in another chapter. This section will describe clinical applications of a few of the other RIA tests, common in developing countries, and amenable to bulk reagent based methodology. However, when referral is limited for a particular assay and as a result, the work load is small, use of ready made kits is more practical and economical (for example PTH, GH).

No special patient preparation is required before withdrawal of a specimen of venous blood for a routine RIA test. Separated serum is most commonly used though heparinized plasma is employed for estimation of cortisol, or renin. Most RIAs are at present "non-extraction" assays and care should therefore be taken to avoid haemolysis especially if ^{3}H or ^{14}C tracers and liquid scintillation counting are used. Unless variations of analyte concentration with time of day are known to exist, such as the diurnal rhythm of cortisol, basal samples may be taken in the morning. Patients need not be fasting but high fat meals which may make serum lipaemic, are better avoided. Instructions for this may become confusing to the patient. A sound principle, in this respect, is to draw basal blood samples in the morning before the first solid meal of the day. This practice would also result in uniform instructions to all patients.

It is important that, before embarking on a clinical service, each laboratory establishes its own normal or reference ranges. Due consideration should be given to the fact that normal values may depend on age (e.g. sex hormones), sex, diet (e.g. Vit B_{12} levels are lower in vegetarians as compared to non-vegetarians), ethnicity, and even socio-economic factors. Where normal values follow a Gaussian distribution, the mean and + or - 2 S.D. may be appropriate to define a normal range but this will not apply when the distribution is skewed (e.g. TSH). Establishment of normal ranges is a more difficult exercise than normally realized. The practice of taking medical students or staff members to determine the normal ranges does not lead to accurate estimation of normal ranges suitable for the population under study. Advice of a statistician on population sampling techniques is worthwhile in establishing representative normal ranges.

In the case of hormones, assay of a single basal blood sample may not always provide adequate information about the functional status of an endocrine gland. Significant functional impairment may not be reflected in a single random sample because physiological compensatory mechanisms come into play. So called "stimulation tests" (challenge tests or dynamic tests) serve an important role of determining functional reserve of the endocrine

gland. Some of them are described below. These tests also help to establish a site of the lesion, whether in the gland or higher up in the pituitary or hypothalamus. Conversely, when the endocrine function is excessive and the basal levels high, suppression tests are useful in finding out whether the endocrine gland is behaving autonomously or whether it is still under the control of normal feed-back mechanisms. Suppression tests may also help in differentiating 'hyperplasia' from neoplastic conditions of the gland. This kind of functional tests bring the in vitro tests in line with the general overall thrust of nuclear medicine that it is more concerned with function than with a static study.

Growth Hormone

Human Growth Hormone (hGH) levels may be low or undetectable in normal subjects. In gigantism and acromegaly, levels of this hormone will be high and the diagnosis is confirmed by levels of more than 2 ng/ml in males and 5 ng/ml hGH in females 60 minutes after a oral glucose load of 100 grams. hGH deficiency needs to be confirmed by measurement 60 minutes after onset of nocturnal sleep (a value of >10 ng/ml will exclude the condition) and an abnormal response to at least two provocative tests such as exercise, arginine, L-Dopa, or glucagon administration, and insulin induced hypoglycemia. A single value of more than 9 ng/ml at any point during the studies excludes the condition. In the arginine stimulation test, a control sample is drawn and 0.5 g/kg body weight arginine infused over a period of 30 minutes. Blood samples are taken at 0, and every 15 minutes thereafter for 1 hour. For the L-Dopa stimulation test, the patient needs to be fasting. L-Dopa is given orally depending on body weight (125 mg if <15 kg, 250 mg if between 15 and 30 kg and 500 mg if over 30 kg) and blood drawn at 0, 20, 40, 60, 90 and 120 minutes. For a glucagon stimulation test, a control sample is drawn, 0.1 mg/kg glucagon given i.m. and blood drawn at 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Reactive hypoglycemia should be watched for during this test.

Cortisol

Normal values for plasma cortisol, as measured by a specific RIA, would be 7-15 μg/100 ml at 8.00 a.m., falling to at least half that value towards midnight. If this diurnal rhythm is disturbed (midnight value high), then hypercorticalism should be suspected and a dexamethasone test performed. For this procedure, 1 mg of dexamethasone is administered at midnight and plasma cortisol measured in a sample taken the next morning. If the value is over 5 μg/100 ml, then 8 mg dexamethasone may be given at midnight and the test repeated. If the cortisol level is above 50% of baseline, then primary adenoma or carcinoma of the adrenal should be suspected. However, malignancy may not be completely excluded by this test because elevated basal cortisol levels due to some ACTH secreting lung or thymic tumours may also be suppressed with 8 mg dexamethasone.

Adrenal reserve capacity in hypocorticalism is determined by an ACTH stimulation test. 0.25 mg synthetic ACTH is given i.m and blood taken at 0, 30, 60, 90 and 120 minutes. Normally, cortisol levels would rise by at least 10 μg/100 ml, at 60 or 90 minutes. A lack of response is suggestive but not diagnostic of primary adrenal disease. An exaggerated

response is seen in bilateral adrenal hyperplasia but a normal response does not exclude the condition. If an exaggerated response is seen in Cushings Syndrome, an adrenal carcinoma should be suspected.

Pituitary reserve ACTH capacity is most often tested by i.v. insulin stimulation test. The patient must be fasting. 0.1 units/kg body weight insulin is given i.v. and plasma cortisol measured at 0, 30, 60, and 90 minutes. Normally, an increment by at least 5 μg/100 ml and a maximal level of more than 20 μg/100 ml would be seen. Blood glucose concentration is also measured to ensure that hypoglycemia below 40 mg/100 ml has been induced. Patients with primary adrenocortical insufficiency (Addisons Disease) are very sensitive to insulin and the test should be performed, if at all, with great caution in these patients. In any event, the test should be terminated at any point if there are signs of severe hypoglycemia.

Metyrapone is a 11-hydroxylase inhibitor and therefore blocks the final stage in the synthesis of cortisol. If the pituitary is normal, lowered cortisol concentration would promote secretion of ACTH and thus increase in the cortisol precursor 11-deoxy cortisol. For the test, medication is withdrawn for 2 days and 30 mg metyrapone given as a single dose orally at midnight. Cortisol and 11-deoxy cortisol are measured the next morning. In a normal response, a marked fall in cortisol and increase of 11-deoxy cortisol in plasma would be seen. This test should not be performed in cases of primary adrenal insufficiency unless a response to ACTH stimulation has previously been obtained.

Certain adrenal enzyme defects such as 21- and 11-hydroxylase deficiency would cause an increase in basal plasma levels of 17 alpha OH progesterone, which is now directly measurable by RIA. ACTH stimulation may be needed in mild cases. An overnight dexamethasone suppression test would exclude adrenal neoplasm.

Aldosterone

In so far as analytes measurable by RIA are concerned, primary hyperaldosteronism is characterized by increase of aldosterone and lowered plasma renin activity (PRA). A 24 hour sample of urine is collected 4 days after the patient has been on a high sodium (120 mEq/day) intake. The collected urine should be stored at pH 5.0. A hormone level over 17 ng/24 hours is suggestive of this condition. Measurement of aldosterone (and PRA) at 8.00 a.m. is useful for distinguishing adenomas from hyperplasia. With adenomas, plasma aldosterone is in excess of 20 ng/100 ml and decreases following 4 hours of normal ambulatory posture. In hyperplasia, on the other hand, it is usually less than 20 ng/ml and increases on 4 hours of ambulant posture. In adrenal carcinoma, plasma aldosterone values may exceed 100 ng/ml.

Parathyroid Hormone

Low parathormone plasma concentrations, also measurable by RIA, are found in "true" hypoparathyroidism and high values in pseudohypoparathyroidism. Hypocalcaemia is found

in both conditions. The diagnosis of hyperparathyroidism is confirmed by the presence of high plasma PTH and hypercalcaemia.

Gonadotrophins

The pituitary gonadotrophins, FSH and LH, are readily measurable by RIA in serum. In combination with estimations of sex hormones (oestrogen and testosterone) they serve to discriminate between primary and secondary hypogonadism. In the former condition, (e.g. Turner's Syndrome), gonadotrophin levels will be high and sex hormones low, whereas the latter is characterized by low sex hormone levels without gonadotrophin (especially FSH) elevation. A luteotrophin stimulation test can also distinguish between primary and secondary hypogonadism. Here, LH-RH is injected i.v. and blood for gonadotrophin assay taken at 0, 30, 60 and 90 minutes thereafter. In a normal response, which indicates that the hypogonadism is not secondary or due to a pituitary defect, LH and FSH levels rise at 30 minutes and 60 minutes respectively. Normal values for these hormones in prepubertal children would be less than 100 ng/ml FSH and 20 ng/ml LH. In adult males, they would range from 120-250 ng/ml FSH and 30-70 ng/ml LH. In adult females in the reproductive age, values in the follicular and late luteal phases of the menstrual cycle would be about the same or slightly higher than in males with marked variations at mid-cycle.

Combined measurements of sex hormones and gonadotrophins (and stimulation tests when indicated) are commonly employed in investigations of infertility to establish whether the primary defect lies in the gonads or in the brain. However, endocrine studies are seldom front-line investigations in infertility as hardly 10% of the couples are likely to have this kind of etiology.

Sex Hormones

In primary male hypogonadism, if mild, testosterone levels may be normal or slightly low and measurement of free testosterone is more useful. High testosterone levels are found in testicular feminization in women. In the polycystic ovary syndrome an LH:FSH ratio of more than 2.5 is considered to be characteristic, while oestrone and testosterone levels may also be elevated. Measurement of testosterone and dihydrotestesterone play some role in the diagnostic protocols of children with various abnormalities of the external genitalia (female or male pseudohermaphroditism, etc.) but chromosome studies are more vital in these conditions.

Progesterone levels in normal females during the follicular phase of the menstrual cycle would be less than 2.0 ng/ml, rising to 12-24 ng/ml one week after ovulation.

Prolactin

Normal levels of Prolactin are usually less than 20 ng/ml. Mild to moderate elevations may be found in many conditions but a level of over 200 ng/ml is considered diagnostic of pituitary adenoma. In doubtful cases, a provocative TRH (500 mg IV) test may serve to

exclude a tumour. If a tumour is present, prolactin levels would not rise to more than twice the basal level.

Insulin and diabetes mellitus

RIA measurements of insulin, combined with glucose estimations, can diagnose hyperinsulinism due to insulin secreting tumours of the pancreas. Insulin (in micro units per ml) to glucose (in mg/100 ml) ratios of more than 0.30 is indicative of this condition.

Measurement of insulin and C-peptide concentrations following arginine stimulation are useful in Type II diabetes mellitus. In this test, 300 ml of a 10% solution of arginine is infused into a fasting patient who has not had insulin, over a period of 30 minutes and blood taken at -15, 0, 15, 30, 45, 60 and 90 minutes. In Type I, insulin dependent diabetes, basal levels of C-peptide would be less than 0.3 ng/ml and there would be no response to arginine. If basal C-peptide is low, say 0.3-1.0 ng/ml, and there is some response to arginine (a 100% rise), the patient is still insulin deficient. In typical Type II diabetes, basal levels would be 2.5 ng/ml or over, and a 100% rise on stimulation would be seen.

RIA for microalbuminuria is becoming popular as a predictor of the development of clinical nephropathy in diabetics.

Tumour markers

Assays for tumour marker detection are likely to become more popular in developing countries as reagents become more freely available but, at present, they consist mainly of measurement of thyroglobulin, hCG, AFP and CEA in a few laboratories. However, certain "eutopic" markers such as calcitonin in medullary carcinoma of the thyroid and ACTH in carcinoma of the bronchus and some other organs may also be included as tumour markers. The value of thyroglobulin measurements by RIA is dealt with in another chapter.

hGG, undetectable in normal circumstances rises to a value, in serum, of 90-2000 miu/ml in the 4th week of pregnancy to 10 000 - 100 000 miu/ml in the 12th week. The hormone is also secreted by trophoblastic tumours and is useful to monitor choriocarcinoma during chemotherapy and to detect the development of this condition in patients who have hydatiform mole.

Elevated AFP levels are found in neural tube defects, in late stage breast carcinoma with metastases and in hepatocellular carcinoma where the values may be grossly elevated, sometimes to over 500 000 mg/ml. Combined measurements of hCG and AFP are useful in the staging of testicular neoplasms.

CEA values would be from 3-10 ng/mg in normal subjects with a mean of about 5 ng/ml. Elevated levels are found in about 50% of cases of colorectal cancer and, to a lesser extent, in breast and lung cancer, and in ulcerative colitis.

RIAs for hCG, AFP, and even CEA are now possible using bulk reagents. Assays for other tumour associated antigens such as OC-125 in ovarian cancer and CA-153 in breast cancer will no doubt become more available to developing countries in time. At present, however, the reagents needed are expensive and not freely available, and their wider use in developing countries awaits an improvement in the situation.

ACKNOWLEDGEMENT

Almost all of the protocols described in the following annexes are taken from the teaching materials used at IAEA organized training courses in the field of RIA over the past 3-4 years. Acknowledgement is made to the experts/lecturers who served at these courses and demonstrated the protocols in question, from the following institutions:

- The North East Thomas Regional Immunoassay Service (NETRIA), St. Bartholomew's Hospital, London, U.K.
- The WHO CCR for Immunoassay, Hammersmith Hospital, London, U.K.
- The National Institute of Health, Nonthanburi, Thailand.

Annex I

THE PREPARATION OF HORMONE (T4/T3) FREE SERUM

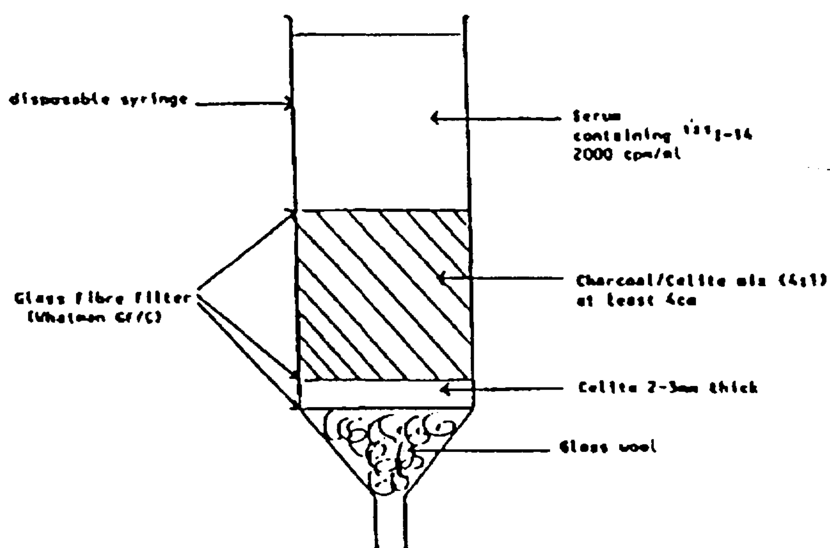
1. Collect a pool of human serum, preferably from many donors. Each donation must be individually tested for Hepatitis B markers and anti-HIV, (antibody to the AIDS virus). Sera found positive should not be used. Note that serum pools may yield false results when tested for virus.

   ALTERNATIVELY, donor horse serum may be used. This avoids the potential infection risk.

2. Filter the serum through a coarse filter (e.g. glass wool), to remove coarse particles.

3. Add ^{125}I-T4 to give approximately 2000 cpm/ml. Allow time for equilibration and remove 1 ml for counting. This yields the initial counts needed to monitor the removal of endogenous hormone.

4. Assemble a 50 ml disposable syringe to serve as a column as illustrated below:



5. Weigh out Celite (Celite Hyflo-Super-Cel, Koch Light) and charcoal (Norit PN5) in the ratio 1:4. Typically, with a 50 ml syringe used, 2 g of celite and 8 g of charcoal would be used.

6. Mix the charcoal and celite in a (covered) beaker or screw cap bottle and add about 25 ml distilled water. Continue to add water in small amounts until a thick slurry just able to be poured into the column is obtained.

7. Using a Pasture pipette, add the charcoal celite mixture to the column and allow to settle until the water is just visible at the top. Do not allow to run dry.

8. Gradually add the serum (approximately 50 ml) to the column.

9. Allow the column to run under gravity and discard the water void volume. The first 1 ml or so of the serum may also need to be discarded.

10. Collect the serum that has passed through the column. When all the serum has entered the column, more water may be added to the top to facilitate the passage of the serum down the column.

11. Count a 1 ml aliquot of the serum to monitor the removal of T4 and T3. Normally, about 98% or more of endogenous hormone is removed by this process.

Annex IA

PREPARATION OF HORMONE FREE SERUM

Adsorption by anion-exchange resin

stripping efficiency

- add ^{125}I T3/T4 1000-2000 cpm/100 μl of serum
- incubate 30 min for equilibration
- count an aliquot before addition of Resin

↓

add AGI-X8 200-400 mesh (Cl Anion-Exchange Resin (Bio-Rad No.140-1451) in the labelled serum (300 mg/ml), stir at 37°C for three hours

↓

filter the serum (coarse glass microfibre filter paper GF/D)

↓

count an aliquot of serum to monitor the stripping efficiency

↓

add further Resin in the same proportions and repeat the operation for another three hours

↓

filter the serum after 2 days incubation with resin, followed by
- coarse filter (GF/D)
- finer filter (GF/B)

↓

add 1:100 dilution of 10% sodium azide, store at -20°C

↓

wash the used resin 2 L of double distilled H_2O
soak the washed resin in double distilled H_2O

↓

check stripping efficiency
- count 100 μl serum for one minute
- repeat the procedure if more than 5% of radioactivity
- stripping efficiency should be more than 95%

Annex II

PREPARATION T3/T4 STANDARD

standard T3	**standard T4**
- weigh T3 3.255 mg	- weigh T4 2.223 mg
(Triiodothyronine, free acid, sigma)	(L-thyroxine free acid, sigma)

dissolve with a little (up to 0.5 ml) of ammonical (2N)-ethanol and dilute in 10 ml volumetric flask with 50% propylene glycol aqueous

spectrophotometric check

For T3		**For T4**
	- dilute stock standard with propylene glycol aqueous	
T3 1:10		T4 1:10
	- check absorbence	
320 nm		325 nm
	- molar concentration (μM)	
OD/4658 = X		OD/6210

- dilute solution X to 1:20 with 0.05 M phosphate buffer and 1:50 in hormone free serum	- dilute stock T4 1:100 with 0.05 M phosphate buffer and 1:10 in hormone free serum

- Final dilute working solution to 1, 2, 4, 6, 10 nM	- Final dilute working solution to 10, 50, 100, 150, 300 nM

REGENERATION OF RESIN

Rinse the resin with double distilled water

stir with 1 N NaOH (1 L NaOH : 300 g resin) 2 hrs., room temp.

filter, discard NaOH , rinse resin until the pH of water returns to 7.0

stir with 1 M HCl (1 L HCl : 300 g resin) for two hours at room temperature

filter, discard HCl, rinse until pH of water returns to 7.0

store the resin dry in a suitable container

CHAPTER 16

Annex III

TYPICAL IMMUNISATION SCHEDULE FOR PRODUCTION OF POLYCLONAL ANTIBODIES TO ANTIGENS

Notes

1. The antigen should be as pure as possible as any impurities present may also produce antibodies. This is specially important if the impurities are structurally similar to the primary antigen (e.g. many steroids) or if the tracer or standard used for the assay is likely to contain the same impurities.

2. High doses of antigen used for immunisation may induce tolerance and a poor antibody response. Do not exceed 100 μg/kg body weight of the animal.

3. The first, and sometimes the second, immunisation is given in Freunds complete adjuvant. Subsequent secondary immunisations are given in Freunds incomplete adjuvant. The complete adjuvant contains inactive TB and other bacteria which are highly immunogenic. To make up the suspension, mix 1 ml of adjuvant and antigen solution in buffer in a syringe. Connect this syringe to another via a three way tap and pass the mixture several times from one syringe to the other until completely emulsified. Good emulsion formation is seen when a drop "floats", rather than disperses, on the surface of the water.

4. Immunogens are always administered subcutaneously or intradermally, never intravenously. The multisite injection technique is preferred. For s.c. injection, 1 ml of emulsion at four to six sites along the back and neck is used. Abscesses may form and, if these are causing great inconvenience, the animal may need to be sacrificed. Where the intrademal route is used, the back of the animal is shaved and 25 - 50 μl of emulsion injected at multiple sites (10 - 100).

5. The more animals immunised, the greater the chances of obtaining antisera. Generally, where rabbits are used, immunising four to six animals may yield at least two good antibody producers.

6. A small test bleed (about 2 ml from an ear vein in the case of rabbits or guinea pigs) is taken two to four weeks after the first injection of immunogen. If three test bleeds betweeen four to twelve weeks do not provide any evidence of antibody formation, it may not be worthwhile to continue and the animal may be discarded.

7. Animals producing good antisera may be bled regularly, at twice weekly intervals. About 20 ml can be collected from the ear vein of a rabbit but, with guinea pigs, cardiac puncture will be necessary. Characteristics of antibodies can change with time and each bleed needs to be separately tested.

8. If an animal producing good antibodies were to become ill, it should be carefully observed and, where there is any possibility of it dying, it should be sacrificed by exsanguination under anaesthesia.

9. At each bleed, blood should be collected in glass tubes and allowed to clot for one to two hours at room temperature and two to six hours at 4°C. The tubes should not be disturbed during the clothing process as haemolysis may result. Centrifuge at 1500 g for 15 minutes, preferably at 4°C. If the clot is then left for a further period, (e.g. overnight) at 4°C, it may further retract and more antiserum may be collected.

Immunisation Protocol

1. For primary immunisation, use 50 - 100 μg/kg antigen in Freunds complete adjuvant. Inject subcutaneously or intradermally as described above.

2. Wait for two to four weeks.

3. First test bleed, taking 2 ml blood and testing for binding activity.

4. First secondary immunisation using same quantity of antigen in Freunds complete adjuvant as before.

5. Wait six to eight weeks, but carry out test bleeds at two to four week intervals as before.

6. Repeat secondary immunisation as in step 4 but using Freunds incomplete adjuvant.

7. Wait two to four weeks.

8. Repeat test bleed.

9. Repeat secondary immunisation and bleeding (20 ml) every two weeks for as long as needed.

Storage of Antisera

1. If a preservative such as 0.1% sodium azide (but not merthiolate) is added, antisera may be stored at 4°C for several years.

2. Antisera are also stable for many years stored forzen at -20°C or -40°C. Rapid (snap) freezing using a mixture of CO_2 and acetone is preferred. The storage should be in aliquots that can be reconstituted just prior to use. Where storage at -20°C is convenient, the antiserum may be diluted 1:10 in buffer containing 0.1% sodium azide before aliquotting. At this temperature, diluted antiserum is more stable.

3. If freeze drying equipment is available, antisera may be lyophylised and stored in aliquots for reconstitution immediately before use.

Annex IV

PROTOCOL FOR PRODUCTION OF IMMUNOGENS FROM HAPTENS USING THE MIXED ANHYDRIDE REACTION

Activation of Hapten (steroid)

1. Add 40 μmol (5ml) of N-methylmorpholine to 40 μmol of the steroid derivate in 250 μl non aqueous solvent (e.g. N, N- Dimethylformamid, DMF or Dioxane).
2. Cool to -15°C. (+ 10°C if using Dioxane).
3. Add 40 μmol (6μl) isobutylchloroformate.
4. React for three minutes at -15°C.

Conjugation to BSA

1. Add the activated steroid prepared as above slowly to 20 mg (0.3 μmol) BSA in 800 μl DMF.
2. React at -15°C for one hour and then at 0°C for three hours.
3. Dialyse the product.

CHAPTER 16

Annex V

CONJUGATION OF T4 TO BSA, USING CARBODIIMIDE

1. Dissolve 20 mg T4 in 5 ml of N, N- Dimethylformamid (DMF).
2. Dissolve 50 mg BSA in 25 ml distilled water.
3. Add 30 mg of carbodiimide derivate (CDI) to the BSA solution and adjust pH to 5.5 with NaOH.
4. Allow to react for ten minutes.
5. Add 10 mg more of CDI, adjust pH to 5.5, if necessary.
6. React overnight at room temperature in the dark.
7. Dialyse and lyophylise product.

Annex VI

ANTIBODY PURIFICATION METHODS

A. Using Ammonium Sulphate

1. Dilute 3 ml of antiserum to 10 ml with 0.9% saline.
2. Add 2.7 g ammonium sulphate with gradual stirring (45% saturation).
3. Mix for one hour at room temperature.
4. Centrifuge at 2000 rpm for 30 minutes at room temperature.
5. Re-dissolve the pellet in the minimum amount of phosphate buffer, pH 7.4, (or other buffer as may be used for antibody coating).
6. Dialyse against phosphate (or other coating) buffer.

B. Using Caprylic Acid (n. Octanoic acid)

1. Dilute 2 ml antiserum to 6 ml with 60 mM acetate buffer, pH 4.
2. Adjust pH to 4.8 using NaOH/HCl.
3. Add 1 ml caprylic acid (= 136 mg) and stir continuosuly for 30 minutes at room temperature.
4. Centrifuge at 3000 rpm for 45 minutes at 20°C.
5. Adjust pH of supernatent to 5.7 using NaOH.
6. Dialyse against three changes of 15 mM acetate buffer pH 5.7 to remove the caprylic acid.

C. Using DEAE-Cellulose Chromatography

1. First prepare purified antibody using ammonium sulphate precipitation as described in A. above.

2. Load the DEAE cellulose column with phosphate buffer pH 6.

3. Add 2 ml of antibody solution in phosphate buffer pH 6.

4. Run column with phosphate buffer, pH 6 and collect 1 ml fractions.

5. The fractions containing purified antibody are located by UV spectroscopy at 280 nM.

Annex VII

DIRECT IODINATION OF PROTEIN USING CHLORAMINE T.

Preparation of ^{125}I-T4 and ^{125}I-T3

1. Suspend 2 mg of T3 in a few ml of phosphate buffer pH 7.4 and add N. NaOH dropwise until the T3 is dissolved. Transfer to a 20 ml flask and make up to volume with phosphate buffer.

2. For iodination, aliquot in 15 ml (1.5 mg) volumes and store at -20°C, in polypropylene vials.

3. To a vial containing 15 ml of T3, add:

   20 μl 0.5 M phosphate buffer, pH 7.4.
   10 μl (1 mCi) of sodium ^{125}I iodine,
   10 μl Chloramine T solution in 50 mM phosphate buffer pH 7.4.

4. Mix for 20 seconds.

5. Add 10 μl (10 μg) of sodium metabisulphite in 50 mM phosphate, pH 7.4, and 100 μl potassium iodide (10 mg/ml) containing 1% BSA.

6. Vortex mix and count vial with contents.

7. Transfer contents to column and count empty vial.

8. The separation column is Sephadex G-25 Fine (approx. 2 g Sephadex) in a column 15 cm x 0.9 cm. Equlibrate and elute with 50 mM NaHCO3, pH 9.0 at a flow rate of 10 - 15 ml per hour.

9. Collect ten minute fractions. Count each fraction and plot counts against fraction number, to yield the chrometagraphic profile. Calculate proportion of radioactivity in each peak eluted: (see examples in Figure).

10. Pool the desired fractions containing T3 and T4 and adjust pH of each of them to 7.5 by dropwise addition of NaHCl. Dilute each to a radioactive concentration of 5 - 10 μCi/ml adding also phosphate buffer (pH 7.4), cysteine hydrochloride and mannitol to give final concentration of 50 mM phosphate buffer, 4% (w/v) mannitol and 0.1% systeine.

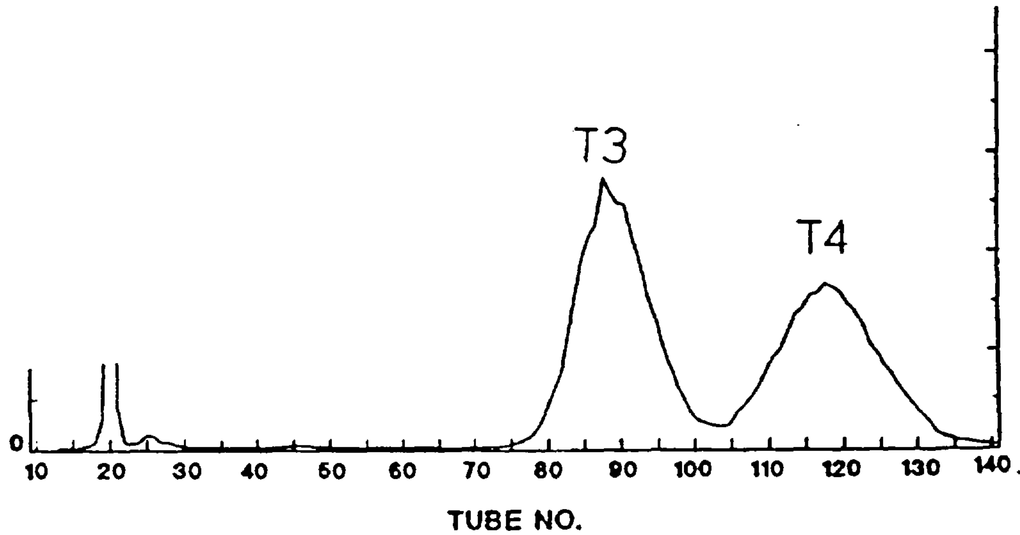
11. Aliquot 0.5 ml volumes and freeze dry.

12. Store at 4°C. The product is stable for at least 4 weeks.

Notes The procedure described above incorporates 40 - 60% of the initial ^{125}I into T4 and 25 - 40% into T3 with only about 5% of the ^{125}I remaining unreacted. Specific activities are about 600 - 1000 μCi/μg for T4 and 200 - 400 μCi/μg for T3.

ERN OF IODINATED T3, T4

Sephadax G-25 Column



Tube no.	Cp/10sec	Tube no.	Cp/10sec	Tube no.	Cp/10sec	Tube no.	Cp/10sec	Tube no.	Cp/10sec	Tube no.	Cp/10sec	Tube no.	Cp/10sec
1		21	413	41	174	61	155	81	11352	101	2193	121	12503
			607		241		191		15306		2182		10503
			1444		360		178		19015		2405		9677
			1249		320		137		21577		3533		8327
5			728		335		143		22831		4254		7216
			707		288		145		27370		5068		6088
			378		209		152		25986		6289		5145
			246		191		177		24897		7969		4233
			188		199		172		24763		9257		3291
10		30	200	50	175	70	186	90	21496	110	10065	130	2574
	124		197		179		205		19154		11860		2004
	111		215		168		257		15487		13550		1480
	133		223		148		309		13337		14171		1181
	212		149		174		524		10527		15590		1074
	283		175		166		824		8359		15707		806
	549		178		181		1312		6172		16780		745
	2819		158		181		2314		4827		16368		545
	47929		169		212		3881		3304		15852		489
	4248		156		188		5730		2687		14720		419
20	678	40	188	60	182	80	7541	100	2442	120	13920	140	341

Annex VIII

PROTOCOL FOR RADIOIODINATION USING SOLID PHASE LACTOPEROXIDASE

1. To 10 mg antigen in iodination vial as in Annex VII, add:

   10 μl 0.5 M phosphate buffer pH 7.4
   1 mCi sodium ^{125}I iodide.

2. Add 10 ml (10-20 ng) solid phase lactoperoxidase, and 5 μl H_2O_2 (0.5 nM).

3. React for ten minutes.

4. Add 5 μl more of H_2O_2 (0.5nM).

5. React 20 minutes.

6. Add 100 μl 0.1% sodium azide.

7. Purify products as in Annex VII.

Annex IX

IODINATION OF PEPTIDES BY IODOGEN METHOD

A. Preparation of Ultrogel column

1. Fill column with water or buffer.
2. Pour in correct amount of swollen Ultrogel using reservoir.
3. Allow column to settle whilst running with water or buffer.
4. Equilibrate column with buffer using high enough reservoir for it to compact.
5. After use store in buffer or water with bacteriostat. Stable at least six months.

B. Preparation of iodogen coated tube

1. Make up a 20 μl solution of iodogen in chloroform or dichloromethane (e.g. by making up a 1 mg/ml solution and diluting 1:50).
2. Pipette 100 μl of the iodogen solution into the bottom of a glass tube (e.g. a glass LP4 tube cut to approx. 4.5 cm deep).
3. Allow to dry in a fume cupboard. This takes approx. two hours.

C. Iodination

1. Mark test-tubes for collection of 400 μl fractions.
   Number the tubes.
2. Set Ultrogel column reservoir at height which gives flow rates of N6 ml/hour.
3. Add 20 μl of peptide solution in 0.05 M phosphate buffer pH 7.2 to the iodogen coated tube.
4. Add 10 μl of low activity $Na^{125}I$.

5. Leave to react for 20 minutes with occasional gentle shaking.

6. Transfer the reaction mixture into another tube containing 200 μl phosphate buffer pH 7.2 and leave for five minutes.

7. Place diluted reaction mixture onto 60 x 0.9 cm column of Ultrogel ACA 54 and run column slowly (6 ml/hr) using Phosphate Buffer containing BSA). Collect about 50 fractions of about 400 μl each.

8. Count fractions and plot elution profile.

Annex X

TYPICAL PROTOCOL FOR CONJUGATE IODINATION OF STEROID

Activation of steroid derivative

2.4 mg steroid in 50 μl dioxane
10 μl 1/5 solution of tri-n-butylamine in dioxane
10 μl 1/10 solution of isobutylchloroformate in dioxane
React 20 minutes 10°C.
3.5 ml dioxane to stop reaction.

Iodination of histamine

220 ng histamine in 10 μl phosphate buffer
0.5 mCi $Na^{125}I$ (5 μl)
50 μg chloramine- T in 10 μl phosphate buffer
React for ~30 seconds
300 μg sodium metabisulphite to stop reaction

Conjugation
Add 50 μl activated steroid to iodinated histamine
10 μl 0.1 M NaOH
React one hour on ice
10 ml 0.1M NaOH
React 1 hour on ice
Acidify 1 ml O.1M HCI
Extract excess histamine etc. with 1 ml toluene/ethyl acetate
Neutralise with 1 ml 0.1M NaOH
Add 1 ml Phosphate buffer
Extract product with 1 ml toluene/ethyl acetate

Purify on TLC

Develop with chloroform/methanol/acetic acid (90/10/1)
Localise by autoradiography and scrape off product band
Dissolve in ethanol.

Annex XI

IODINATION OF ANTIBODY (RABBIT IgG) BY N-BROMOSUCCINIMIDE METHOD

The antibody must be pure for iodination. Purification can be done by ammonium sulphate or caprylic acid precipitation, followed by isolation of IgG using a protein-A sepharose column or DEAE chromatography.

The optimal specific activity for iodinated IgG is approx. 12 μCi/μg. The final specific activity of the product can be altered by adjusting the amount of protein added, the amount of $Na^{125}I$ added, the amount of N-bromosuccinimide added, and the time of reaction.

1. Equilibrate a small Sephadex G25 column with 0.05M phosphate buffer.

2. Make up a solution of N-bromosuccinimide (200 μg/ ml) in 0.05 M phosphate buffer.

3. To a small tube (eg. an Eppendorf tube) add:

   10 μg IgG (eg. 10 μl of a 1 mg/ml solution)
   10 μl of 0.5 M phosphate buffer
   10 μl of the low activity $Na^{125}I$ provided.

4. Add 5 μl of N-bromosuccinimide solution, mix and react for 20 seconds.

5. Add 200 μl 0.05 M phosphate buffer to dilute reaction mixture (some workers add excess tyrosine to, in effect, stop reaction).

6. Immediately apply the mixture to the chromatography column and run with 0.05M phosphate buffer. Collect about 30 fractions of ten drops each.

7. Count each fraction for one second. Plot elution profile.

8. Add about 5 - 10 mg of BSA to the fractions saved (eg. 20 μl of 30% BSA).

9. Calculate specific activity of label:

$$\text{specific activity} = \frac{\text{labelled counts} \times \mu Ci\ Na^{125}I}{(\text{labelled} + \text{free counts}) \times \text{mass IgG}(\mu g)}\ \mu Ci/\mu g.$$

Annex XII

PREPARATION OF ^{125}I THYROXINE WITH PRODUCT SEPARATION BY HPLC

Note: This is a typical protocol, obtained through the courtesy of Dr. R. Edwards, Director, NETRIA, St. Bartholomews Hospital, London, U.K. which describes the preparation of ^{125}I Thyroxine including details of the HPLC procedure.

A. Apparatus & Procedure

High pressure pump, e.g. Altex model 110A, Rheodyne no. 7125 syringe loading injector, flow through radioactivity detector, and recorder, fraction collector, column, ultrasphere 5m ODS, 4.6 mm x 4.5 cm (Beckman), 1.5 ml conical microfuge tubes, snap top volumetric glassware, pipettes and tips, disposable plastic ware.

A manual (e.g. Mini-assay type 6-20) counter is used for radioactive counting; the bottom of the sample holder should be 17 cm above the bottom of the well.

Assemble the column and detector into the HPLC system, pump water through the system for 15 minutes at 1 ml/min., open the sample loop so this is also washed through.

THIS WATER WASH IS MOST IMPORTANT

Transfer to pumping the eluant for a further ten minutes, including the sample loop, then reduce flow to 0.5 ml/min and leave running to waste.

Load the fraction collector with tubes, set to collect 30 drop fractions, switch on and bring arm to start position, set the ratemeter to 3 x 10^4 cps, linear, time constant 3.3 sec and the recorder to 15 cm/hr.

B. Perform the iodination (using chloramine T), as described in Annex VII, using T3, free acid, as the starting material for iodination.

C. Purification

1. Insert the column outlet into the fraction collector drophead, start the eluant flow at 0.5 ml/min and observe the fraction collector to see if it is operating correctly,

2. switch on the chart recorder and check that it is operating,

3. load approx. 0.2 ml of eluant buffer into a 1 ml disposable syringe fitted with the injection needle, followed by the iodination mixture and another 0.2 ml (approx.) of eluant,

4. with the sample loop injector in the inject position, slowly load the sample from the syringe into the sample loop,

5. count the vial again to check residual activity and record the counts,

6. mark the chart recorder and turn the injection valve to the load position, watch for the first peak to appear on the chart recorder, then turn the loop back to the inject position, leave the pump running,

7. using a Pasteur pipette, rinse the vial with water into the disposal sink and again count the vial for recording solid waste activity,

8. after 20-30 minutes when all product peaks should have been eluted, stop the pump. Transfer the column outlet back to waste and the eluant back to water, continue washing with water for at least 30 minutes, open the sample loop so this is also washed,

9. remove the syringe and injection needle, wash with water, also wash through the channels of the injection valve,

10. transfer the column wash to methanol, wash for 15 minutes before switching off.

D. Dispensing and drying

1. Count each of the collected fractions for 1 sec. in the holder of the mini-assay, calculate the percentage of radioactivity in the iodine, T3, T4 peaks,

2. for pooling, use the fractions corresponding to T4 but omit one fraction from the beginning and one from the end of the peak. Calculate the total activity,

3. pool the selected T4 fractions into diluent buffer and dilute to give a radioactive concentration of 10mCi/ml,

4. count 10 ml of the diluted T4 solution for 10 seconds in the well of the mini-assay. There should be approx. 25,000 counts/10 sec./10 ml (= 10 mCi/ml at 70% efficiency) but no less than 20,000 counts.

E. Aliquot into 0.5 ml or 1.0 ml fractions (5-10 mCi) and freeze dry.

Annex XIII

ANTIBODY COATED TUBES/WELLS

1. Dispense 300 μl/tube or 200 μl/well of a ~1, 10, 100 μg/ml IgG solution in phosphate buffer pH 7.4. For blanks dispense 300 μl/tube or 200 μl/well of buffer.

2. Enclose in a container in a humid atmosphere and leave at 4°C overnight.

3. Aspirate IgG solution from tubes/wells.

4. Dispense 500 μl/tube or 250 μl/well wash buffer, and aspirate again.

5. Dispense 500 μl/tube or 250 μl/well 1% BSA solution to block remaining binding sites. Two hours at room temperature is sufficient time for blocking, but for convenience leave the tubes/wells containing 1% BSA overnight at 4°C.

6. Aspirate or decant the 1% BSA solution.

7. Add 300 μl/tube or 200 μl/well iodinated rabbit IgG in assay buffer and incubate at room temperature for 2 hours.

8. Aspirate and wash with 1 ml assay buffer.

9. Count.

Annex XIV

ANTIBODY COATED CELLULOSE

The Activation procedure

5 gm of Sigmacell is weighed into a 50 ml conical flask fitted with a ground glass stopper. 0.61 gm 1,1' -Carbonyldiimidazole (CDI) and 25 ml acetone is added and the mixture left to react one hour at room temperature with shaking. The activated imidazole-carbamate, cellulose is recovered by filtration over a glass microfibre filter, washed with three 100 ml aliquots of acetone, and allowed to air dry. The cellulose may be used immediately or stored dry at -20°C.

1. Weigh 200 mg activated cellulose into a polystyrene tube

2. Add 1 ml of N10 mg/ml IgG solution in barbitone buffer pH 8, and vortex briefly to form a slurry

3. Leave tube rotating overnight at room temperature

4. Centrifuge at 2500 rpm for five minutes at room temperature

5. Retain the supernatent for use again and to test for protein concentration

6. Resuspend the cellulose in 10 ml 0.5 M bicarbonate buffer pH 8 and rotate for 20 minutes

7. Centrifuge at 2500 rpm for five minutes at room temperature

8. Resuspend the cellulose in 10 ml 0.5 M bicarbonate buffer pH 8 and rotate for 20 minutes

9. Centrifuge at 2500 rpm for five minutes at room temperature

10. Resuspend the cellulose in 10 ml 0.1M acetate buffer pH 4 and rotate for 60 minutes

11. Centrifuge at 2500 rpm for 5 minutes at room temperature

12. Resuspend the cellulose in 10 ml 0.1 M acetate buffer pH 4, sonicate for 30 seconds and rotate for overnight at room temperature.

13. Centrifuge at 2500 rpm for five minutes at room temperature (adding assay buffer for constant volume)

14. Resuspend the cellulose in five ml assay buffer

15. Pipette 5,10, 20, 50, 100, 200 μl cellulose in duplicate into assay tubes

16. Add 100 μl labelled rabbit IgG solution and leave at room temperature for 2 hours with occasional shaking to keep the cellulose in suspension.

17. Add 1 ml wash solution, centrifuge at 2500 rpm for five minutes at room temperature, decant the supernatent and count the cellulose pellet.

18. Plot a dilution curve for the solid-phase antibody.

Annex XV

ANTIBODY COATED MAGNETIC PARTICLES

The Activation procedure

Roll the bottle containing the magnetic particles for 30 minutes at room temperature at N30 rpm. Pipette or pour out the required amount (20 ml = 1 g = sufficient for IgG isolated from ~ 1ml serum). Sediment the particles on a magnetic block and aspirate the supernatent. Wash the particles three times with 20 ml water, by mixing gently with water, sedimenting, and aspirating the supernatent. Wash the particles five times with acetone. Adjust volume to 10 ml with acetone and add 0.12g 1,1' -carbonyliimidazole (CDI). Mix gently by rolling one hour at room temperature. Sediment the particles and wash four times with 40 ml acetone, four times with 40 ml water and four times with 40 ml bicarbonate buffer pH 8.

Day 1

1. Dispense 0.5 g magnetic cellulose into a polystyrene tube

2. Add 0.5 ml ~ 10 mg/ ml IgG solution in barbitone buffer pH 8, and adjust volume to 10 ml with bicarbonate buffer pH 8.

3. Leave tube rolling overnight at room temperature

Day 2

4. Sediment the particles and wash twice with 20 ml bicarbonate buffer

5. Sediment the particles and wash with 20 ml bicarbonate buffer containing 3 ml/L ethanolamine

6. Sediment the particles and resuspend in 20 ml bicarbonate buffer containing 3 ml/l ethanolamine and roll for 30 minutes at room temperature

7. Sediment the particles and resuspend in 20 ml acetate buffer pH 4 and roll for 30 minutes at room temperature

8. Sediment the particles and wash twice with 20 ml assay buffer

Day 3

9. Pipette 5, 10, 20, 50, 100, 200 ml magnetic cellulose suspension in duplicate into assay tubes (adding assay buffer for constant volume)

15. Add 100 ml labelled rabbit IgG solution and leave at room temperature for two hours with occasional shaking to keep the particles in suspension

16. Add 1 ml wash solution, sediment the particles and aspirate the supernatent. Wash the particles with a further 1 ml wash solution and count the magnetic pellet.

17. Plot a dilution curve for the solid-phase antibody.

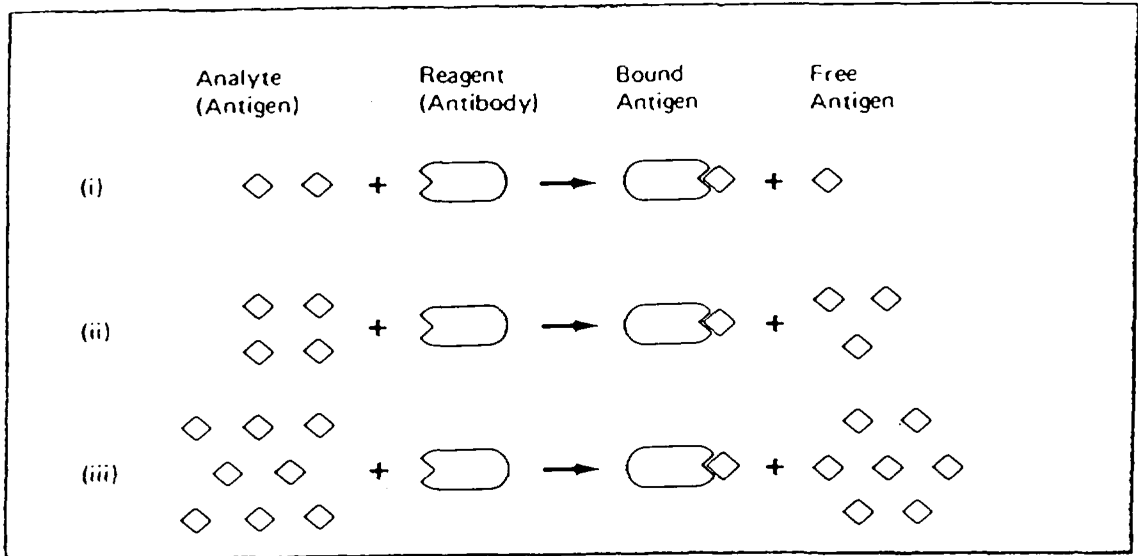


Fig. 16.1 "Limited" reagent RIA. Increasing concentrations of analyte give rise to increasing proportions of analyte in the free fraction.
(Taken from Edwards R.: Immunoassay; An Introduction. See ref. [4])

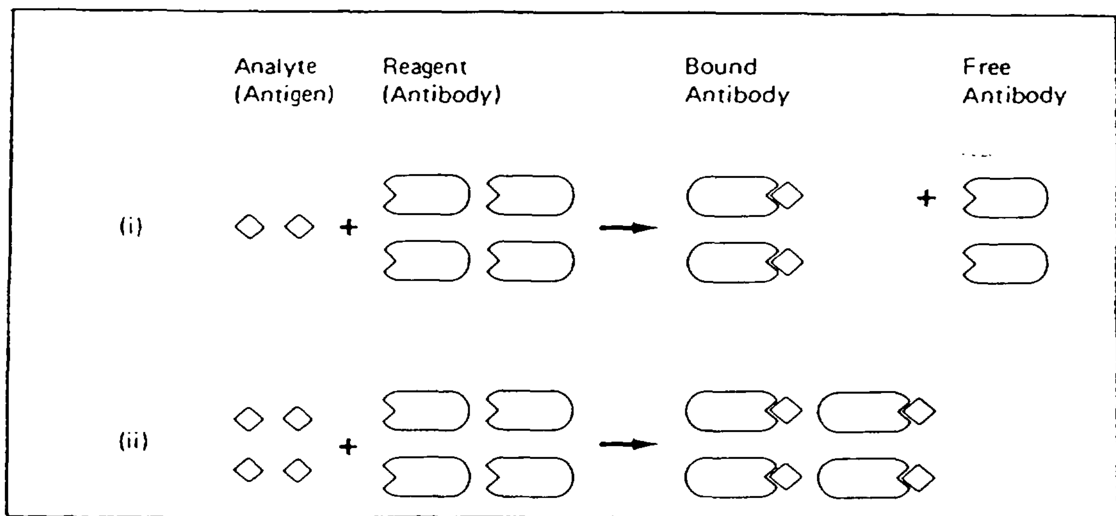


Fig. 16.2 "Excess" reagent IRMA. Increasing concentrations of analyte give rise to a corresponding increase in bound antibody.
(Taken from Edwards R.: Immunoassay; An Introduction. See ref. [4])

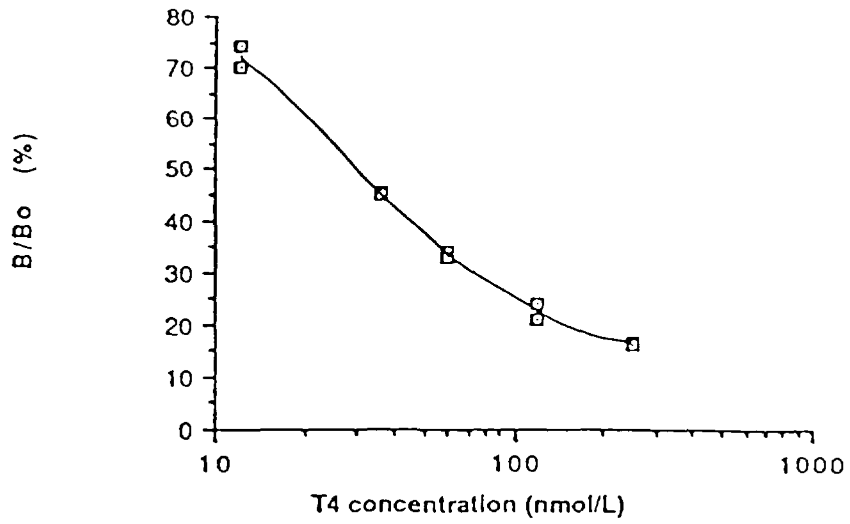


Fig. 16.3 A typical RIA standard curve for T_4.

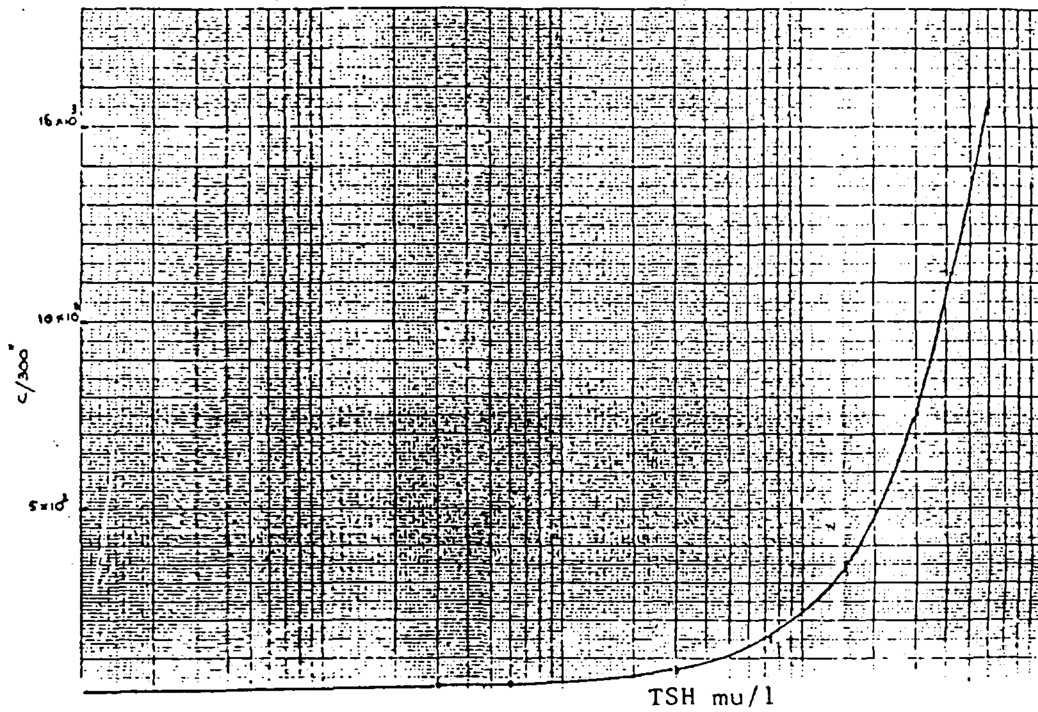


Fig. 16.4 A typical IRMA standard curve for TSH.

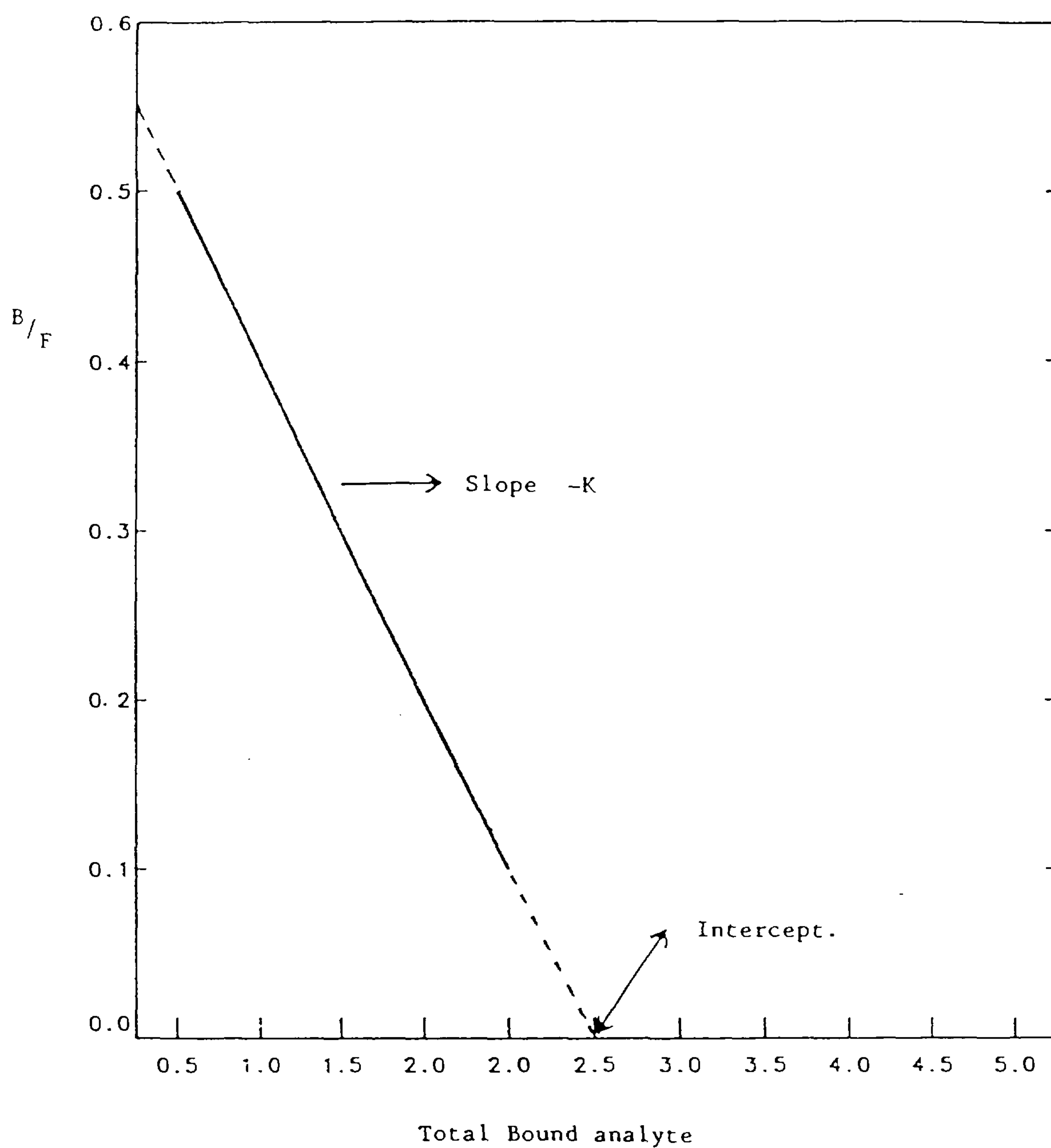


Fig. 16.5 A Scatchard Plot, relating analyte concentrations B/F vs. total bound. The slope yields the value of K and the intercept on the x-axis the concentration of binding sites, q.

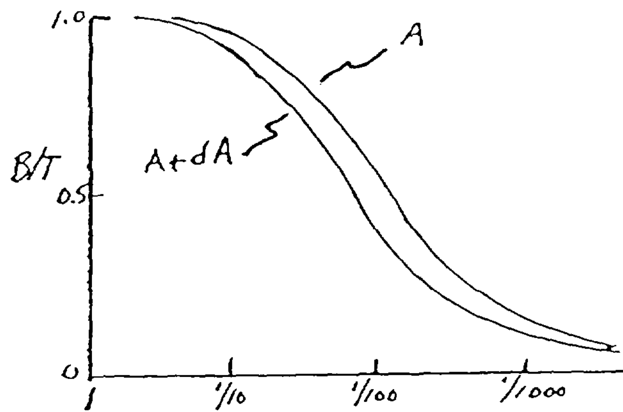


Fig. 16.6 Antibody dilution curves. The upper curve is with tracer only (A) while the lower is with tracer plus unlabelled analyte (dA). That dilution of antiserum at which there is maximal displacement between the two curves is chosen.

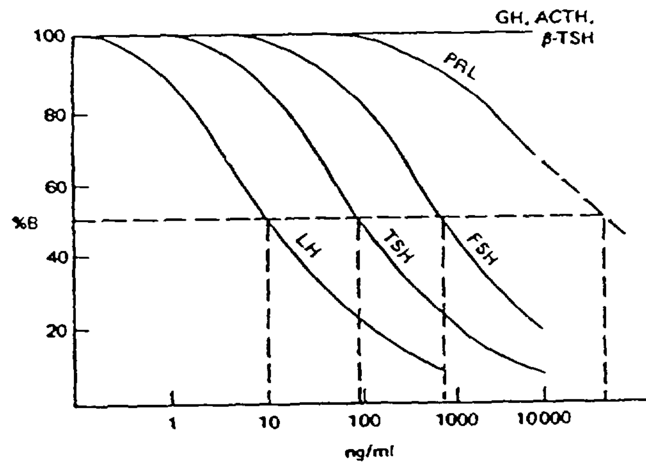


Fig. 16.7 The specificity of an LH assay as assessed by cross reactivity curves. (Taken from Laboratory Training Manual on RIA in Animal Reproduction. IAEA Technical Report Series 233.)

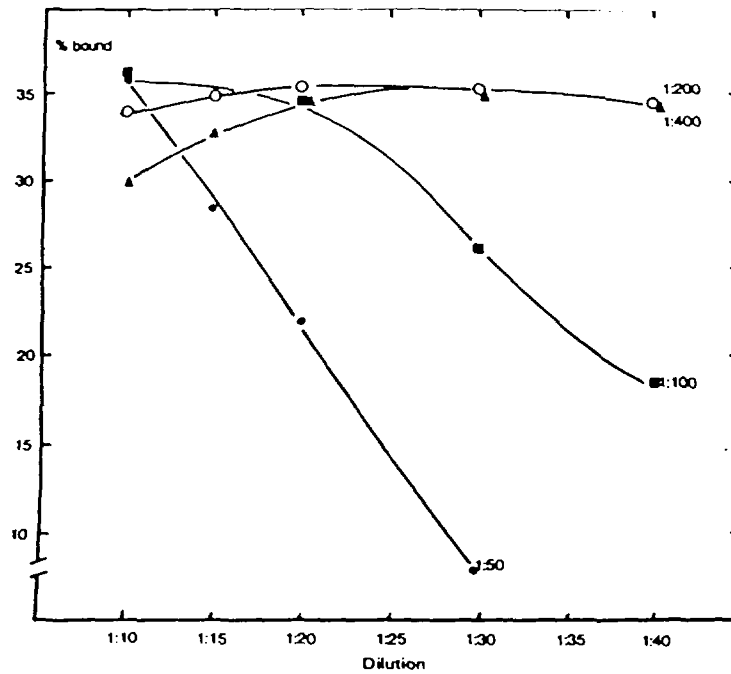


Fig. 16.8 Precipitation characteristics of a second antibody used with non-immune serum. A 1:30 dilution of second antibody and a 1:200 to 1:400 dilution of non-immune serum yields maximal precipitation and a wide plateau. (Taken from "Immunoassays for Clinical Chemistry", ed. W.M. Hunter and J.E.T. Corrie, 1983, pg. 463. Churchill Livingstone.)

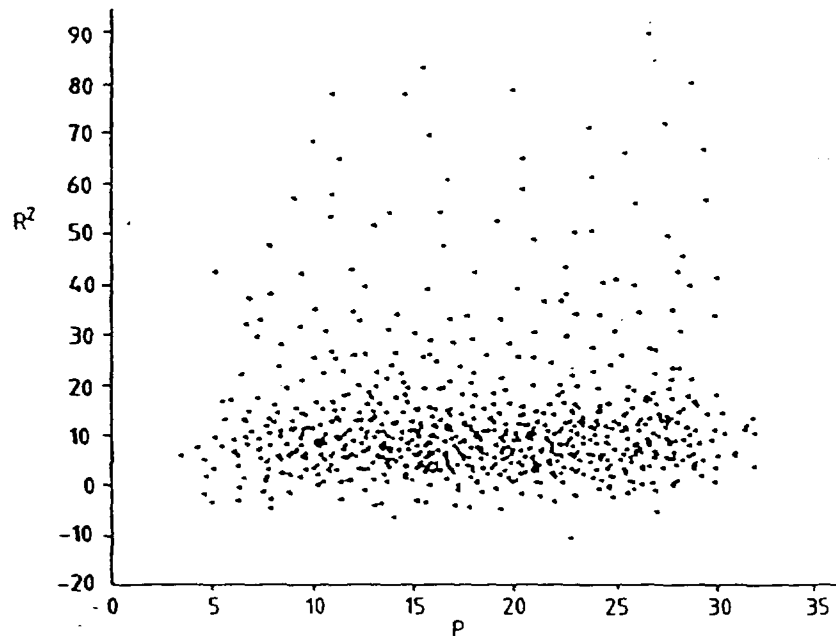


Fig. 16.9 A response error relationship (RER). The error (as CV) at each response point is plotted against the mean response to yield a "snowstorm" of points through which a smooth curve may be drawn.

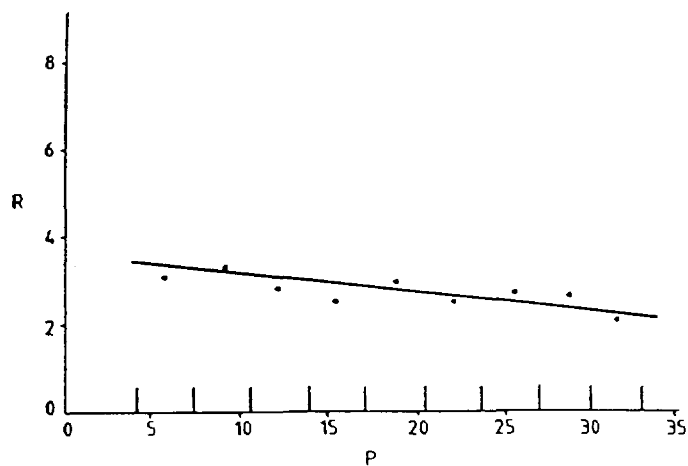


Fig. 16.10 A final RER curve, as error in the response vs. Mean Response.

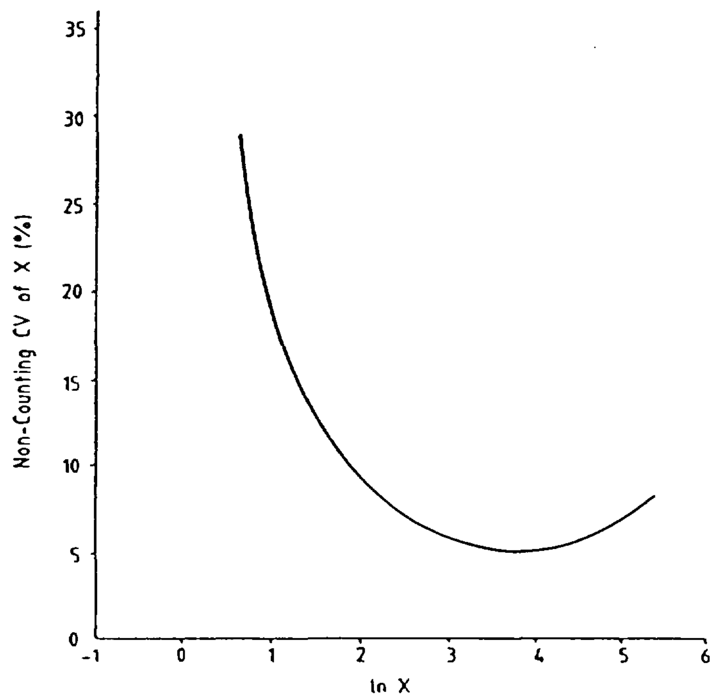


Fig. 16.11 An imprecision profile relating error (as CV) to analyte concentration.

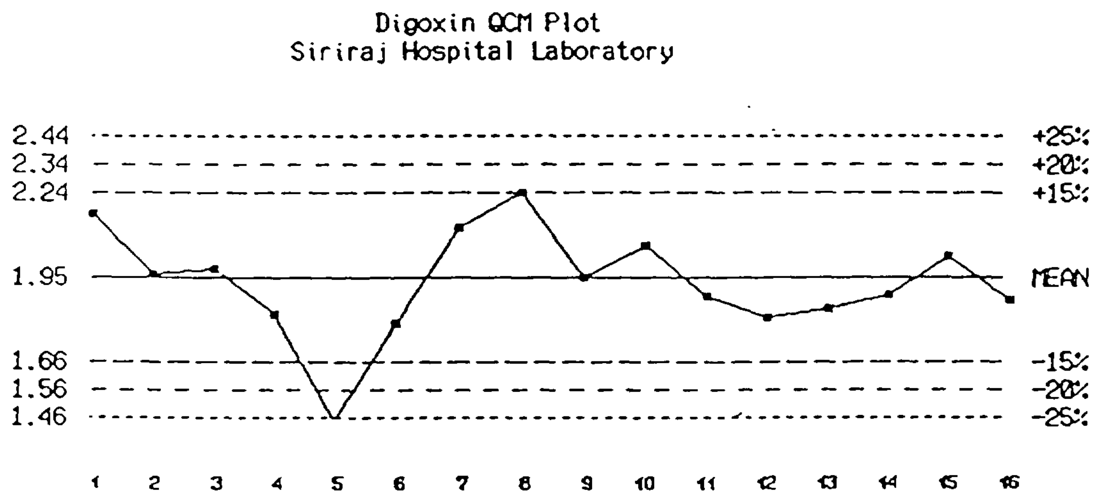


Fig. 16.12 A typical Shewhart (Levy-Jennings) laboratory internal quality control chart.

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Chapter 17

IN VITRO THYROID TESTING IN POPULATIONS WITH LOW THYROXINE BINDING GLOBULIN CAPACITY

A. Cuarón

Total thyroxine (T_4) concentration in serum is a reliable indicator of thyroid function in most individuals, but it is affected by altered concentrations of thyroxine-binding globulin (TBG) in serum. Within certain limits, the variations in total TBG binding capacity (TBG_{TOTAL}) caused by the fluctuations in the concentration of this binding globulin in serum can be modulated by calculating the free thyroxine index (FT_4I) as the product of T_4 and the in vitro uptake of triiodothyronine by a secondary binder (T_3U). This calculation is empirically based on the facts that free TBG binding capacity (TBG_{FREE}) is inversely related to T_3U and that T_4 and T_3U show opposite behaviour when measured in sera with altered TBG: a low T_4 in serum with reduced TBG_{TOTAL} is compensated by a high value for T_3U, while an elevated T_4 in serum with increased TBG_{TOTAL} is compensated by a low value for T_3U. In both cases the product of T_4 and T_3U renders a normal FT_4I value, showing a certain association with the concentration of free T_4 in serum (FT_4). In fact, this index has been shown to be superior than several FT_4 assay systems in the assessment of thyroid status in clinically euthyroid subjects with relatively high or low T_3U.

However, TBG_{TOTAL} is a direct function of the concentration of TBG in serum, which can be severely decreased in various clinical conditions (Table I). Besides, although not affecting TBG_{TOTAL}, certain drugs are also bound to this protein and have the virtue of reducing TBG_{FREE} and T_4 and increasing T_3U. Most of these factors are present in severely ill cardiac patients, especially in the developing countries where the prevalence of malnutrition and severely decreased TTBG is extraordinarily high. The imbalance is so great in these cases that calculation of the FT_4I usually renders a very low misleading value.

TABLE I. CAUSES OF DECREASED TOTAL AND FREE THYROXINE-BINDING GLOBULIN BINDING CAPACITY.

Reducing mechanism	Clinical situation
Decreased intake / anabolism:	Severe malnutrition.
Decreased anabolism:	Hepatic insufficiency Congestive heart failure Hypoadrenalism Severe illness
Increased excretion / catabolism:	Nephrosis Severe illness
Competitive binding to TBG:	Salicylates Hydantoin derivatives

These misleading results of the in vitro thyroid function tests and the corresponding FT_4I has made endocrinologists uneasy for many years. They have been ascribed to a reduction of the intrinsic sensitivities of the T_3U and T_4 assays when TBG is oversaturated by endogenous T_4, as in sera from euthyroid individuals with abnormally reduced TBG_{TOTAL}. This explanation is, however, farfetched, since the interactions between T_4 and its protein carrier should be in compliance with the strict rule of the physico-chemical law of mass action, which means that on a theoretical basis the oversaturation of TBG is not possible without a corresponding increase in serum FT_4 concentration and the consequent appearance of the characteristic signs and symptoms of thyrotoxicosis.

The uncommonly high prevalence of low TBG_{TOTAL} in the patients of the Instituto Nacional de Cardiologia de Mexico led the author to explore the means to obtain a more accurate indicator of thyroid function than the usual FT_4I, which in this peculiar population tended to give values of hypothyroidism in euthyroid patients and normal results in thyrotoxic individuals. Our first step was to measure TBG_{TOTAL} by electrophoresis and FT_4 concentration by equilibrium dialysis in every sample assayed for T_4 and T_3U for their proper interpretation. This approach increased the clinical value of in vitro thyroid function testing, since TBG_{TOTAL} and FT_4 assays were by far more accurate than T_3U and T_4 concentration, but resulted impractical because TBG_{TOTAL} measurements made by electrophoresis and FT_4 assay by dialysis are time consuming and cumbersome to comply with an increasing clinical demand.

This problem was overcome by developing a table where T_3U and T_4 values were correlated with TBG_{TOTAL} as calculated by using a empirical equation derived by Nusynowitz and Benedetto by using the very same in vitro systems for T_3U (Tri-Tab, Nuclear Medical Laboratories. Normal values: 35 - 45%) and T_4 assays (Tetra-Tab RIA, Nuclear Medical Laboratories. Normal values: 5.5 - 11.5 μg/dl). This equation, is:

$$\mathbf{TBG_{TOTAL} = 15.35\,[\,40 / T_3U\,]^{1.5} + 14.96\,[\,T_4 / 8\,]^{0.5} - 9.7} \qquad (1)$$

By applying this equation in sera from a population with a high prevalence (0.571) of TBG abnormalities, we have found a highly significant linear correlation between this estimate of TBG_{TOTAL} and the actual values measured by electrophoresis. The resulting regression line closely approximated the identity line of the graph and the test demonstrated to be highly sensitive (0.93) and specific (0.97) for TBG alterations. Free TBG binding capacity could be easily calculated as the difference between TBG_{TOTAL} and T_4:

$$\mathbf{TBG_{FREE} = TBG_{TOTAL} - T_4} \qquad (2)$$

With this two new parameters included in our reports, the referring physician was able to know if the alterations in the in vitro thyroid function tests were due to thyroid disfunction or to changes in TBG_{TOTAL}, increasing both sensitivity and specificity of the procedures.

Then, we tested the hypothesis regarding the misleading FT_4I as due to a reduction of the intrinsic sensitivities of T_4 and T_3U assays when TBG_{TOTAL} is reduced and the binding

protein is oversaturated by endogenous T_4. This was done by correlating T_4, T_3U and FT_4I with TBG_{TOTAL} in sera from normal individuals and patients with altered thyroid function and/or abnormal TBG_{TOTAL}. The results showed that the three parameters, T_4, T_3U and FT_4I, were able to efficiently separate the serum samples according to the actual thyrometabolic status of their donors at any TBG_{TOTAL} included in the study (7.3 - 43.6 μg T_4/dl), but that their normal ranges varied with TBG_{TOTAL}. Significant shifts in their respective normal ranges were observed even within the normal spectrum for TBG_{TOTAL} (15 - 28 μg T_4/dl), but these effects were more significant in sera with abnormal TBG_{TOTAL}. It is clear that even at the extremes of the normal range for TBG_{TOTAL} these procedures would render erroneous results according to their conventionally established values.

The peculiar features of the normal band for T_4 along the full scale of TBG_{TOTAL} **(Fig. 17.1A)**, means that the normal range for T_4 is lower and narrower (3.7 - 7.5 μg/dl) when TBG_{TOTAL} is at its lower normal value (15 μg T_4/dl) than when it is at its higher normal level (28 μg T_4/dl). In the later case, T_4 should be between 7.0 and 14.0 μg/dl in order to sustain a euthyroid state. This behaviour explains the low sensitivity of T_4 for thyrotoxicosis and its low specificity for hypothyroidism at relatively reduced levels of TBG_{TOTAL} when the diagnostic decision is based on its fixed conventional normal values (4.5 - 11.5 μg/dl).

The conduct of T_3U in relation to TBG_{TOTAL} is the inverse of that of T_4 **(Fig. 17.1B)**: its normal spectrum narrows and reduces as TBG_{TOTAL} is increased. This is a clear consequence of the increase of free binding sites in the protein and the consecutive reduction of the fraction of radioactive T_3 available to the secondary binder. At relatively low TBG_{TOTAL} levels (15 μg T_4/dl), the normal range for T_3U is 43 - 52%, but it shifts to 30 - 36% at relatively high levels of TTBG (28 μg T_4/dl). This behaviour explains its low sensitivity for hypothyroidism and its low specificity for thyrotoxicosis when TBG_{TOTAL} is relatively reduced and the fixed conventional normal values (35 - 45%) are used in the interpretation.

The normal spectrum for the FT_4I is also affected by TBG_{TOTAL} in that it rises and widens as TBG_{TOTAL} is increased, reaching asymptotic values when TBG_{TOTAL} is well beyond its physiological levels **(Fig. 17.1C)**. As it is, the normal limits for the FT_4I are 1.45 - 3.85 at relatively low values of TBG_{TOTAL} (15 μg T_4/dl), but they shift to 2.15 - 5.20 when TBG_{TOTAL} is relatively high (28 μg T_4/dl). These variances explain why, when interpreted according to its conventionally fixed limits (2.2 -4.7) the FT_4I shows a low sensitivity for thyrotoxicosis and a low specificity for hypothyroidism at relatively low values of TBG_{TOTAL}.

These results demonstrated that the limitations of T_4, T_3U and the FT_4I when TBG is seriously altered by nonthyroidal illnesses cannot be ascribed to a reduction of the intrinsic sensitivities of the in vitro testing systems, as proposed by others, but to an erroneous choice of their normal values according to the corresponding TBG_{TOTAL}. Besides, these results confirm that the FT_4I is based on an inverse relationship between T_4 and T_3U as TBG_{TOTAL} is increased, and that T_3U is a value inversely related to TBG_{FREE}:

$$TBG_{FREE} = 1 / T_3U \qquad (3)$$

As mentioned before, this kind of relationship is ruled by the law of mass action according to the equation:

$$FT_4 = (T_4) / (K_{tbg})(TBG_{FREE}) =$$

$$= (T_4) / (K_{tbg})(1 / T_3U) = \quad (4)$$

$$= (T_4)(T_3U) / (K_{tbg})$$

As can be observed in these equations, the calculation of the FT_4I as the product of T_4 and T_3U oversimplifies the relationship between TBG_{FREE} and T_3U, since it does not take into account the needed association constant (K_{tbg}), thereby introducing an error that becomes significant when there are wide variations in TBG_{TOTAL}. Furthermore, the FT_4I is the product of T_4, measured in $\mu g/dl$, and T_3U, which is a simple ratio, while the law of mass action rules the use of molar concentrations for all parameters. All this means that in theoretical basis the misleading FT_4I should not be considered as a surprising event in sera with abnormal TBG_{TOTAL}.

Since TBG_{TOTAL} is totally independent of the thyroid status of the individual, it was decided to combine T_4 and TBG_{TOTAL} in a single equation to render a value more related to the thyrometabolic state of the individual. Since all evidences pointed toward the well known importance of TBG as a regulator of FT_4, the only hormonal fraction in serum able to diffuse into the tissues and hence be available for their metabolism and action, the selected index was the ratio between T_4 multiplied by 100 and TBG_{TOTAL}, which represent the fractional saturation of TBG by endogenous T_4 (Sat%):

$$Sat\% = [T_4 \times 100] / TBG_{TOTAL} \quad (5)$$

The FT_4 concentration in serum, as measured by equilibrium dialysis, appeared to be closely related to the Sat% of TBG by endogenous T_4, as estimated by this equation (**Fig. 17.2**), according to a positive nonlinear function. It seems that FT_4 concentration reaches thyrotoxic levels when more than 50% of the binding sites in TBG are saturated by T_4, while FT_4 becomes defective when less than 25% of the total TBG binding capacity is occupied by the hormone, independently of the particular TBG_{TOTAL} in the sample. It is important to note that Sat% should not be mistaken with the ratio between T_4 and the serum TBG concentration measured by radioimmunoassay, previously suggested by Szpunar as another free thyroxine index. In order to be comparable with Sat%, this ratio should be calculated by using the molar concentrations of the two molecules in serum. Otherwise, this ratio could yield meaningless data, as those reported by Szpunar with TBG saturations higher than 100% in euthyroid sera.

Precise knowledge of total T_4, FT_4, and TBG_{TOTAL} on every single sample assayed, allowed the individual calculation of the association constants between T_4, on the one side,

and TBG (K_{tbg}) and albumin (K_{alb}), on the other, by following the general equation of the law of mass action:

$$K_{prot} = [\text{Protein-bound } T_4] / [\text{Protein}_{FREE}][FT_4] \quad (6)$$

where:
K_{prot} = association constant for the binding of T_4 to the carrier protein,
Protein-bound T_4 = concentration of binding sites occupied by T_4 in the carrier protein,
Protein_{FREE} = concentration of unoccupied binding sites in the carrier protein,
FT_4 = concentration of FT_4.

The calculated value of K_{tbg} on all triplicate samples was $2.336 \pm 0.075 \times 10^{10}$ M_{-1}, with a coefficient of variability of 3.21%. The associated constant between T_4 and albumin (K_{alb}) was four orders of magnitude lower than K_{tbg}, $0.85 \pm 0.06 \times 10_6$ M_{-1}, with a coefficient of variability of 7.06%, demonstrating once more that although albumin is more abundant than TBG and has a higher binding capacity, its affinity for thyropid hormones is much more lower. The product of the ratio K_{alb}/K_{tbg} and the free albumin binding capacity (Albumin_{FREE}) resulted sufficiently small and was discarded as an influential factor on the interactions between T_4 and TBG. The association constant between T_4 and pre-albumin (K_{tbpa}) was not estimated since this binding is selectively inhibited by Veronal (barbital) ions, which were basic in the used buffer solutions, and because there are many evidences that seem to indicate that T_4 binding to TBPA is an artefactual effect of certain buffer systems and has no pathophysiological significance. These observations showed again that TBG is by far the most prominent carrier protein for T_4 in our assay system.

Since FT_4 concentration is directly related to the degree of saturation of TBG, it follows that it is inversely proportional to its unsaturated fraction (100 - Sat%). By combining these two factors with K_{tbg}, a new equation was derived to calculate FT_4 concentration from the information on T_4 and TBG_{TOTAL}:

$$FT_4 = (Sat\%) / (K_{tbg})(100 - Sat\%) \quad (7)$$

The results obtained by using this equation showed a close linear correlation with the actual values of FT_4 concentration measured by equilibrium dialysis, with a regression line approaching the identity line of the graph and a very significant correlation coefficient (Fig. 17.3).

By using these equations based on the law of mass action, we were able to improve to optimal levels both the operating characteristics (sensitivity, specificity and accuracy) and the predictive values of in vitro thyroid function testing in populations with a high prevalence of TBG abnormalities, such as those usually found in cardiological populations with malnutrition. By performing only two in vitro thyroid function tests, T_4 and T_3U, these equations made possible information on several significant parameters involved in the peripheral physiology of thyroid hormones (Table II), allowing a clearer than usual pathophysiological insight.

TABLE II. PHYSIOLOGICAL DATA OBTAINED FROM IN VITRO THYROID FUNCTION TESTING (T_4 and T_3U) BY USING EQUATIONS BASED ON THE LAW OF MASS ACTION	
Parameter.	**Units**
Measured:	
T_4 concentration in serum.	μg / dl
In vitro uptake of T_3 by a secondary substrate.	%
Estimated:	
Total TBG binding capacity.	μg T_4 / dl
Free TBG binding capacity.	μg T_4 / dl
Fractional saturation of TBG by endogenous T_4.	%
Free T_4 concentration in serum.	ng T_4 / dl

This set of data renders a clear physiopathological profile to determine if the abnormal in vitro thyroid function tests are due to primary disorders in thyroid function (Table III), or because primary abnormalities on TBG are affecting thyroid function in order to maintain the euthyroid level of FT_4 in serum (Table IV).

TABLE III. TYPICAL RESULTS IN DYSTHYROID PATIENTS WITH NORMAL T.B.G.

Parameters	Hypothyroidism	Thyrotoxicosis
T_4	Low	High
T_3U	Low	High
FT_4I	Low	High
TBG_{TOTAL}	Normal	Normal
TBG_{FREE}	High	Low
Sat%	Low	High
FT_4	Low	High

TABLE IV. TYPICAL RESULTS IN EUTHYROID INDIVIDUALS WITH ALTERED T.B.G.

Parameters	Hypothyroidism	Thyrotoxicosis
T_4	Low	High
T_3U	High	Low
FT_4I	Low to Normal	Normal to High
TBG_{TOTAL}	Low	High
TBG_{FREE}	Low	High
Sat%	Normal	Normal
FT_4	Normal	Normal

At this point, it is important to notice that thyroid hyperfunction is not a synonym for thyrotoxicosis, as neither thyroid hypofunction has necessarily the meaning of hypothyroidism. Thyrotoxicosis and hypothyroidism are terms referred to the thyrometabolic state of the individual, dependent on the peripheral level of FT_4 in blood and tissues, while thyroid hyper- and hypofunction are terms used to express the levels of activity of the thyroid gland, not necessarily related to thyrotoxicosis and hypothyroidism. When TBG_{TOTAL} is decreased, the thyroid gland needs to reduce its function to avoid the oversaturation of this protein by T_4, the consequent rise on serum FT_4 concentration, and the appearance of thyrotoxicosis. The contrary events occurs when TBG_{TOTAL} is increased: thyroid function is increased to evade hyposaturation of the binding protein, the resulting reduction of FT_4 in serum and the signs and symptoms of hypothyroidism. In fact, TBG plays a very important role in the peripheral control of FT_4 in serum and of the thyrometabolic state of the individual.

This approach has the added advantage of being in favour of cost/benefit since it limits the use of more expensive tests. We have restricted the T.S.H. assays to define the etiology of hypothyroidism and thyrotoxicosis, and confined the radioimmunoanalytical assay of T_3 to the very infrequent study of T_3-thyrotoxicosis.

It is unfortunate, however, that the equations herein derived apply only to the particular in vitro thyroid testing systems used by the author (Tetra-Tab RIA and Tri-Tab, Nuclear Medical Laboratories), and only if the normal ranges for T_4 and T_3U are the same. The equations need to be revised accordingly with other in vitro thyroid testing systems, but the measurements of TBG_{TOTAL} by electrophoresis and of FT_4 concentration by dialysis might be avoided by using the very same commercial kits herein employed for the calculation of these parameters and perform the needed correlations. In any case, as in any other procedure of nuclear medicine, to obtain truthful results it is compulsory to follow the rigid rules of quality control when performing the assays for T_4 and T_3U.

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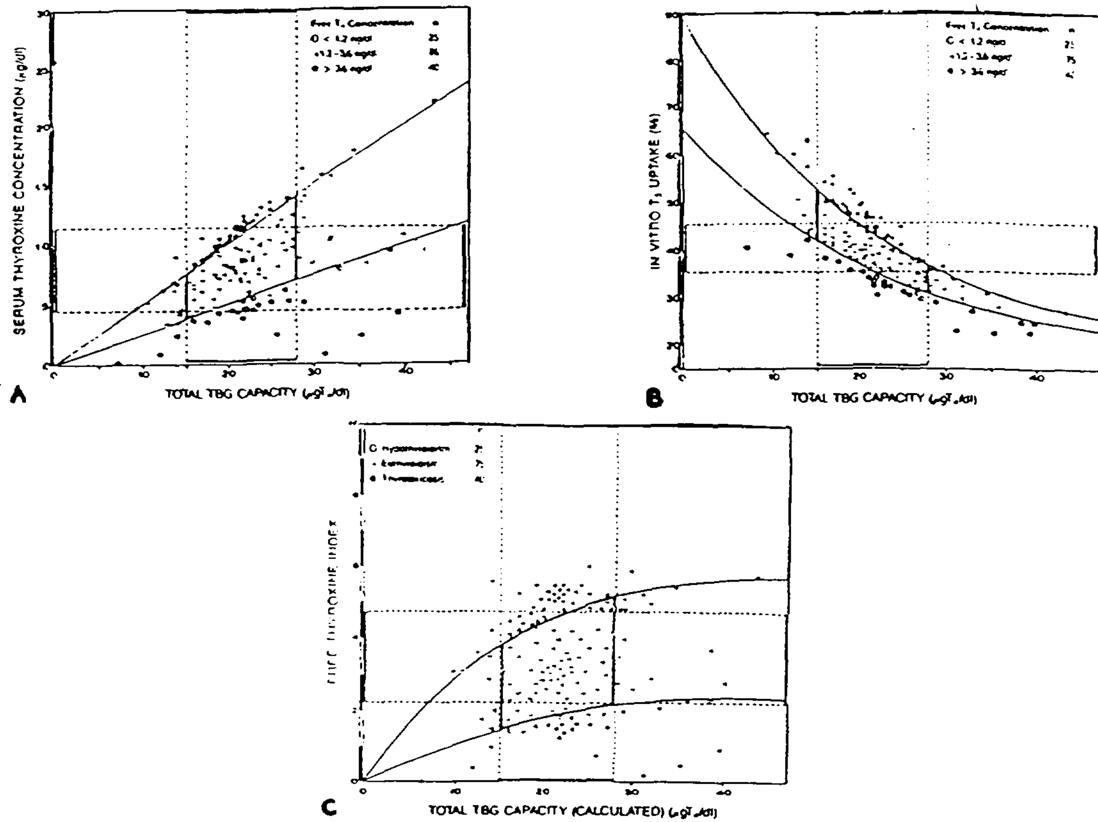

Fig. 17.1 Correlation of in vitro testing results in 141 sera with their corresponding value for total TBG binding capacity (TBG_{TOTAL}):

A. Serum thyroxine concentration (T_4).

B. In vitro uptake of T_3 by silicate (T_3U).

C. Free thyroxine index (FT_4I). Fixed conventional normal limits are shown at their respective coordinate.

Their variant normal limits according to total TBG binding capacity, are:

A. For serum T_4.
Upper normal limits = 0.5 TBG_{TOTAL};
lower normal limits = 0.25 TBG_{TOTAL}.

B. For T_3U.
Upper normal limits = $69\ e^{-TBGTOTAL/18} + 21$;
lower normal limits = $43\ e^{-TBGTOTAL/18} + 21$.

C. For FT_4I.
Upper limits = $5.9\ (1 - e^{-TBGTOTAL/12})$;
lower normal limits = $2.4\ (1 - e^{-TBGTOTAL/13})$

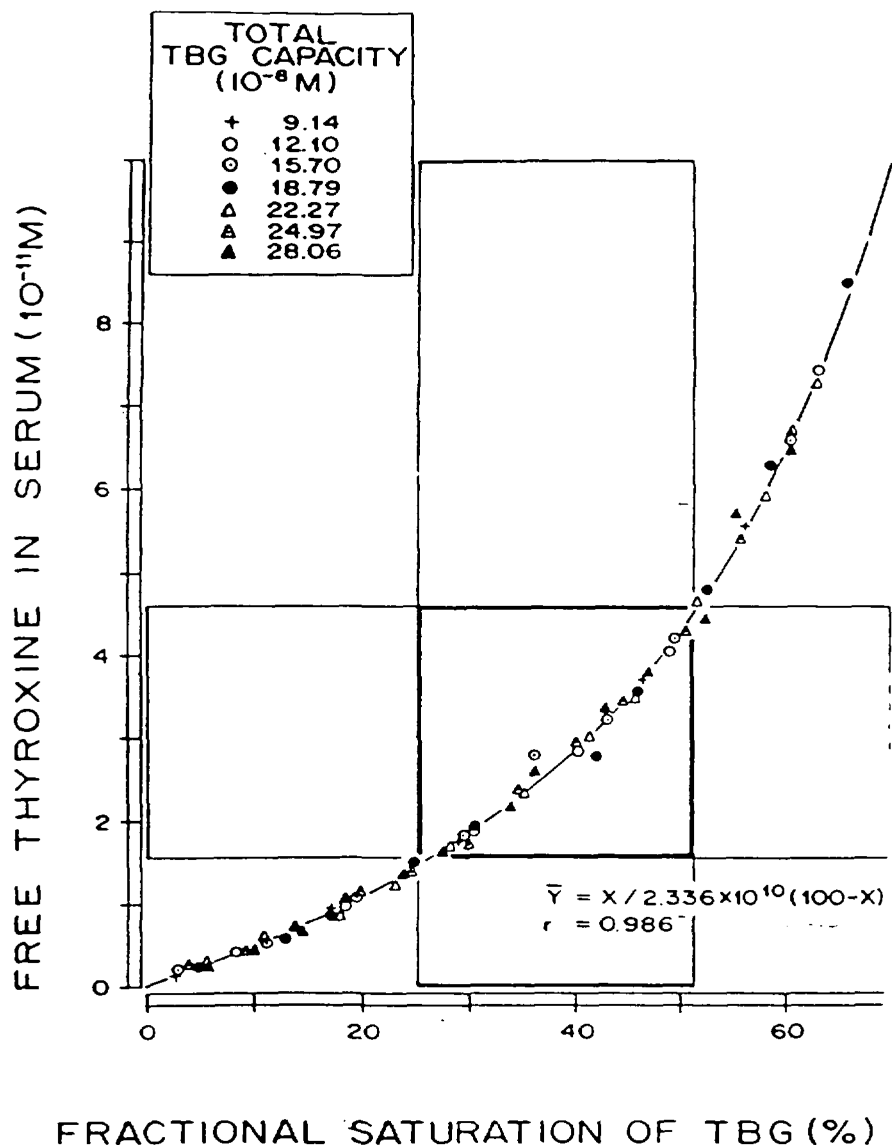

Fig. 17.2 Correlation between the fractional saturation of thyroxine-binding globulin by endogenous thyroxine, calculated from the in vitro thyroid tests (Equation 5), and the concentration of the free hormone in serum measured by equilibrium dialysis.

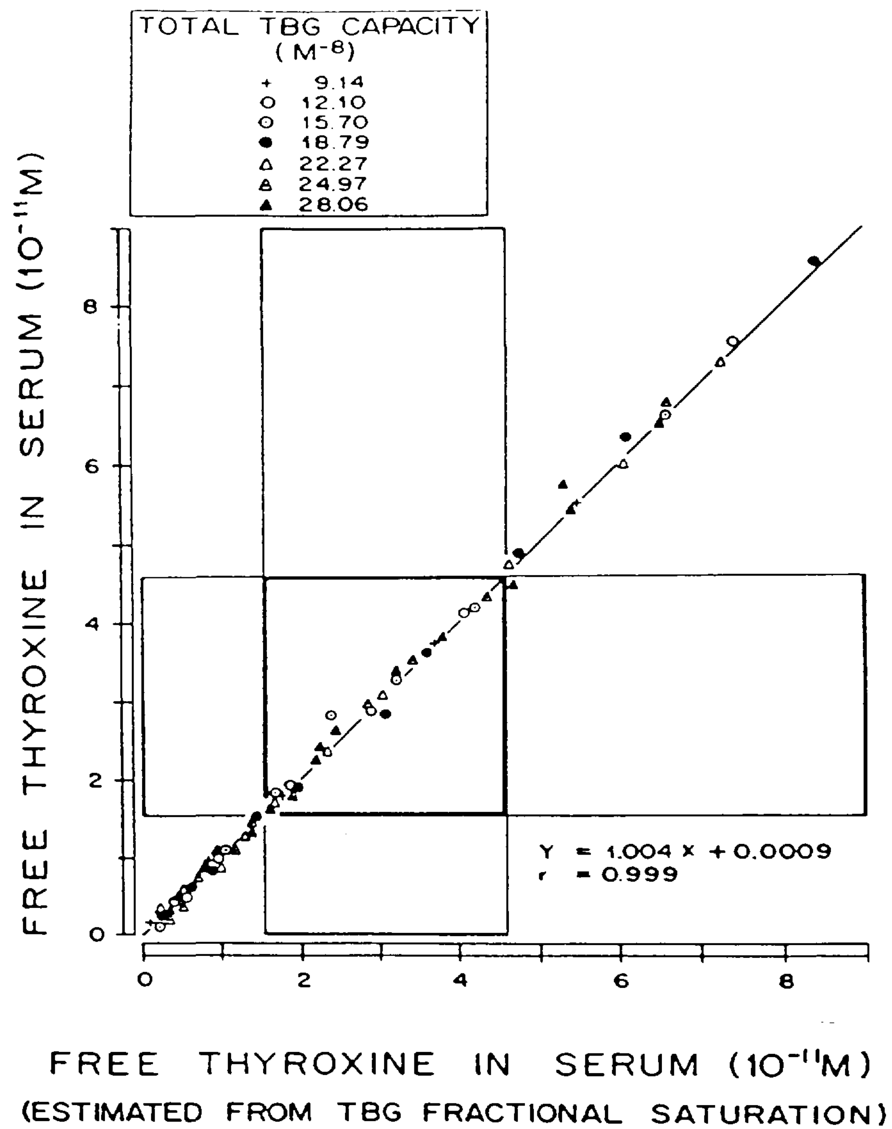

Fig. 17.3 Correlation between free thyroxine concentration in serum estimated from the fractional saturation of the thyroxine-binding globulin by the hormone (Equation 7) and its actual values measured by equilibrium dialysis.

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Chapter 18

DNA PROBES

J. Castelino

Introduction

There is no doubt that radioimmunoassays have contributed a lot to the diagnosis and understanding of many diseases. There are two problems with them:

(a) they use radioisotopic label and

(b) they are difficult to automate because of the separation step, which is indispensable.

To bring the immunoassays in the fold of clinical chemistry, a large amount of effort is now directed towards developing non-isotopic assays.

On the other hand, In vitro nuclear techniques are gradually moving in new directions opened up by modern molecular biology. Two approaches are promising in this respect:

(a) DNA probes and

(b) proteins of biological interest produced by recombinant techniques.

The creation of DNA probes for detection of specific nucleotide segments differs from ligand detection in that it is a chemical rather than an immunological reaction. Complementery DNA or RNA is used in place of the antibody and is labelled with ^{32}P. So far, DNA probes have been successfully employed in the diagnosis of inherited disorders, infectious diseases, and for identification of human oncogenes.

Recombinant techniques have already provided a number of pure human proteins in large quantities including insulin, growth hormone, lymphokines etc. Immunoassays, mostly RIA, have been critical in monitoring therapeutic blood transfusion.

The latest approach to the diagnosis of communicable and parasitic infections is based on the use of deoxyribonucleic acid (DNA) probes. The genetic information of all cells is encoded by DNA and the DNA probe approach to identification of pathogens is unique because the focus of the method is the nucleic acid content of the organism rather than the products that the nucleic acid encodes. Since every properly classified species has some unique nucleotide sequences that distinguish it from every other species, each organism's genetic composition is in essence a finger print that can be used for its identification. In addition to this specificity, DNA probes offer other advantages in that pathogens may be identified directly in clinical specimens; the method does not depend on the expression of an antigen, and organisms that may have undergone spontaneous mutation can still be identified

since single mutations rarely result in a major change in nucleic acid composition. In order to monitor the end result of the DNA probe test, a label is incorporated into the probe. The most common label is the radionuclide ^{32}Phosphorus, that is detected in the end product of the test either by autoradiography or with a scintillation counter.

Clinical problems both diagnostic and therapeutic are being solved using new approaches made possible by DNA techniques. These techniques are over a decade old, yet many clinicians and biomedical personnel are unaware of the potential impact of these techniques on their ability to diagnose diseases rapidly and accurately. During the next decade, these techniques are expected to become the backbone of diagnostic laboratories. Preliminary reports from laboratories already using commercially available DNA probe kits are encouraging. This Chapter tries to introduce these technology to the physicians, describe their potential for medical diagnosis and point out how relevant and practicable they are for the developing countries.

The DNA Molecule

The basis of molecular biology is deoxyribonucleic acid or DNA. DNA is a double-stranded helical molecule composed of pairs of nucleotide bases: adenine (A) guanine (G) thymine (T) and cytosine (C). Within a strand of DNA, the bases are linked by a sugar-phosphate backbone. The DNA molecule resembles a twisted ladder with the A and T and G and C linked together by hydrogen bonds. The two strands can be separated by heat (thermal denaturation) or by raising the pH or lowering the ionic strength of the DNA solution. The DNA molecule is most stable in its native double-stranded state. Thus when single-stranded DNA is placed in solution, under appropriate temperature and salt conditions, the complementary strands will recombine to form a duplex molecule **(Fig. 18.1)**

A region of DNA, which encodes a protein is termed a gene. The genetic information is encoded by a sequence of bases via a non-overlapping code in which three bases (a triplet) determine a particular amino acid. For a gene to be expressed, an enzyme, RNA polymerase II, copies or transcribes one strand of the DNA into mRNA (messanger RNA), which is then decoded or translated by the protein synthesis machinery in the cytoplasm. The mRNA comprises of a single-stranded polynucleotide chain with a sugar-phosphate backbone in which the order of bases is the complement of the transcribed DNA strand of the gene. In RNA, thymine (T) is replaced by a closely related base uracil (U) which will also pair with or hybridize to Adenine (A).

DNA probes, then, are pieces of nucleic acid, labeled in some fashion, that can seek out and bind to stretches of DNA or RNA that have complementary sequences (adenine opposite thymine (or uracil), cytosine opposite guanine. The two strands of nucleic acid must be in contact and have sufficient complementary base sequences so that a stable double-stranded molecule is formed. Complementary sequences of DNA can bind to RNA counterparts. Thus probes may also be labeled RNA strands directed towards DNA targets or labeled DNA sequences directed towards RNA targets.

Nucleic acid molecules will recombine only when the two strands are composed of complementary sequences. It is this property of nucleic acids that is the basis of the DNA or RNA probe reaction. The site of action is shown in **Fig. 18.2.**

The hybridization reaction consists of four components: the probe, the target (which is contained in the sample), the reporter molecule, and the hybridization method. The sample serves as a source of nucleic acid to be analysed and can consist of a suspension of an unknown organism, (for culture confirmation) or a clinical specimen such as sputum, or serum. The nucleic acid in the sample is referred to as the target DNA or RNA, and the radionuclide label on the probe, the reporter molecule.

Designing a Probe

All organisms contain some unique sequences of DNA or RNA within their genome that distinguish them from all other organisms. The key to developing a nucleic acid probe (i.e. either a DNA or a RNA probe) is to isolate these sequences, reproduce them in large quantities, and attach a reporter molecule to them so they can be incorporated into a hybridization reaction. Hybridization is a process whereby two single strands of nucleic acid come together to form a stable double-stranded molecule. As long as the sequences of bases along each stretch of nucleic acid are complementary, they will bind and stay together.

To produce the unique sequences, cloning vectors are used. The most commonly used cloning vectors are Plasmids. These plasmids are co-valently closed circular pieces of DNA that replicate independently of the bacterial chromosome. They are not required for cell replication but often give the host cell some advantage such as antimicrobial resistance, etc. Plasmids range in size from a few thousand base pairs (bp) to as large as 400 000 bp (400 kilobases or kb). One set of plasmids often used in recombinant DNA technology is called cloning vectors. Cloning vectors are small plasmids often just two to five kb that contain a selectable marker such as ampicillin resistance and a stretch of DNA that can be cleaved by many different restriction endonucleases. These are enzymes, found in bacteria, which cut DNA at specific sequences. For example, the enzyme EcoRI cuts the DNA chain between G and A in the sequence GAATTC. Each time a particular DNA is cleaved by an enzyme, precisely the same set of fragments is generated. Many such enzymes are now available.

Examples: pßr322, a 4.6 kb plasmid with ampicillin and tetracycline resistance; pUC18 and pU19 a pair of plasmids with ampicillin resistance, and multiple restriction sites and an indicator system utilizing a ß galactisidase gene where plasmids with foreign DNA inserts produce colourless colonies, while plasmids with no inserts produce blue colonies **(Figs. 18.3 and 18.4).**

The usual method of isolating and reproducing the unique sequence that will become the probe begins by cleaving that stretch of nucleotide bases away from the remaining nucleic

acid in the cell, using a set of enzymes known as restriction endonucleases. These enzymes provide the molecular scissors to cleave DNA at specific sequences of nucleotide bases. For instance, the enzyme Bam H1, obtained from Bacillus amyloliquefaciens, will cleave the DNA molecule between the Guanine - Guanine bases, Hae III, from Haemophilus aegyptius, between guanine and cytosine, and the ECORI, from Escherichia coli, between Guanine and adenine. Endonucleases are named by the first letter of the genus, the first 2 letters of the species, the strain and the number indicates whether it is the first, second etc. enzyme discovered in the organism. The single-stranded segments produced by restriction endonuclease cleavage are referred to as sticky ends because they can recombine with any other piece of DNA that has been cleaved with the same enzyme, regardless of the source of that DNA. Thus DNA from a virus such as herpes simplex (HSV) type 2 can be cleaved and inserted into a small plasmid from E. coli if the plasmid has been cleaved with the same enzyme. The sticky ends are then sealed with a second enzyme known as DNA ligase, to produce a double-stranded circular molecule and introduced into E. coli by transformation (addition of $CaCl_2$). The plasmid containing the DNA insert of HSV type 2 will replicate in E. coli making hundreds of copies. This process is referred to as cloning. In the simplest terms, cloning is the process of isolating a piece of DNA and placing it in a vector that allows hundreds of copies of that DNA sequence to be produced, when the plasmid replicates **(Figs. 18.5 and 18.6)**.

The plasmid now greatly amplified in copy number can be purified from the bacterial cell by centrifugation or filtration or by column filtration. The plasmid can be labeled directly by random primer method or the foreign DNA sequence isolated by restriction endonuclease digestion followed by centrifugation and the probe labelled by nick translation.

The unique sequence that will constitute a diagnostic probe need not be a whole gene nor need it be from within a sequence that actually encodes a protein.

Hybridization Reactions

After a double-stranded DNA molecule is denatured to single strands, it is capable of reassociating with either a DNA or a RNA strand of complementary sequence. The degree and specificity of binding depends on temperature, pH, use of a denaturant such as formamide, and salt concentration of the reaction buffer. Nucleic acid molecules can tolerate a certain number of mismatched base pairs (such as adenine molecules lining up opposite cytosine or guanine molecules opposing thymines) and still form stable duplexes as long as a significant number of base pairs do match and form bonds. However, the greater the degree of mismatched bases along the strands of nucleic acid, more likely that the two molecules would come apart. The degree of mismatch that can be tolerated in a hybridization reaction and still maintain a double-stranded molecule (and produce a positive hybridization signal) is referred to as the "stringency" of hybridization.

The concept of stringency is very important in understanding the specificity of DNA probe reactions. If the salt concentration or temperature of hybridization is altered, the specificity of the probe will change. The range of conditions that can be tolerated without

affecting the specificity of a probe vary depending on the length of the probe and the percentage of guanine and cytosine residues in the probe. The shorter the probe, the more narrow the range of temperature and salt concentration that can be tolerated.

Under conditions of high stringency, only exact matches of DNA will anneal and stay together. Under conditions of low stringency (i.e. reactions carried out at low temperature, in high salt concentration, or in low concentration of formamide) two DNA strands that are only 80 to 90% homologous may bind together and result in a positive hybridization signal. For example, under conditions of high stringency, a probe developed to Campylobacter jejuni would hybridize only to DNA from that organism while under conditions of low stringency the same probe would bind to DNA from Campylobacter coli and C. fetus but still not bind to DNA from E. coli or Shigella flexneri.

Formats for Hybridization Reactions.

Hybridization reactions can be performed in four formats: on a solid support (filter), in solution, in situ, or by using the Southern Blot hybridization procedure after gel electrophoresis. The majority of DNA probes reported in the literature have used the solid support format. However, both solution and solid support formats are utilized by the new commercially available probe kits.

To carry out the hybridization using the solid support format, the sample, which can be purified DNA, a suspension of a microorganism isolated in culture, or a clinical specimen, is either spotted directly on the filter or placed in a vacuum manifold which concentrates the specimen in a small area. The sample is lysed and the DNA denatured by the addition of NaOH or by steaming the filter above a beaker of ammonium acetate. Acid-fast organisms may require pretreatment with anti-microbial agents or other reagents to aid in disrupting their cell wells. Once denatured, the DNA is attached to the filter by baking it in a vacuum oven at 80°C for two hours. The filter is prehybridized with a non-homologous DNA such as salmon sperm DNA or calf thymus DNA, to prevent the non-specific binding of probe to the filter. After hybridization, the filters are washed at various temperatures determined by the stringency of the reaction. Although nitrocellulose filters are traditionally used for such assays, Whatman No. 541 paper and synthetic nylon filters are also frequently used **(Figs. 18.7 and 18.8)**.

The second format is to carry out the hybridization reaction in solution. In this format, both the target and the probe nucleic acid are free to move, maximizing the chance that complementary sequences will align and bind. Solution hybridizations go to completion 5 to ten fold faster than on solid supports. In several of the commercially available solution hybridization reactions samples are incubated at high temperature (72°C) in a sonicating water bath to disrupt the cells and cause them to release their nucleic acids. The addition of glass beads to the sample often aids in the disruption of the cell wall. After the hybridization step, the nascent duplexes are removed from solution by the addition of hydroxyapatite, which selectively binds duplex nucleic acid leaving single-stranded nucleic acids in solution. In some procedures the duplex DNA is removed from the hydroxyapatite by increasing the salt

concentration or by heating, while in other procedures the hydroxyapatite-bound doulbe-stranded nucleic acid is collected by centrifugation and the pellet is washed and quantitated by scintillation counting.

Polymerase chain reaction (PCR)

At times the amount of target nucleic acid present in a sample may be extremely small and thus beyond the limits of detection for conventional hybridization reactions. The recent development of the polymerase chain reaction is one of the most substantial technical advances in molecular genetics in the past decade. The procedure amplifies DNA by chemical proliferation in vitro of a predetermined stretch of DNA. It is possible to amplify specific DNA sequences, from as short as 50 base pairs to over 2000 base pairs in length, more than a million fold in only a few hours.

The PCR method was developed by Saikai and his co-workers at Cetus Corporation. It is based on the repetitive cycling of three simple reactions, the conditions of which vary only in the temperature of incubation. All three reactions occur in the same small tube and with temperature stable reagents. The repetitive cycle is therefore self contained and fully automated.

In addition to the target DNA to be amplified, the important reagents are two single stranded oligonucleotide (Primers), synthesized to be complementary to known sequences of the target DNA; larger amounts of the four deoxyribonucleoside triphosphates, and the heat stable Taq DNA polymerase, isolated from the thermophilic bacterium Thermus aquaticus.

The first step in the procedure is the heat denaturation of native (target) double-stranded DNA, which can be used virtually straight from any clinical, laboratory, or forensic specimen. The target DNA melts at high temperature (90 - 100°C) to liberate single-stranded DNA, which can subsequently reanneal to any other DNA that has complementary sequences. Recent experience suggests that amplification may begin in a sample containing only a single target molecule of DNA, making the PCR the most sensitive detection technique for specific DNA sequences.

In the second step of the cycle, performed at reduced temperatures, two short DNA primers are annealed to complementary sequences on opposite strands of the target DNA. These primers are chosen to encompass the desired genetic material; they define the two ends of the amplified stretch of DNA. The two primers must not anneal to each other and their sites of annealing must be sufficiently distant from each other to allow the subsequent synthesis of new products. The specificity of the PCR method is derived from the precision of this DNA-DNA annealing reaction.

The cycle's third step is the actual synthesis of a complementary second strand of new DNA, which occurs through the extension of each annealed primer by Taq polymerase in the presence of excess deoxyribonucleoside triphosphates. A new single strand of DNA is synthesized for each annealed primer. Each new strand consists of the primer at its 5' end

trailed by a string of linked nucleotides that are complementary to those of the corresponding template. An essential feature of the PCR procedure is that all previously synthesized products act as templates for new primer extension reaction (i.e. DNA synthesis) in each ensuing cycle. The result, aptly named "chain reaction" is the geometric amplification of new DNA products **(Fig. 18.9)**.

Since the primers form the kernals of all new DNA strands, each of the two different primers, as well as the four deoxyribonucleoside triphosphates must initially be present in massive amounts relative to the quantity of target DNA.

After extension of the primers, the cycle is repeated first by raising the temperature to convert double-stranded to single-stranded DNA, and then by lowering the temperature to allow the steps of annealing and extension.

The success of the procedure depends on knowing the sequence of the target DNA, at least at the site of primer annealing to allow the synthesis of appropriate complementary primers. Synthesizing the olignucleotide primers is itself an automated procedure and relatively inexpensive. If a RNA sequence is to be amplified a cDNA copy of it must be synthesized first using reverse transcriptase, before the PCR is begun.

A PCR reaction cycle typically takes five to seven minutes and is repeated 30 to 40 time to give a million copies.

Oligonucleotide Probes

Some of the currently available probe kits utilize very small stretches of nucleotides as probes. These short probes (oligonucleotide probes) are synthesized de novo in the laboratory on one of the several instruments designed for this purpose. Normally, only 14 to 40 base pair in length, these probes display exquisite specificity. Under stringent conditions they may be capable of detecting a change in a single base pair of a DNA or RNA sequence, which is enough to prevent binding of the probe to the targets.

Olinucleotide probes are very stable over time, and relatively simple to prepare. One batch of oligonucleotide probes is often enough to last in a laboratory for several years since only picogram amounts of probe are required for hybridization. Because of their small size and low complexity (base ratios) these short probes hybridize to target DNA at very rapid rates often with reaction times of less than 30 mins. This is in contrast to long probes that often require 4 to 16 hours.

The disadvantage of short probes is that each oligonucleotide can be labelled with only a single reporter molecule. Thus they are often 10 to 100 fold less sensitive than long probes.

Labelling Probes

Traditionally the most commonly used detection system is the ^{32}P label directly incorporated into the probe by nick translation. Other methods and other labels may also be used (Table I).

TABLE I: COMMON RADIOLABELS FOR DNA PROBES

Isotope	Emission Type	Energy	T½	dpm/mol (100% isotopic enrichment)
^{32}P	ß	1.71 MeV	14.3 d	2.02×10^{19}
^{125}I	γ	0.035 MeV	60 d	3.94×10^{18}
^{3}H	ß	0.0181 MeV	12.3 d	6.39×10^{16}
^{35}S	ß	0.167 MeV	87.4 d	3.33×10^{18}

Nick translation

Incorporation of labelled bases into long probes by nick translation involves nicking one of the two strands of double-stranded DNA with DNASE 1, and then excising stretches of the single strands at the nicks with the 5'-3' exonuclease activity of E. coli DNA polymerase I. In the process the polymerase enzyme also adds nucleotides to the 3' OH. The native bases are replaced with labelled bases in the reaction mixture as the nick moves along the DNA strand. When ^{32}P-labelled bases are incorporated, specific activities of 5 x 108 dpm/μg can be obtained with labelled strands 400 to 800 nucleotides in length. Probes synthesized with ^{3}H labelled bases have specific activities of 0.5 x 108 to 1.5 x 108 dpm/μg. Labelling by nick translation is a fairly simple reaction resulting in uniformally labelled probes with high specific activity (**Fig. 18.10**).

Random Primer Method.

This method is based on the hybridization of the DNA to be labelled with a mixture of all the possible hexanucleotides. The complementary strand is synthesized from the 3' end of the primer hexanucleotide by the Klenow fragment of E. coli DNA polymerase I. The unique sequence need not be separated from the plasmid. The plasmid DNA containing the unique sequence to be used as a probe is heated to separate into single-stranded DNA. The solution is then placed on ice and two to five μl of random primers are added. These primers will anneal to portions of the two single-stranded circular DNAs. Then 5 μl of ^{32}P dATP and unlabelled dCTP, dGTP and dTTP are added with DNA polymerase I. The primers thus extend and a new strand of DNA is produced incorporating the ^{32}P label. The unbound ^{32}P dATP is removed by passing through a spin column (a small sephadex bead column placed on top of a centrifuge tube and spun). The DNA comes through the ^{32}P dATP and other triphosphates remain on the column.

The labelled plasmid DNA is boiled shortly before the hybridization reaction and a drop placed on the spotted target DNA on nitrocellulose paper.

Detection Systems

Autoradiography

The extent of hybridization with radiolabelled probes on filters and in situ is monitored with autoradiographic methods. The procedure is sensitive with good resolution and does not destroy the hybridization support matrix. It lacks the exact quantitative ability of liquid scintiliation methods, but it can reveal artifacts not seen in counting.

The hybridized filter is exposed to X-ray film (Kodak XAR 5 or X-OMAT AR) in a light tight cassette. Exposures range from hours to days, depending on the level of radioactivity present. In general, an area of a dot 3 mm across with about 100 dpm of ^{32}P. The sensitivity of the method can be increased with the use of intensifying screens (Du Point Cronex Quanta III, Cronex Lightning Plus, or Fugi Mach II) by eight to ten fold with two screens.

The film, placed between the hybridized filter and screens, is exposed at -70°C in order to prolong the fluoresence emitted in response to a decay event. As little as 5dpm/7mm^2 area can be visualized overnight.

Scintillation Counting

If the hybridized support is sufficiently radioactive, the area of interest can be cut out and counted in a liquid scintillation counter.

Probes in a Diagnostic Laboratory

Current DNA probes require one to three hours to complete. This is still considered an advantage when compared to culture methods that may take four to six weeks. Probes enable the direct detection of pathogens in clinical specimens although often not the sensitivity of the pathogen to drugs. In certain cases, e.g. P. falciparum and S. mansoni, Neisseria gonorrhoeae sensitivity to pyrimethamine, oxaminiquine and pencillin can be determined by using probes designed for this.

Tests using probes when used in a batch format are relatively inexpensive, although use of probes for individual specimens can be expensive (**Table II**).

TABLE II. COMPARISON OF COSTS FOR 5 AND 50 SAMPLES IN THE FILTER HYBRIDIZATION ASSAY FOR CAMPYLOBACTER JEJUNI			
Cost Factor		**Cost per**	
		5 samples	**50 samples**
DNA probe kit	($ 16)	3.20	0.32
Reagents	($ 5)	1.00	0.10
Labour	($ 19.50)	3.90	0.39
Total		8.10	0.81

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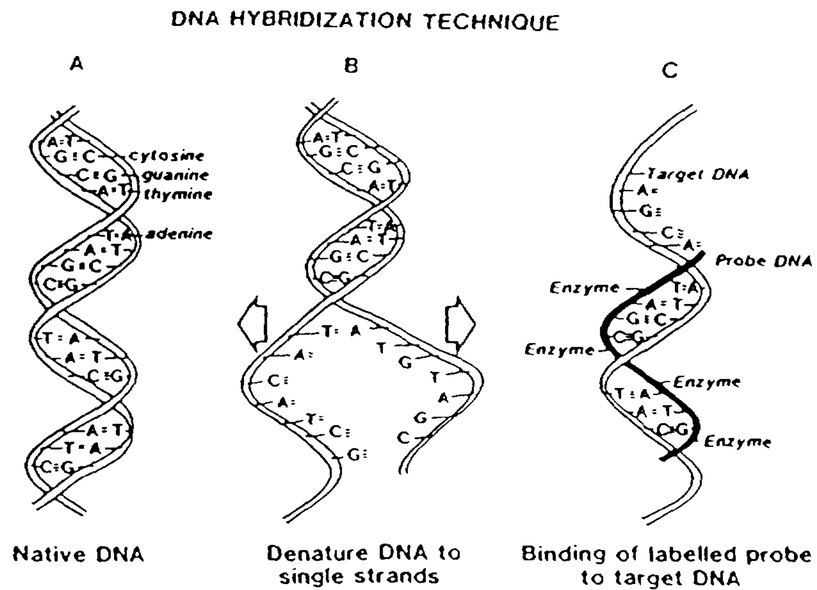


Fig. 18.1 Structure of deoxyribonucleic acid, effects of denaturation and binding of probe to target DNA [from Tenover, F.C., Clin. Microbiol. Rev. 1, 83 (1988)].

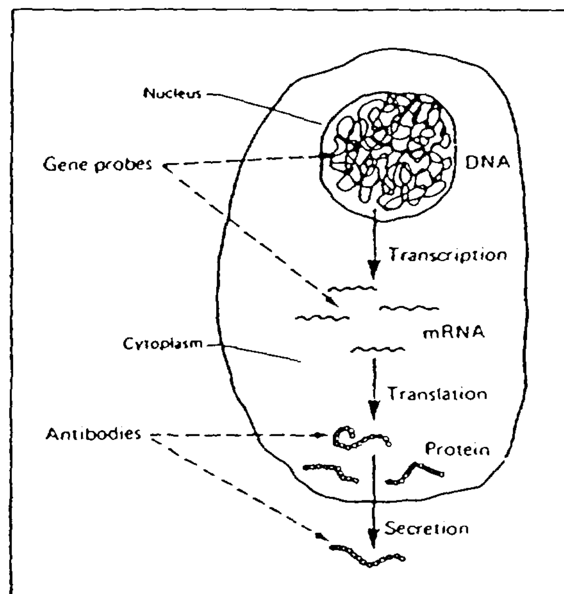


Fig. 18.2 Site of action of gene probes.

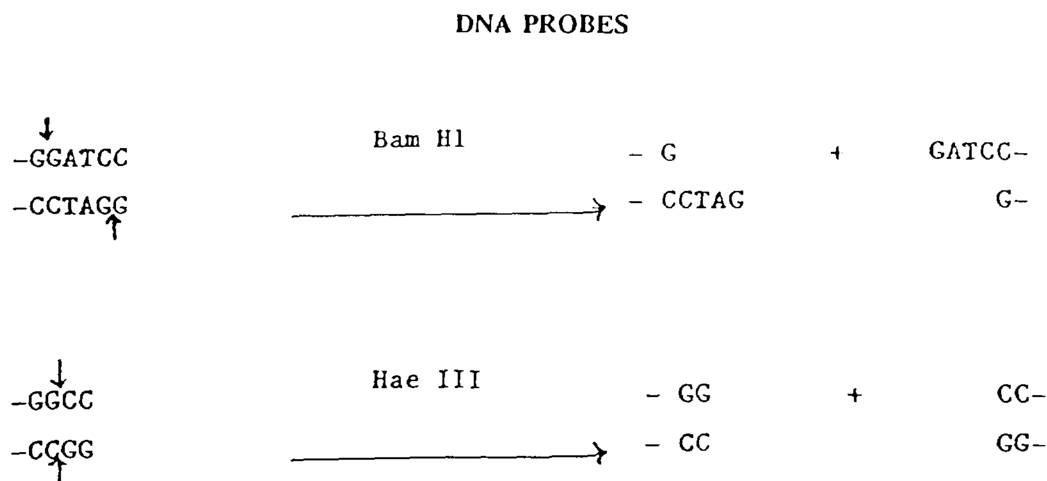


Fig. 18.3 Sequences cleaved by the restriction enzymes Bam H1 and Hae III.

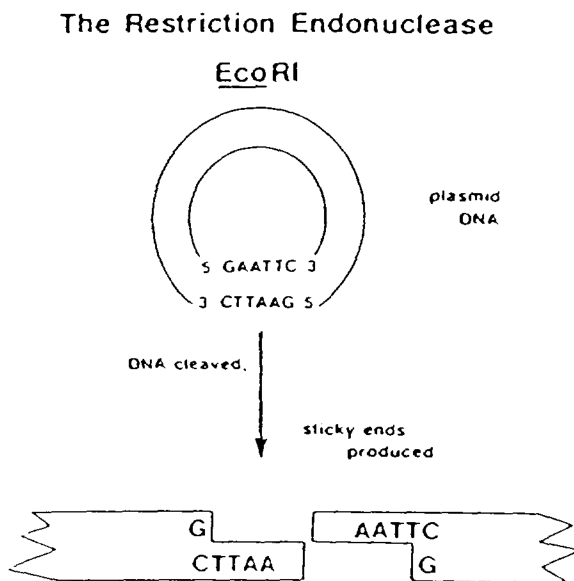


Fig. 18.4 The restriction endonuclease Eco RI. [From Tenover, F.C., Clin. Microbiol. Rev. 1, 83 (1988)].

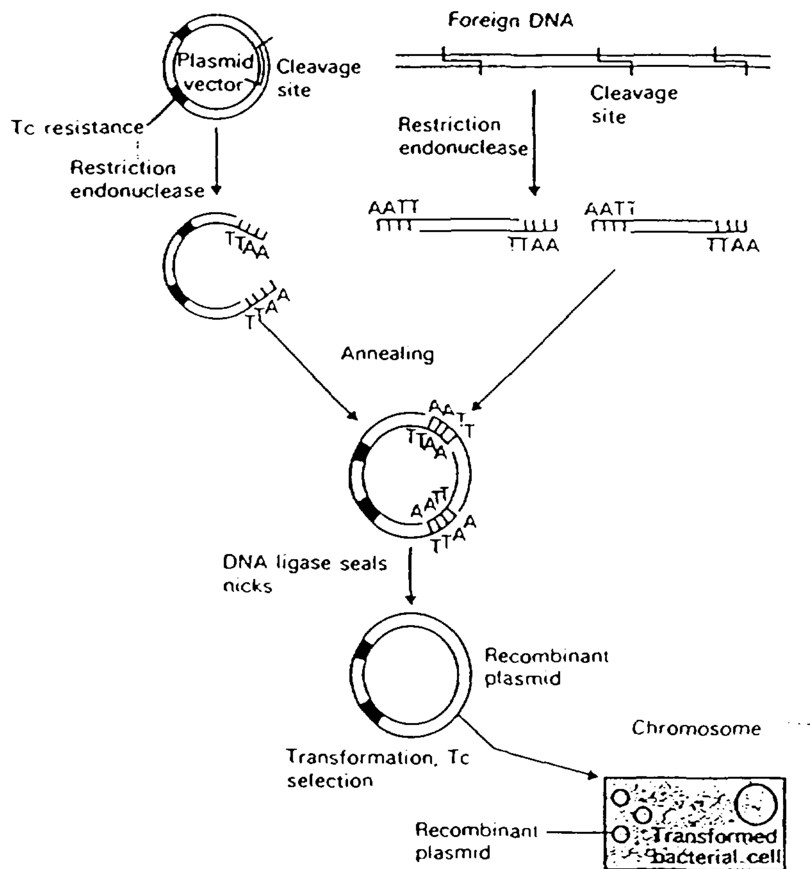


Fig. 18.5 Use of DNA ligase to create a covalent DNA recombinant joined through association of termini generated by Eco RI.

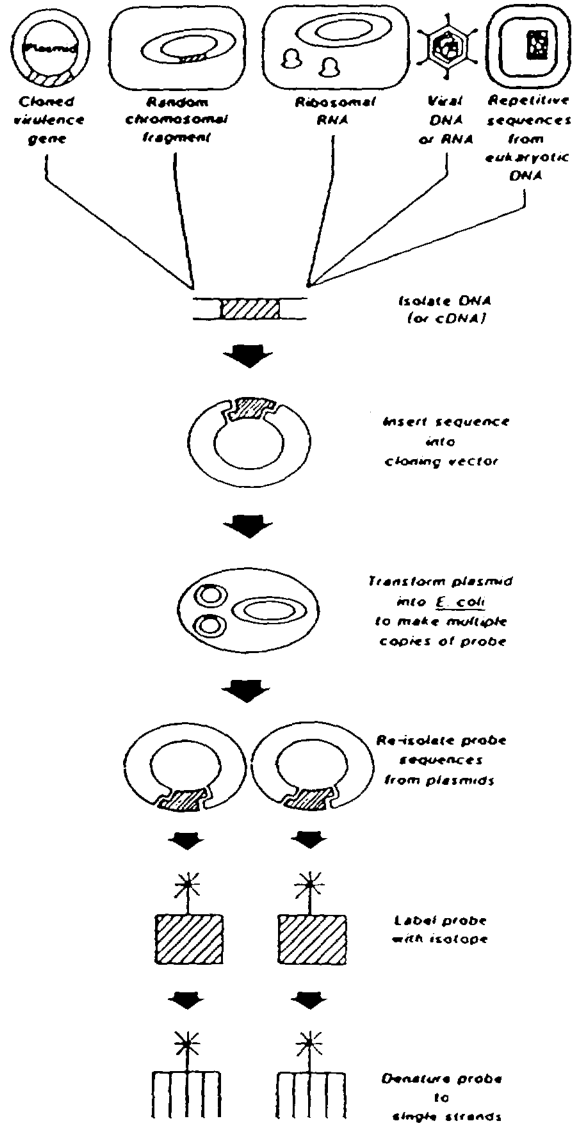


Fig. 18.6 The development of probes from diverse infectious agents. [From Tenover, F.C., Clin. Microbiol. Rev. 1, 83 (1988)].

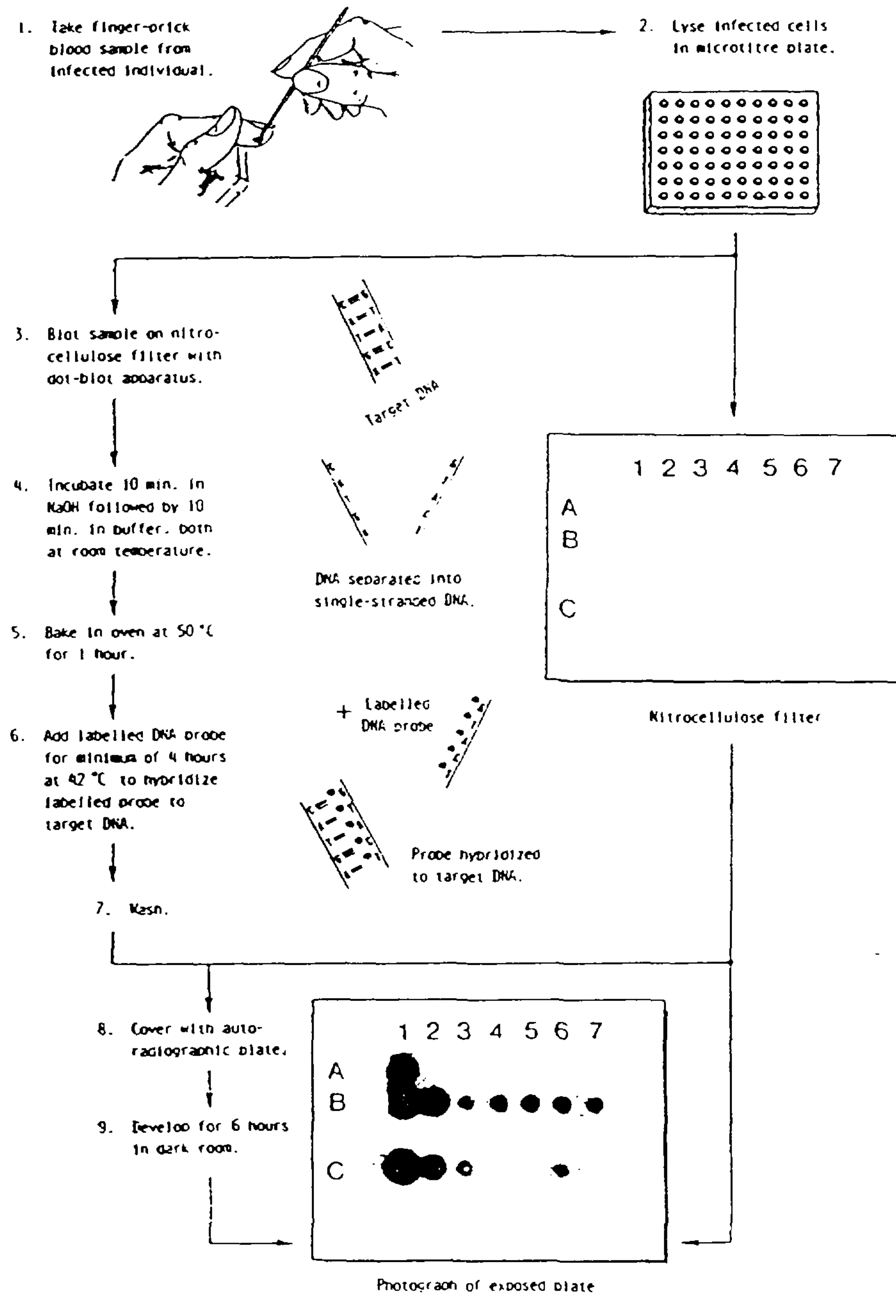


Fig. 18.7 Method used for filter hybridization of DNA probes to P. falciparum DNA.

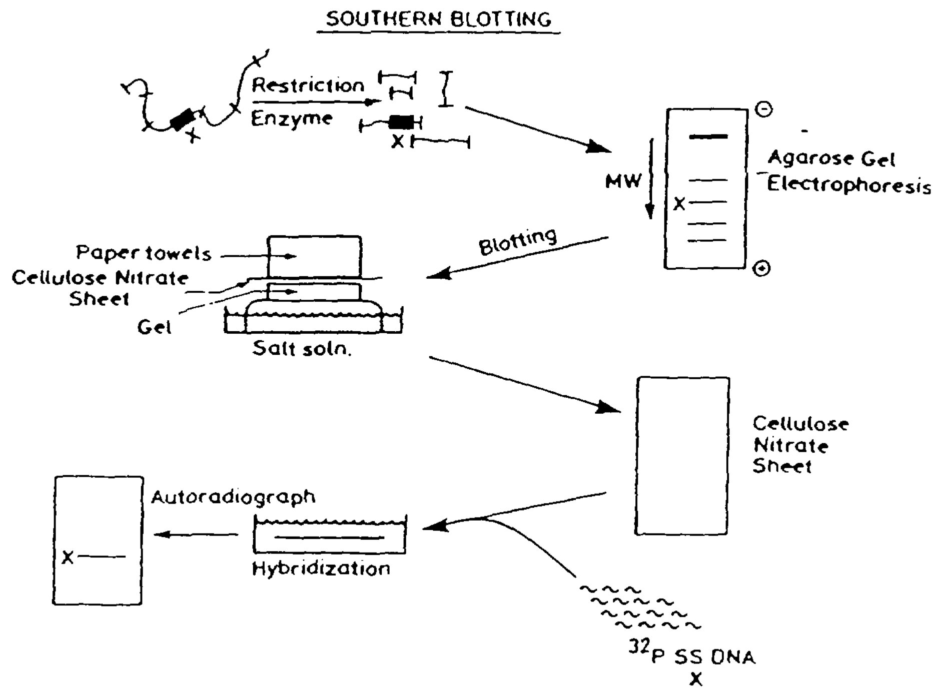


Fig. 18.8 Technique of southern hybridization.

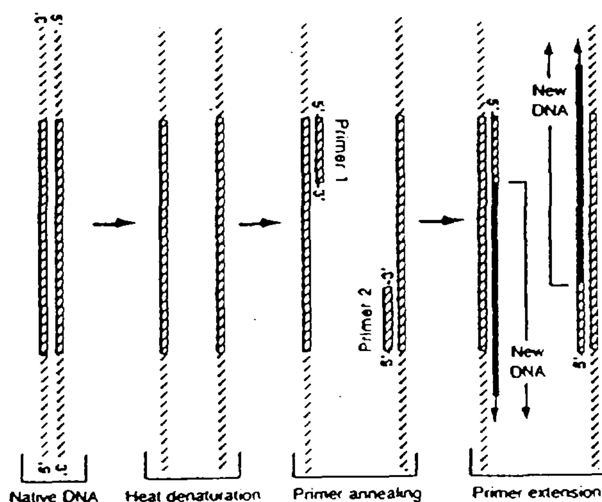
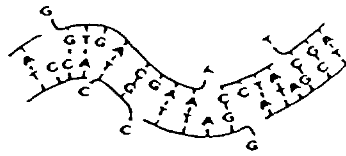
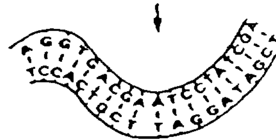


Fig. 18.9 First Round of the Polymerase Chain Reaction. The basic polymerase-chain-reaction cycle consists of three steps performed in the same closed container but at different temperatures. The elevated temperature in the first step melts the double-stranded DNA into single strands. As the temperature is lowered for the second step, the two oppositely directed oligonucleotide primers anneal to complementary sequences on the target DNA, which acts as a template. During the third step, also performed at a lower temperature, the Taq- polymerase enzymatically extends the primers covalently in the presence of excess deoxyribonucleoside triphosphates, the building blocks of new DNA synthesis. The native DNA target sequences, which will be massively amplified as "short products" in the ensuing cycles, are boxed. The vector of action of the DNA polymerase is denoted by the arrows projecting from the newly synthesized DNA, indicated by the dark bars.



DNA nicked or broken with deoxyribonuclease

^{32}P-labelled nucleotides A CGT added
with DNA polymerase I



NICKS repaired with new nucleotides replacing damaged ones

Fig. 18.10 Nick translation to make a DNA fragment sequence highly radioactive.

CHAPTER 18

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Chapter 19

DNA TECHNOLOGY FOR DIAGNOSIS AND VACCINES FOR INFECTIOUS DISEASES

N.K. Notani

Introduction

As far as the infectious diseases are concerned the outlook for the developing countries looks grim. In India, for instance, during last few years, there have been at least three killer epidemics - from encephalitis virus, leishmania and malarial parasite. Besides, leprosy is also endemic in certain parts of the country. These four infectious diseases have been chosen for discussion either because these are currently the major health problems or they have some interesting features making them attractive for research.

All the four diseases have a complex life history and some of the infectious agents have more than one host for multiplication. Secondly, these agents interact with the human system in a very intricate way. A useful generalization may be to bear in mind that on an average a viral genome may code for about a dozen to 300 genes, a bacterial genome for 2000 or so genes and a eukaryotic genome from 4000 genes and up. On the other hand, mutations may occur with a very high frequency, as for example, they do in AIDS virus and influenza virus - which creates new problems for diagnosis, vaccines and drug design.

Three or four general strategies are adopted for the control of infectious diseases. Early diagnosis, vaccination and chemotherapy. In the situations where there is transfer through mosquitoes or ticks from alternate hosts, control of the vector and of the infection in the alternate host are additional measures to be taken. This Chapter looks at the problems of disease control from the perspective of genetics, since molecular genetics now provides powerful tools in the form of radiolabelled DNA probes and clones of selected segments, useful for diagnosis as well as for vaccine design.

Molecular techniques in DNA diagnostics

DNA (Southern) blot technique is a very versatile one. In this technique, DNA is first digested with a restriction nuclease which may yield a number of fragments but of fixed size. These can be separated by agarose gel electrophoresis and blotted on to a membrane-filter. These are denatured and form the target sequence for a ^{32}P-labelled or S-labelled DNA probe. The probe or the detector DNA molecule is usually a segment of cloned DNA. Its hybridization with the target molecules indicates the presence of sequences homologous to the probe. Sensitivity of detection is so high that the DNA of a single hair or a small volume of blood, lysed and bound to filter membrane, can be used as the target for diagnostics.

DNA sequence analysis is accomplished either by the so-called dideoxy-nucleotide method or by chemical breakage induced at specific nucleotide sites. In the dideoxy method, a synthetic end-labelled oligonucleotide is used as a primer. Four different chain-extending reactions are set-up, each containing the identical primer and the template to be sequenced mixed with three deoxynucleotides mixed with one of the chain-terminating

dideoxynucleotides. When separated on polyacrylamide gels by electrophoresis, chain lengths are separated lengthwise differing in a single nucleotide length forming a kind of zigzag ladder from which the sequence can be read after autoradiography.

Japanese Encephalitis (JE)

It has a fairly wide distribution and is prevalent in Asia, southern USSR, much of China, Japan, Taiwan, Philippines and India. It has a mortality rate of 8%, neurological residual effect on 30% and persistent mental disturbance in 10% of the cases.

JE virus belongs to Flavivirus arthropod - borne virus group B. This group includes also the Dengue and Yellow Fever virus. These viruses can replicate both in invertebrate (mosquito/tick) and vertebrate (man) hosts. It is a RNA virus. The invertebrate host of the JE virus is generally a species of Culex mosquito. Man - mosquito - man cycle probably does not occur on any significant scale, and although swine are implicated as an alternate host, it has been proposed that the 'Basic Cycle' goes through birds in India. The inactivated Nakayama strain of the virus grown in mouse brain, is used as a vaccine of which only about two million doses are being produced in India. Inactivation with radiation of JE virus is being tried at present.

Leprosy

The prevalence worldwide of leprosy has been estimated to be between ten and twelve million cases, the disease being endemic in Asia, Africa and Latin America. The causative organism *Mycobacterium leprae* can develop resistance to the commonly used chemotherapeutic drug Dapsone, and thus a vaccine would be useful. Although both humoral and cellular immune responses to the infection occur, cellular immunity apparently determines the outcome of the infection. In lepromatous leprosy patients, there is a specific deficit in Cellular Immune Response (CIR) which then allows an unlimited replication and spread of the mycobacteria. Tuberculoid leprosy patients suffer from enhanced CIR resulting in the destruction of nerve endings.

An important 65 kD antigenic protein of *M. leprae* has been characterized. It is somewhat similar to heat shock proteins. This protein contains an *M. leprae* B cell epitope and a number of T cell epitopes. A HLA-DR2 restricted, *M. leprae*-reactive T cell clone 2F 10 has been isolated from a human tuberculoid leprosy patient which seems to proliferate in an in vitro situation specifically in response to *M. leprae*. At low concentrations only Dharmendra lepromin or Armadillo- derived *M. leprae* induce a good proliferation response. Only *M. vaccae* at high concentration induces a fair response. None of the other 18 mycobacterium species tested including *M. tuberculosis* induced any response.

In order to characterize the epitopes involved in inducing this response, 65 kD protein of *M. leprae* produced from *E. coli* was tested. It gave a good response along with Armadillo-derived *M. leprae*. However, 65 kD protein from *M. bovis* BCG from *E. coli* gave no response. Sequence of the minimal peptide (the so-called peptide 17) for stimulation

of clone 2F, 10 has been determined and shown to be L Q A A P A L D K L. It is flanked by leucine at either end and omission of either leucine results in the loss of stimulatory activity. A specific monoclonal antibody III E9 also inhibits this activity. Similarities have been noted between peptide 17 and an HLA DR2 peptide. It is from the third hypervariable region of the DR2 B3 chain that stimulates proliferation of 2F10 although with a 100-fold lower efficiency. DR2 allele is apparently present only in about one third of most populations. Its role in pathogenesis of tuberculoid leprosy is suggestive.

Leishmaniasis

Leishmaniasis is caused by at least 14 different species or subspecies of leishmania. There are three types of leishmaniasis:

(a) simple cutaneous, which is often self-limiting,

(b) mucocutaneous, involving destruction of nasal tissue and

(c) visceral disease, a systemic infection which is fatal if not treated. The parasite is transmitted by a sandfly vector. Generally the diagnosis is done by direct examination of tissue biopsy or by a delayed type of hypersensitivity reaction (Montenegro test). Direct treatment requires that the strain is identified.

DNA probes are useful in that first of all it eliminates the need for culturing of the parasite. The major new world species of leishmania can be distinguished by DNA probes of total kinetoplast DNA (kDNA). Sample from the lesions can be directly applied to a nitrocellulose filter and processed further. In a comparison of tests (Montenegro, DNA hybridization, culture and histopathology), DNA hybridization test was more sensitive than either culturing or histopathology tests. It was positive in about 80% of the patients detected by Montenegro test. Montenegro test is an immunological test. So there is a possibility that it is positive even in patients with previous infection or cross-reacting antigen. On the other hand, kDNA probes used may not be appropriate for all patients. For the old world leishmania, even the species cannot be distinguished. Subcloning or some other constructs may help in distinguishing old world species and subspecies. Sandflies can be directly squashed on nitrocellulose filters and checked for the presence of leishmania by suitable DNA probes.

Malaria

Malaria parasite *Plasmodium falciparum* has a complex life cycle with stages in the female anopheles mosquito and in the liver and blood (erythrocytes) of the intermediate host (man). Symptoms are caused by asexual parasites and these proliferate in erythrocytes. Sporozoites released by mosquito in the human system enter hepatocytes and form schizonts

which release merozoites into the blood. Merozoites infect erythrocytes. Merozoites multiply in erythrocytes and rupture them. Some of these differentiate into male and female gametocytes. These are picked up by the mosquito and these develop into male and female gametes. Fertilization occurs and the zygotes develop into ookinetes which invade the gut where sporozoites form within oocyte. Sporozoites next migrate to the salivary gland of the mosquito and will infect humans during the next blood meal.

The detection of malaria is possible by microscopic examination of a blood smear. This is adequate for diagnosing acute malaria but is time-consuming and requires a trained technician when low numbers of parasites are present. ELISA is being developed but has the disadvantage that antibodies might be present even after disappearance of the malarial parasites.

DNA probes have been developed which detect the current infection in the blood. Even parasite density is measurable and the method is adaptable to field use. Blood sample obtained by a digital puncture can be directly applied to nitrocellulose filter.

A *Plasmodium falciparum* genomic DNA library has been constructed which was screened by colony hybridization using nick translated *P. falciparum* DNA. From one thousand colonies, seven were selected for their hybridization intensity. DNA of these was digested, electrophoresed and transferred to nitrocellulose. Filters were probed either with human DNA or *P. falciparum* DNA. pPF14 showed intense hybridization suggesting the presence of highly repeated DNA sequences. *P. falciparum*, *P. vivax* and *P. cynomolgi* DNAs were all probed with pPF14. Of these, only *P. falciparum* hybridization was positive, indicating specificity of the probe. The detection level was down to 10 pg which is equivalent of about 100 malarial parasites. It is estimated that whereas one microscopist can read 60 slides per day, with DNA hybridization, almost 1000 samples can be examined per day.

Making of malarial vaccines has been greatly facilitated by the development of technology for culturing of *P. falciparum.* Antigens involved in protective immunity could be identified, and the immunity is both species- and stage-specific. Several ideas for making vaccines have been tried, three of which are discussed. The first one is a vaccine prepared by synthesis of a peptide consisting of four amino acids Asp - Ala - Asp - Pro (NANP) repeated three times. It is a dominant epitope of *P. falciparum* and monoclonal antibodies to sporozoite react with it.

$(NANP)_3$ sequence is repeated 37 times in circumsporozoite (CS) protein of Brazilian *P. falciparum* but only 23 times in Thai *P. falciparum.* This vaccine is being tried in humans in Africa.

Yet, another potential vaccine has been prepared by fusing the immuno - dominant repetitive epitope of CS protein to the pre-S2 region of the surface antigen of hepatitis B virus. Such hybrid proteins assembled into particles induce a high-titre antibody response.

Finally, a model oral vaccine has been prepared and shown to induce cellular immunity in mice. A *Salmonella typhimurium* WR 4017 avirulent strain with impaired ability to multiply in macrophages is transformed with plasmid pMGB2 which consists of a plasmid with *P. berghei* CS protein expressed constitutively. Female BALB/c mice 6-8 weeks old were immunized by the subcutaneous (10^4 bacteria) or oral (10^9 - 10^{10} bacteria) route. Only subcutaneous treatment induced low levels of antibodies at four weeks but not at five weeks. Mice that were given bacteria orally made no antibodies. Mice challenged between four to nine weeks with sporozoites (apparently) induced cell immunity. Thus, this technique is also important for T-cell epitope identification.

Comments

Life cycle, DNA diagnostics and potential vaccines has been reviewed for four infectious diseases. These are Japanese Encephalitis, leprosy, leishmania and malaria. They are quite common in many developing countries and need the attention of researchers and health authorities. There is very little or no characterization of pathogenic strains that cause encephalitis. Vaccines can be effective only when these are specific to the particular strain. Thus, initial effort on a large-scale should be directed towards identification and characterization of pathogenic strains.

Leishmania is currently raging in North India. Although, the new world species can be distinguished by kinetoplast DNA (kDNA) probes, this is difficult for the old world species. Probably a set of subclones of kinetoplast DNA or RFLP (Restriction Fragment Length Polymorphism) analysis of genomic DNA may be of help.

Research on malaria is quite advanced. Specific probes like pPF14 are already available and perhaps that identify drug resistance strains probes can also be made. The malaria parasite's epitopes consisting of repetitive amino acid sequence are intriguing. Their number can vary as has been shown for Thai and Brazilian strains. The significance of this is not clear nor is the mechanism of generation understood.

With regard to malaria vaccines, perhaps the induction of cell - mediated immunity may turn out to be more efficacious. Although there are two other vaccines, viz. synthetic and as fused protein, their usefulness remains to be demonstrated. The mouse model system has the added advantage that some antigen genes may be introduced to make transgenic mice and their expression seen.

Leprosy is endemic in quite a few developing countries. Recent researches have provided new insight that cell-mediated immunity may be more important. Specificity of peptide 17 (or a ten amino acid epitope of it) of 65 kD protein in inducing proliferation of T-cell 2F10 cell line derived from tuberculoid leprosy patients suggests that for diagnosis, DNA sequence corresponding to it may provide a useful probe. Because ten other mycobacterium species failed to stimulate the proliferation of 2F10 cells, it may be assumed that it will also be a distinguishing probe. The similarity of this epitope to HLA-DR2 B3

hypervariable region suggests that this may have a role in the pathogenesis of tuberculoid leprosy. Possibilities for further sophisticated research are abundant both in malaria and in leprosy.

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Chapter 20

RADIONUCLIDE BRAIN SCANNING

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Introduction

Medical imaging plays an important part in the diagnostic work-up of a patient as it can provide both anatomical and physiological visualization of different organs. Since the early part of this century, when X-rays were introduced for imaging, brain imaging has attracted lot of interest because visualization of the brain has been a challenging task and there are hardly any other investigations which can provide an insight of what is going on in the brain. During this period, brain imaging has seen continuous development and today's physician has several alternatives that he can choose for looking inside the brain. Advances in neuro-imaging moved from pneumoencephalography in the thirties to carotid angiography in the fifties. Radionuclide brain scanning was introduced in the late fifties, but it was only after $^{99}Tc^{m}$ was available for clinical studies in the mid-sixties that brain scanning became the most frequent neuro-imaging study. In the early seventies it was largely replaced, specially in developed countries by X-ray computerized tomography (CT). In the eighties nuclear magnetic resonance became another advanced imaging modality competing with and complementing CT. All these developments have been increasingly complex and exorbitantly expensive and usually out of the reach of many of the developing countries.

At one stage on this road to development, radionuclide brain scanning was the only technique available for imaging of the brain. Advent of CT and MRI pushed it to the background. It regained some of the grounds lost to "allied advances" with the introduction of brain perfusion radiopharmaceuticals. Positron emission tomography is a promising functional imaging modality that at present will remain as a research tool in special centres in developed countries. However, clinically useful developments will gradually percolate from PET to SPECT. The non-nuclear imaging methods are totally instrument dependent; they are somewhat like escalators, which can go that far and no further. Nuclear imaging has an unlimited scope for advance because of the new developments in radiopharmaceuticals. As the introduction of a radiopharmaceutical is less costly than buying new instruments, the recent advances in nuclear imaging are gradually perfusing through the developing countries also. Therefore, it is essential to follow very closely PET developments because what is research today might become routine tomorrow.

Historical review

In the early part of this century, it was observed that fluorescein crosses the abnormal blood brain barrier (BBB) and localizes in malignant tissues. This discovery was used to detect brain tumours during surgery under ultraviolet light. Later, fluorescein was labelled with ^{131}I and was used for detecting brain tumours by the help of Geiger-Muller tube. ^{32}P as sodium phosphate was also utilized to detect brain tumours at surgery. ^{131}I human serum albumin also behaved in the same way and because of its gamma emission, it can be used for

external counting. ^{203}Hg (later replaced by ^{197}Hg) Neohydrin had better physical and chemical properties. $^{113}In^{m}$ binds to plasma proteins after intravenous injection and was used also to localize brain tumours. Since the mid-sixties $^{99}Tc^{m}$-pertechnetate became the radiopharmaceutical of choice. It was replaced later by other $^{99}Tc^{m}$ radiopharmaceuticals with more rapid clearance from the blood; namely $^{99}Tc^{m}$-glucoheptonate (GHP) and $^{99}Tc^{m}$ diethylene triamine penta acetic acid (DTPA).

All the above radiopharmaceuticals had the property of crossing the blood brain barrier (BBB) when there was a pathological lesion in the brain and they always seeped into the focal lesions and not in the normal brain which remains as impervious to radiopharmaceuticals as to new ideas.

In the early eighties, freely diffusible compounds were introduced for the study of cerebral perfusion; first ^{123}I amphetamine followed by $^{99}Tc^{m}$-labelled hexamethyl propylene amine oxime (HMPAO). Recently $^{99}Tc^{m}$ ethyl-cysteine dimer (ECD) is under clinical trial. ^{123}I brain receptor radiopharmaceuticals are currently under development.

These developments of radiopharmaceuticals were *pari passu* with improvements in instrumentation leading to higher sensitivity and better resolution. Of special interest and importance was the development of single photon emission tomography (SPECT). Without SPECT, brain perfusion imaging would not have been possible.

Positron emission tomography advances were also simultaneously on both the fronts: instrumentation and radiopharmaceuticals. The detectors changed from single to multiple gantries, the size of the individual crystals became smaller, electronics and computers improved and accordingly the resolution improved from 16 mm to less than 6 mm, with the hope of having 3 mm in the near future. The radiopharmaceutical developments were remarkable using either ^{15}O or ^{13}N Ammonia for brain perfusion measurements, ^{18}F deoxyglucose for metabolic studies, ^{18}F spiperone and other numerous drugs for neuro-receptors and pharmacological studies.

Radiopharmaceuticals for brain imaging

Radiopharmaceuticals used for brain imaging can be classified as:

1. Lipophilic or hydrophilic:

   (a) Hydrophilic compounds cross the abnormal BBB, localize at the pathological site and not in the normal brain tissue.

   (b) Lipophilic compounds cross the normal BBB, localize in the normal brain cells by any of the following mechanisms:

(i) active transport for metabolic functions e.g. ^{18}F deoxyglucose or for neuro-receptor function e.g. dopamine, acetylcholine or opiate receptors.

(ii) passive transport proportional to blood flow, however, the compound stays in the brain cells due to changes either in the compound structure as with $^{99}Tc^{m}$ HMPAO or because of differences in pH between the intracellular and extracellular compartments as with ^{123}I HIPDM (hydroxymethyl iodobenzyl propane diamine). Lipophilic compounds will show brain scans which will look opposite to the hydrophilic ones; i.e the normal brain tissue will show no uptake while the pathological site will concentrate the radioactivity. The blood vessels and the venous sinuses will retain activity equal to that of the background activity; i.e reflects the blood clearance.

2. Another way of classifying brain radiopharmaceuticals is according to their metabolic characteristics:

(a) Those that become protein bound after intravenous injection as $^{99}Tc^{m}$ HSA (human serum albumin), ^{131}I-HSA, ^{197}Hg- and ^{203}Hg-labelled Neohydrin.

(b) Those that distribute extracellularly as $^{99}Tc^{m}$-pertechnetate, $^{99}Tc^{m}$-DTPA and $^{99}Tc^{m}$-glucoheptonate.

(c) Those that localize intracellularly as ^{42}P, ^{201}Tl and ^{84}Rb.

The **ideal radiopharmaceutical** for brain imaging should have the following characteristics:

(a) can be easily prepared for direct and safe administration to the patient.

(b) reasonable cost.

(c) physical half life of less than 24 hours, preferably 12 hours.

(d) relatively short effective half life that permits optimum imaging.

(e) monoenergetic gamma rays from 150 - 250 KeV.

(f) no particulate radiation in its decay so as to minimize the radiation absorbed dose.

(g) should have high lesion specificity.

The following are the characteristics of the commonly used radiopharmaceuticals for brain imaging:

1. $^{99}Tc^{m}$ pertechnetate:

It is bound to serum proteins, crosses the abnormal BBB, the concentration in the lesion is proportional to its protein content and is variable according to the histology of the lesion. The peak concentration in the lesion may be delayed and this delay may exceed its effective half life, making it difficult to visualize the lesion. $^{99}Tc^{m}$-pertechnetate is taken up by the choroid plexus. Therefore it is essential to block the choroid plexus by giving potassium perchlorate 400 - 800 milligrams orally at least half hour before intravenous injection of technetium pertechnetate. It is also taken up by the thyroid, gastric mucosa as well as by the salivary glands. Salivary gland uptake is a problem for vertex views of the brain. Salivary and gastric uptake can be blocked by the intravenous injection of one milligram of atropine 15 minutes before intravenous injection of $^{99}Tc^{m}$-pertechnetate. However, this is not routinely recommended because of the side effects of atropine specially in glaucoma or glaucoma susceptible patients. Technetium pertechnetate has a relatively slower blood clearance than other technetium radiopharmaceuticals. Therefore brain scan images has to be delayed for two hours after the intravenous injection. This delay allows for blood clearance and a lower background activity. Early images after the intravenous injection helps to differentiate vascular from nonvascular lesions when compared with the delayed images.

2. $^{99}Tc^{m}$ DTPA or glucoheptonate:

Both have faster blood clearance than $^{99}Tc^{m}$-pertechnetate, therefore both have the advantage of higher target to non-target ratio. Both are not taken by the choroid plexus, thyroid, salivary glands or gastric mucosa. Therefore there is no need for patient preparation by giving oral potassium perchlorate or intravenous atropine. Because of faster clearance, delayed images are usually obtained 30 - 40 minutes for DTPA and 45-60 minutes for glucoheptonate after the intravenous injection.

Doses for $^{99}Tc^{m}$ radiopharmaceuticals:

For all three $^{99}Tc^{m}$ radiopharmaceuticals the dose is the same:

For children : 200 μCi per kilogram body weight with a minimum dose of 3 mCi.

For adults: 250 - 300 μCi per kilogram body weight.

3. $^{99}Tc^{m}$ Hexamethyl propylene amine oxime (HMPAO):

It is a lipophilic compound, that crosses the normal BBB. The uptake in the normal brain

is proportional to the blood flow with an extraction efficiency of around 60 % at normal blood flow rates, and a lesser extraction at higher blood flow rates and the reverse at lower flow rates. However in spite of these variations in extraction at different blood flows, it correlates well with PET measurements and is useful for brain perfusion imaging in various neurological disorders. It is supplied in lyophilized kit form. It should be prepared according to manufacturers recommendations using freshly eluted $^{99}Tc^{m}$-pertechnetate. It must be injected within 20 minutes after preparation. Quality control for labelling efficiency usually takes about that time. Usually it is injected first and quality control results are known after the injection. A delay in injection beyond 20 minutes is not advisable because the compound is changed gradually from a d- isomer to a l-isomer. The l-isomer crosses back to the vascular component in the brain and is not retained by the normal brain tissue. The usual recommended dose is 200 - 250 μCi per kilogram body weight with a minimum dose of three mCi for children. The start of imaging should be delayed for 20 minutes after the I.V. injection to allow for blood clearance. The compound is cleared by the hepatobiliary system and the kidneys. Recent recommendation is to wait for two hours after the I.V. injection for higher target to non-target ratio.

Normally cerebral perfusion images are most informative with SPECT. Ordinary cerebral images with a planar gamma camera are not much help.

Other lipophilic compounds used for radionuclide brain scanning include:

(a) ^{123}I N-Isopropyl amphetamine "IMP"

(b) ^{123}I HIPDM

(c) ^{201}Tl Diethyl-dimethyl chlorine "DDC".

All three compounds are not suitable for routine clinical use especially in developing countries either because of high cost, short half life of ^{123}I or the need for in-house labelling of HIPDM or the new ligand DDC. ECD (d-$^{99}Tc^{m}$ ethyl-cysteine dimer) is a new brain perfusion compound that is still currently in phase III clinical trials. It has faster blood clearance and therefore better target to non-target ratio than $^{99}Tc^{m}$ HMPAO.

Imaging techniques

Review of the patient's clinical history is important in order to optimize the imaging technique. There are general recommendations that have to be observed for the highest quality of images. These include patient preparation, selection of the proper position either anterior, posterior or vertex views for the cerebral flow study, use of high specific activity in the smallest possible volume for intravenous injection which has to be given as a bolus injection, by using Oldendorf technique.

The cerebral angiogram is a dynamic depiction of the intracranial circulation. It is

acquired every one second for digital acquisition and displayed or acquired every 2-3 seconds for analogue formatter. The anterior view is the one most commonly used. In children, the posterior view may be considered in cases of suspected congenital anomalies. The vertex view is used infrequently for lateralisation of the lesions. Computer analysis by generating time activity curves over both carotid and middle cerebral hemispheres may be helpful **(Fig. 20.1)**.

1. Static acquisition

Usually four projections are obtained; anterior, posterior, right and left laterals. The anterior view is obtained with the head in flexion in order to see the frontal areas. It is important to exclude facial activity as far as possible without excluding the temporal regions or the occipital lobes so that most of the counts are acquired mainly from the brain area. The total number of counts for the anterior view under these conditions should be at least 500 000 counts, if using $^{99}Tc^{m}$ pertechnetate, 400 000 for $^{99}Tc^{m}$ glucoheptonate and 300 000 for $^{99}Tc^{m}$ DTPA. The time for the total counts for the anterior view should be used as the fixed acquisition time for the other views. Occasionally a vertex view is helpful in localizing the lesions. In this case, shielding of facial and shoulder activity is necessary by using lead apron. Occasionally orbital view is obtained. It is acquired with the head in 30 degrees extension. In children a converging collimator might be helpful, however attention should be given to the geometric disfigurement that it might produce. The converging collimator is of special value in the posterior view to enlarge the posterior fossa. In all other acquisitions, the parallel hole, low energy, preferably high resolution collimator should be used **(Fig. 20.2)**.

2. SPECT Acquisition

SPECT imaging has been recognized to be useful for better definition of the lesions in the brain, to separate superficial lesions of the cranial vault from deep seated lesions and for better resolution of lesions in the posterior fossa. In order to improve the resolution for SPECT images the distance between the face of the collimator and the head should be as small as possible. Sometimes the shoulders of the patient limits this positioning. In order to overcome this problem, modifications have been made either in the detector shielding or in the collimators. Developments in the collimators involve either fan beam collimators, slanting holes or long bore collimators **(Fig. 20.3)**.

Quality control of the imaging device is essential before starting any SPECT imaging. Most important procedures are the centre of rotation, field uniformity and stability of the system.

Acquisition recommendations for SPECT are usually as follows: a full circular rotation of 360 degrees, 64 projections, 64 x 64 x 8 matrix, time of acquisition varies according to the radiopharmaceutical used. It varies from 20 - 25 seconds per projection for $^{99}Tc^{m}$ HMPAO and 40 - 45 seconds for ^{123}I IMP. Usually a high resolution collimator is recommended, if HMPAO or IMP is used. A general purpose collimator will be

satisfactory, if using $^{99}Tc^{m}$ pertechnetate, glucoheptonate or DTPA. For data processing, images are usually prefiltered and processing parameters varies from instrument to instrument. In general, more smoothing is needed, the lower the number of counts per projection. Usually processed data are displayed in one pixel thick transaxial, sagittal and coronal slices.

Clinical indications of radionuclide brain scanning

Before the introduction of C.T. on a wide scale in modern hospitals, radionuclide brain imaging constituted a large portion of the workload of nuclear medicine departments. At present, radionuclide brain scanning can be divided into

(a) old-fashioned type done with $^{99}Tc^{m}$ pertechnetate, DTPA or glucoheptonate and

(b) the new-fashioned type done with cerebral perfusion compounds like ^{123}I IMP, ^{123}I HIPDM or $^{99}Tc^{m}$ HMPAO and, in future, $^{99}Tc^{m}$ ECD. ^{123}I IMP and HIPDM are not likely to be easily available in the developing countries and currently even in developed countries, they are largely replaced by $^{99}Tc^{m}$ HMPAO. In developing countries, due to the unavailability of CT or MRI imaging, radionuclide brain scanning still plays a substantial role in the investigations of patients, suspected of having brain pathology. For these reasons, this Chapter would mostly describe current applications with $^{99}Tc^{m}$ pertechnetate, DTPA or glucoheptonate and $^{99}Tc^{m}$ HMPAO SPECT studies.

1. Brain tumours

(a) Gliomas: They arise from primitive glial cells, forms 50 % of all intracranial tumours in adults and children, has a variety of cell types and a wide range of malignancy. The highly malignant astrocytomas (grade III and IV) are characterized by vascularity, edema, and necrosis; rarely spreading outside the brain and is usually fatal within few months. In radionuclide angiogram, these tumours are noted by early vascularity, intense uptake in the arterial phase lasting through the venous phase. The delayed images with $^{99}Tc^{m}$ hydrophillic compounds show avid concentration of the radiopharmaceutical, the speed and intensity of which are proportional to the grade of malignancy, vascularity of the tumour as well as the degree of disruption of the BBB. Other gliomas, especially grade I and II astrocytomas, are not easily detected due to low vascularity and less intense concentration of the radiopharmaceutical. With $^{99}Tc^{m}$ HMPAO, these tumours are usually seen as areas of less concentration (cold areas) of radioactivity. CT and MRI imaging are better suited for the early diagnosis of tumours with low grade malignancy. Their sensitivity varies from 90% - 95% versus 80% - 90% for radionuclide brain scanning.

(b) Meningiomas. Meningiomas occur usually in adults, characterized by high vascularity, slow growth, usually located close to the cranium, sometimes accompanied by bone erosion or reactive sclerosis and large superficial draining veins. It is easily diagnosed on the brain scan. It is characterized by intense arterial uptake, progressively increasing and stabilizing in the venous phase and remaining intense in the delayed images **(Fig. 20.4)**. $^{99}Tc^{m}$ cerebral perfusion compounds will show hyperaemia in the angiogram phase that fades away in the delayed images, because of the blood clearance. Perfusion images are not useful in the diagnosis of these patients. Although the sensitivities of CT, MRI and radionuclide brain scanning are equal yet, CT and MRI are preferred for their three dimensional visualization which is helpful in surgical evaluation.

(c) Metastatic Tumours. They constitute about 25 % of all intracranial tumours. Most common sites for primaries are lungs, breast, prostate, malignant melanomas, kidneys and gastrointestinal tract. They are usually multiple and there are no preferred sites for their location. They are usually fatal within a year but can remain silent for several months. Therefore it is important to look for brain metastasis in asymptomatic patients with high risk for brain metastasis (e.g. small cell type lung carcinoma, breast and kidney malignancies) before contemplating surgery for the primary. The intensity of the uptake of the hydrophillic type of radiopharmaceuticals in the brain scan is variable depending on their size, vascularity, grade of malignancy, rate of growth and the impairment of the BBB. They are usually not detected in the radionuclide angiogram. Brain metastasis in $^{99}Tc^{m}$ HMPAO scans will appear as areas of less radioactive uptake and are not very useful. The sensitivity of $^{99}Tc^{m}$ hydrophillic radiopharmaceuticals for detecting brain metastasis varies from 75% to 87% versus 85 to 95 % for X-ray CT or MRI. These figures were reported with planer imaging for the radionuclide procedures. However the sensitivity for SPECT imaging has not been carefully looked at. The resolution of the radionuclide techniques is not expected to be less than one cm, even with SPECT, versus a few millimetres with CT or MRI. The latter would be preferred whenever they are available.

(d) Other brain tumours such as primary sarcomas, fibrosarcomas or lymphomas are rare. Pituitary tumours, cranial nerve tumours, brain stem gliomas and pinealomas are better diagnosed by CT or MRI. Primary vascular brain tumours as hemangiopericytomas or hemangioblastoma are very rare, and are usually located in the posterior fossa. In these situations also, usually CT or MRI plays a major role in the diagnosis.

Other radionuclides of special interest for radionuclide imaging of brain tumours and metastasis are ^{67}Ga citrate and ^{201}Tl. Gallium is non-specific because it cannot differentiate between tumour tissue and infection. Thallium on the other hand is specific because in our experience it is taken up by viable tumour tissue and not by acute inflammatory lesions. Chronic granulomatous lesions such as tuberculosis might take up ^{201}Tl. However the intensity of uptake will be much less than tumour uptake. Recent approach of quantitating

thallium uptake in brain tumours has shown that the ratio of uptake in the tumour to the contralateral normal side is proportional to the grade of malignancy of the tumour and might reflect the prognosis and the future behaviour of the tumour. ^{201}Tl imaging for brain tumours is also helpful in differentiating recurrent tumour from tumour necrosis or post-operative changes. In this regard it might be superior and, at least, supportive to CT and MRI.

2. Cerebrovascular disorders

(a) Cerebrovascular occlusion. It has a complex pathogenesis; thrombosis is the cause in 70% of the cases, embolism and haemorrhage being less common. It leads to cerebral hypoxia which might be poorly recognized in the early phase of the disease. Cerebrovascular disease can manifest as episodes of transient ischemic attacks (TIA) due to microembolism from fragile atheromas mostly in the carotid or vertebral arteries in the neck or base of the skull. These ischemic episodes might vary in severity from transient unconsciousness to complete hemiparesis. The symptoms are variable according to the branches involved; which might be motor, sensory, visual or auditory in nature. In other words, signs and symptoms depend on the artery involved, location and type of occlusion and extent of brain damage.

The sequence of events in ischemic infarct is characterized by initial central necrosis with perivascular engorgement and local edema. During this period, which lasts for 10 - 14 days, the brain scan is normal, although the radionuclide cerebral angiogram might show an area of decreased perfusion in the arterial, capillary and venous phases. After the third week, there is increased focal uptake in the ^{99}Tc^{m} pertechnetate, DTPA or the glucoheptonate brain scan. This increased uptake plateaus in three to four weeks and decreases gradually to normal in six to eight weeks. If the developments of collaterals is inadequate, this increased uptake might persist up to one year. In such a case, it might be difficult to differentiate cerebral infarct from brain tumour. However, an infarct can be recognized by lack of growth, its location in the territory of a vessel and its usual flame like shape with irregular margins. The earlier the resolution of this uptake, the better is the prognosis. If there is an abnormal brain scan in one to seven days, it is usually due to emboli leading to haemorrhagic infarct. It is postulated that sometimes in embolic infarct, the embolus becomes fragmented and the blood flow can pass through the site of the obstruction into the ischemic infarct. Infarcts of the brain stem, internal capsule and basal ganglia are difficult to visualize on the ^{99}Tc^{m} pertechnetate brain scan.

The radionuclide angiogram is very important and should always be done whenever there is a suspicion of vascular abnormality. It is important to decide before the study which positioning will be most informative depending on where the lesion is suspected. In 75% of the cases the anterior position is most useful because the middle or the anterior cerebral branches and their territories are commonly

involved. If there is suspicion that posterior cerebral or the vertebral artery is involved, the posterior view should be used.

During the first week of the incident, the radionuclide angiogram will show ischemic area with normal brain image in the delayed scans. After the first week, the brain scan will show what is called as the "flip flop" appearance, i.e early ischemia at the side of the infarct followed by delayed venous washout and increased uptake in the later part of the angiogram and in the delayed images.

$^{99}Tc^{m}$ bone seeking radiopharmaceuticals are known to show positive uptake in the area of the infarct. This is helpful in differentiating infarct from other lesions. The mechanism of this uptake is not clear. It may be due to avidity of these compounds for necrotic tissues.

The radionuclide cerebral angiogram and the brain scan correlate well with CT, clinical findings and remain to be cost effective. However there is delay of days or weeks before both of them become positive. The brain scan takes at least seven days while the CT shows changes in affected tissues after 48 hours. On the other hand radionuclide brain scanning with brain perfusion radiopharmaceuticals as $^{99}Tc^{m}$ HMPAO or ^{123}I IMP have higher sensitivity for detecting changes in perfusion early after cerebrovascular accident (CVA).

During the first 24 hours brain scanning with $^{99}Tc^{m}$ HMPAO is POSITIVE in 95% of the cases in defining the location of the occlusion versus 70 - 75% with CT **(Fig. 20.5)**. There is a definite advantage with the radionuclide brain studies with $^{99}Tc^{m}$ HMPAO, HIPDM or ^{123}I IMP over CT or MRI in transient ischemic attacks in defining the degree of stenosis and the status of the collaterals in symptomatic patients and in deciding whether they can benefit from surgical treatment of establishing communication between the external and the internal carotid circulation. Further information in this respect can be obtained by doing semiquantitative cerebral perfusion studies before and after intravenous administration of Diamox, a drug which produces vasodilatation of the normal intracranial vessels. Atheromatous vessels show a lesser or no response to this drug.

(b) Cerebral haemorrhage

(i) **Intracranial haemorrhage**: It can be due to trauma, ruptured aneurysm, inflammatory vascular lesions, A-V malformations, tumours or blood dyscrasia. The signs and symptoms are variable according to the extent, location and underlying pathological state. The brain scan is usually normal in the early images but abnormal in the delayed images. Radionuclide brain scanning with cerebral perfusion compounds, on the other hand, is positive right from the early phase. The lesion is seen as an area of decreased

perfusion. In addition it has the advantage over X-ray and MRI of showing the extent of associated vascular spasm in the neighbouring territory which is usually predominant in the early period. This information might be of value in the management and prognosis of the patient.

(ii) **Subarachnoid haemorrhage:** The most common cause is rupture of an arterial berry aneurysm. It is usually diagnosed by cautious lumber puncture, contrast angiogram and CT. Blood in the subarachnoid space leads to arterial spasm which may persist up to three weeks and might lead to cerebral infarct. Therefore it is important to do a brain scan using $^{99}Tc^{m}$ HMPAO in order to determine the time for surgical intervention, if needed. The regular radionuclide brain scan with $^{99}Tc^{m}$ pertechnetate is of little value in these patients.

(iii) **Arteriovenous Malformations:** A-V malformations are usually congenital or acquired, can be single or multiple, usually located in the cerebral cortex and have a tendency to bleed and/or calcify. Clinically they produce focal seizures. They are easily diagnosed by CT with contrast enhancement or by MRI. The radionuclide angiogram is characteristic with increase in the arterial phase which decreases in the venous phase. This differentiates them from meningiomas which are usually superficial in location and which show in the angiogram increased arterial phase with further increase in the venous phase. The brain scan shows increased activity in the early part within minutes after the injection of the radiopharmaceutical. It becomes faint or almost absent in the delayed scan taken two hours after the injection **(Fig. 20.6)**.

3. Verification of brain death

With the current progress in organ transplant and with the possibility of maintaining unconscious patients on life sustaining equipment for long periods of time, there is need for an accurate diagnosis of acute brain death. There are several requirements laid down for this in the American and the European practice. Among these is a proof of absent intracranial circulation. This could be done by either a contrast angiogram which is an invasive procedure and has several risks. The radionuclide angiogram is a valid alternative that is non-invasive. It can be done with a mobile gamma camera at the bedside. $^{99}Tc^{m}$-pertechnetate is the radiopharmaceutical most commonly used for this. The success of the test depends on injection of a good bolus with total dose in as small a volume as possible injected according to Oldendorf's technique. If for any reason, the injection is not proper or there is a failure in acquisition of the angiogram due to camera or computer failure, the physician can still rely on the delayed images of the brain scan which show absence of radioactivity in the venous sinuses. However delayed images might show slight radioactivity in these sinuses due to communication between the extracranial and the intracranial venous circulation through the communicating diploic venous channels through the cranial vault.

Some investigators recommend the use of a tourniquet over the scalp to suppress such a communication between these two circulations.

In our experience, $^{99}Tc^m$ HMPAO provides more accurate information for this purpose and should replace $^{99}Tc^m$ pertechnetate. Normally $^{99}Tc^m$ HMPAO is taken up by the normal brain tissue, if there is any intracranial circulation, no matter how diminished it is. If there is no intracranial circulation there will be NO activity inside the calvarium in the brain. There is no need for the angiographic phase of the study. If a mobile gamma camera is available, static images can be obtained at the bed side. There is no need for SPECT images. The study is very easy to interpret. If there is no activity inside the cranial vault in the brain tissue, the diagnosis of brain death can be safely made. If there is any activity seen in the brain, it would be advisable to avoid the diagnosis of brain death in spite of other clinical evidences. The study can be repeated whenever death is suspected later.

4. Intracranial infections

Intracranial infections such as brain abscess, ventriculitis or specific infections as tuberculosis or coccidiomycosis are usually diagnosed by CT or MRI. The role of the radionuclide brain scan is very limited. $^{99}Tc^m$ pertechnetate brain scan will show areas of increased uptake in the delayed images in the lesion. In ventriculitis, it will take the shape of the ventricles. In other diseases, it could be solitary or multiple. There is no role for cerebral perfusion brain scan in these entities. A gallium scan will show increased uptake in the areas involved although it is rarely necessary.

In the following five conditions of encephalitis, epilepsy, dementia, psychological disorders and acute head injury there is no role for the radionuclide brain scan with $^{99}Tc^m$ pertechnetate, DTPA or glucoheptonate. Brain scanning with cerebral perfusion compounds plays an important role and is more sensitive than CT or MRI in delineating the location of damage to the brain. Its role in acute head injury is supplementary to CT and is superior to it specially in the first 24 - 48 hours.

5. Encephalitis

Herpes encephalitis is difficult to diagnose. The only definitive test for its diagnosis is brain biopsy. The clinical picture is usually confusing. Peripheral blood picture, lumber puncture and CT are usually normal. The cerebral perfusion brain scan with $^{99}Tc^m$ HMPAO is characterized by marked hyperaemia of the involved part of the brain. The intensity of uptake is usually so high in the early part of the disease that it can be easily seen on planer images and there is no need for SPECT images. The intensity of uptake subsides with the regression of the disease or with successful treatment. Therefore it is essential to do the test as soon as possible once the diagnosis is suspected. Follow up scans will help in evaluating the regression of the disease **(Fig. 20.7)**.

6. Dementia

The differentiation of senile dementia from multi-infarct dementia, Alzheimer disease or other psychological disorders or depression is important for early diagnosis, management planning and medicolegal advice. Certain types of dementia are characterized by special pattern on the positron emission tomography scans using either ^{18}F deoxyglucose, ^{13}N ammonia or labelled receptors. $^{99}Tc^{m}$ HMPAO or ^{123}I IMP cerebral perfusion scans are comparable to PET scans, easier to perform and likely to be available in many developing countries. Multi-infarct dementia is characterized by multiple small areas of decreased perfusion scattered over the cerebral cortex with no preference between the frontal or parietal lobes. Occasionally areas of luxury perfusion are detected. These are related to the possible etiology of this disease as infarcts due to micro-emboli followed within few days by luxury collateral circulation. The areas of luxury perfusion show as areas of increased perfusion sometimes detected at the periphery of small areas of decreased perfusion. They are usually transient and clears within few weeks.

Alzheimer disease has a characteristic pattern of bilateral symmetrical areas of decreased perfusion involving both temporal and parietal lobes. It rarely affects the frontal lobe.

Depression is characterized by decreased perfusion of the frontal lobes. This pattern is reversible with successful treatment.

7. Psychological disorders

PET studies have proved helpful for identifying certain abnormalities in glucose metabolism, neuro-receptors and cerebral perfusion in some psychological and neurological disorders. Recent developments were able to label some dopamine receptors with ^{123}I. It is too early to evaluate the results with them. Cerebral perfusion studies with $^{99}Tc^{m}$ HMPAO have been reported to be comparable to those acquired by PET radiopharmaceuticals in patients with schizophrenia, drug abuse, and other psychological disorders. We have shown that schizophrenia is accompanied by increased perfusion to the basal ganglia, usually bilateral in the majority of the cases. There is also associated ventricular dilatation in more than half of the cases. Areas of decreased perfusion due to cerebral damage have also been recognized and are thought to be secondary to electro-convulsive therapy.

In other centres cerebral perfusion studies done in association with and without psychometric testing had shown that $^{99}Tc^{m}$ HMPAO is a reliable and reproducible test for monitoring cerebral perfusion changes and effect of treatment in many psychological disorders.

8. Epilepsy

Epilepsy is a complex disorder, which could be primary or secondary to brain damage due to intracranial trauma or infection. Certain types are resistant to medical treatment and requires surgical approaches. In these cases it is essential to localize accurately the focus of

epilepsy and to confirm it by more than one test in order to plan the right surgical approach. CT and MRI as well electroencephalogram have low sensitivity varying between 25-35%. Invasive electrode electroencephalographic monitoring is the most accurate technique. PET studies with ^{18}F deoxyglucose proved to correlate well with invasive EEC in more than 90% of the occasions. Cerebral perfusion studies with ^{99}Tc^{m} HMPAO also proved to correlate well with PET studies. Areas of increased or decreased perfusion correlated well with areas of increased or decreased glucose uptake.

The findings in epilepsy of changes in cerebral perfusion depends on the timing of the injection, whether in the interictal or in the preictal or in the ictal phase. In the interictal phase, the area of the epileptic focus is usually recognized as an area of decreased perfusion, rarely as an area of increased perfusion. If the injection is made in the preictal or in the ictal phase, the area of the focus of the epilepsy is characterized by increased perfusion (Fig. 20.8).

9. Head injury

Previously ^{99}Tc^{m} was frequently used for the diagnosis of subdural haematoma following head injury. This indication has decreased after the use of CT. This is mainly because there is a delay of seven to ten days before a brain image shows an appearance characteristic of subdural haematoma. A membrane has to form around the haematoma which eventually shows increased uptake of the radiopharmaceutical in the delayed static images. The usual location of the haemorrhage is on the surface of the brain. The flow study shows an area of decreased flow on the surface of the brain with a concavity to the outside.

Acute head injury is currently considered as the most important clinical indication for cerebral perfusion imaging using ^{99}Tc^{m} HMPAO especially in the immediate period following the trauma. It has also proved very helpful in the follow up in defining residual damage to the brain. Soon after the trauma when the patient is in coma with more than one medical and surgical problems, the most important question facing the physician is the assessment of the damage to the brain and based on that, what is the prognosis of the patient.

Changes in cerebral perfusion could be focal or non-focal. Non-focal lesions have diffuse decreased perfusion, or asymmetric perfusion between right and left cerebral hemispheres or decreased fronto-occipital slope. Normally the frontal lobes should have an equal or greater perfusion than the occipital lobes. Reversal of the fronto-occipital ratio is noticed in cases of increased intracranial pressure. Diffuse decreased cerebral perfusion is presumably due to diffuse axonal injury due to the sudden deceleration induced by the trauma. Focal lesions are usually due to cerebral contusion, cerebral haemorrhage or pressure from a haematoma on the surface due to subdural haemorrhage or fracture. Subarachnoid haemorrhage causes diffuse spasm with big focal defects. Usually the defects are those of decreased perfusion. The lesions might vary in size. The small lesions are less than half the size of one cerebral lobe, and the larger are bigger than the size of half a cerebral lobe. These lesions can involve any part of the cerebral hemisphere but rarely the cerebellum.

In addition to the focal and non-focal lesions trauma can induce surface or meningeal lesions. These can be detected on the brain scan, on the surface of the brain and causing pressure on the underlying cerebral cortex. Non-focal lesions usually resolve and bear good prognosis. The prognosis of focal lesions depends on their location, size and number. Brain stem, cerebellar, temporal and parietal lesions have worse prognosis than frontal or occipital lesions. Obviously the smaller the number and the size of the lesions, the better the prognosis. There is no relation between the period of coma, the nature of the lesions and the time of recovery.

Radionuclide cisternography

The study of the pathophysiological changes in the pathways of the cerebrospinal fluid (CSF) is possible by injection of a radiotracer in the subarachnoid space. Normally the CSF is produced from the choroid plexuses in the lateral and third ventricles. It is transported probably by vascular pulsations to the fourth ventricle. Through foramina of Luschka and Magendi, it passes to the subarachnoid space in the posterior fossa. It ascends over both sides of the cerebral hemispheres along the Sylvian fissures to the vertex of the brain where it is absorbed by the arachnoid villi to the venous circulation. In the spinal cord, there is a two directional flow of CSF, downwards and upwards. There is a balance between CSF production and absorption. Any obstruction in the flow of the CSF will cause proximal dilatation and stasis.

Radiopharmaceuticals used to study CSF flow should have special characteristics. They should not be irritant to central nervous system (CNS) membranes. They should not be proteins which can cause reactions. The physical half life of this isotope should be long enough to continue the study up to three or four days. It should not be absorbed from the subarachnoid space but should be absorbed from the arachnoid villi. Once it is in the venous circulation, it is excreted by the kidneys. In the early days, ^{131}I Human Serum albumin was used. It did cause occasional reactions in the meninges producing signs of meningism. At present, ^{169}Yb (T½ 32 days), and ^{111}In (T½ 28 days) DTPA are the radiopharmaceuticals of choice.

Current indications for Radionuclide Cisternography are the following:

(a) Differentiating obstructive from communicating hydrocephalus.

(b) Diagnosis of CSF leak from the nose, sphenoidal or middle ear regions.

For these indications, the radiopharmaceutical is injected in the subarachnoid space at the level between L2-3 vertebrae. Images are taken immediately after the injection to verify the accuracy of injection. Planar images are taken of the head - posterior, anterior and both laterals - at 2-6, 24, 48 and 72 hours. Tomographic images give better resolution and definition of sites of radionuclide accumulation.

In cases of CSF leak, the placement of a cotton swab for several hours in the nose or ear on the side of suspected leak and subsequent counting of the swab increases the accuracy of the detection of the CSF leak **(Fig. 20.9)**.

Although contrast dynamic X-ray CT cisternography is competing with radionuclide cisternography, it is more difficult to do and interpret and not suitable for use in developing countries for the detection of the leak.

(c) evaluation of patency of CSF shunts by direct injection in the tube or in the ventricle and dynamic imaging every few seconds for the shunt and the site of drainage in the peritoneal, pleural or venous sites.

The role of radionuclide cisternography to evaluate congenital anomalies in the CSF flow is now completely taken over by X-ray CT and MRI.

We have not covered in this chapter a number of items such as quantitation of cerebral perfusion studies, the use of ^{133}Xe gas etc. Further readings in these areas are recommended.

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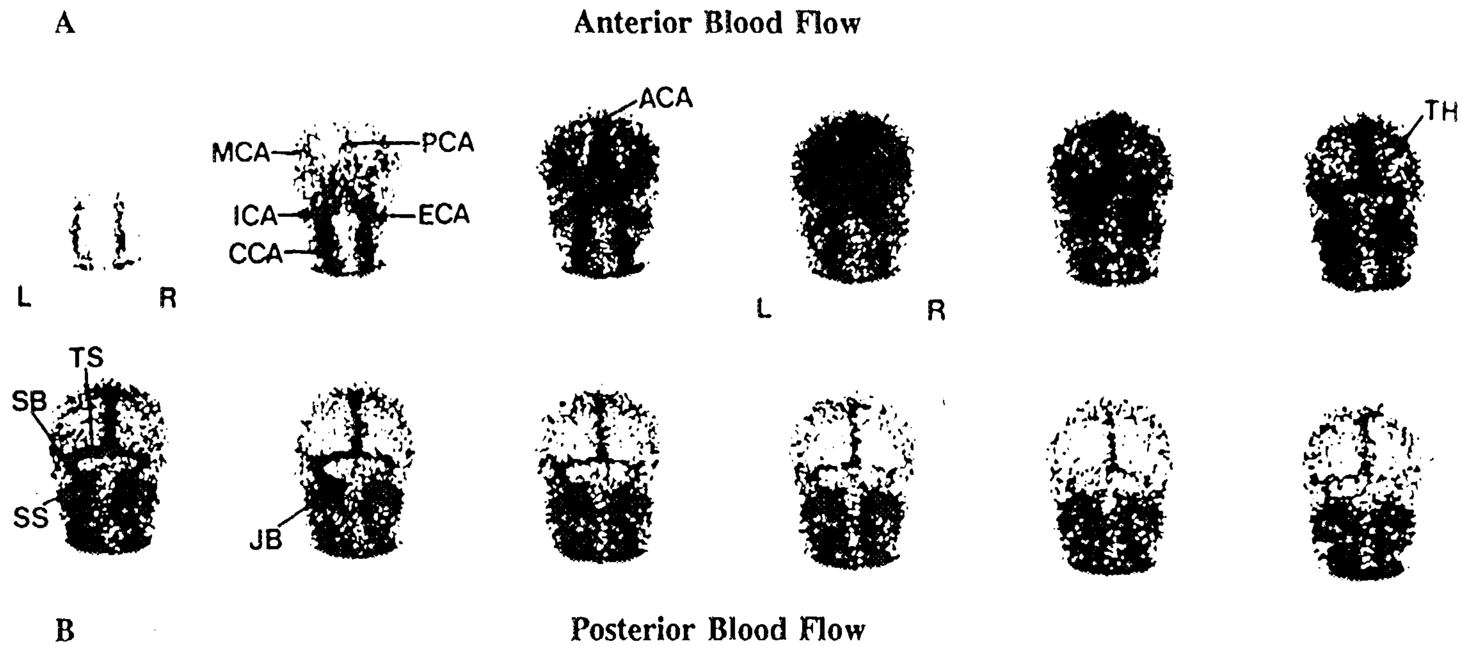

Fig. 20.1 Radionuclide cerebral angiogram (Posterior View) arterial, capillary and venous phases are seen in sequence. CCA - common carotid artery, ICA - internal carotid artery, ECA - external carotid artery, MCA - middle cerebral artery, PCA - posterior cerebral artery, ACA - anterior cerebral artery, TS - Transverse Sinus, SS - Sigmoid Sinus, JB - jugular bulb.

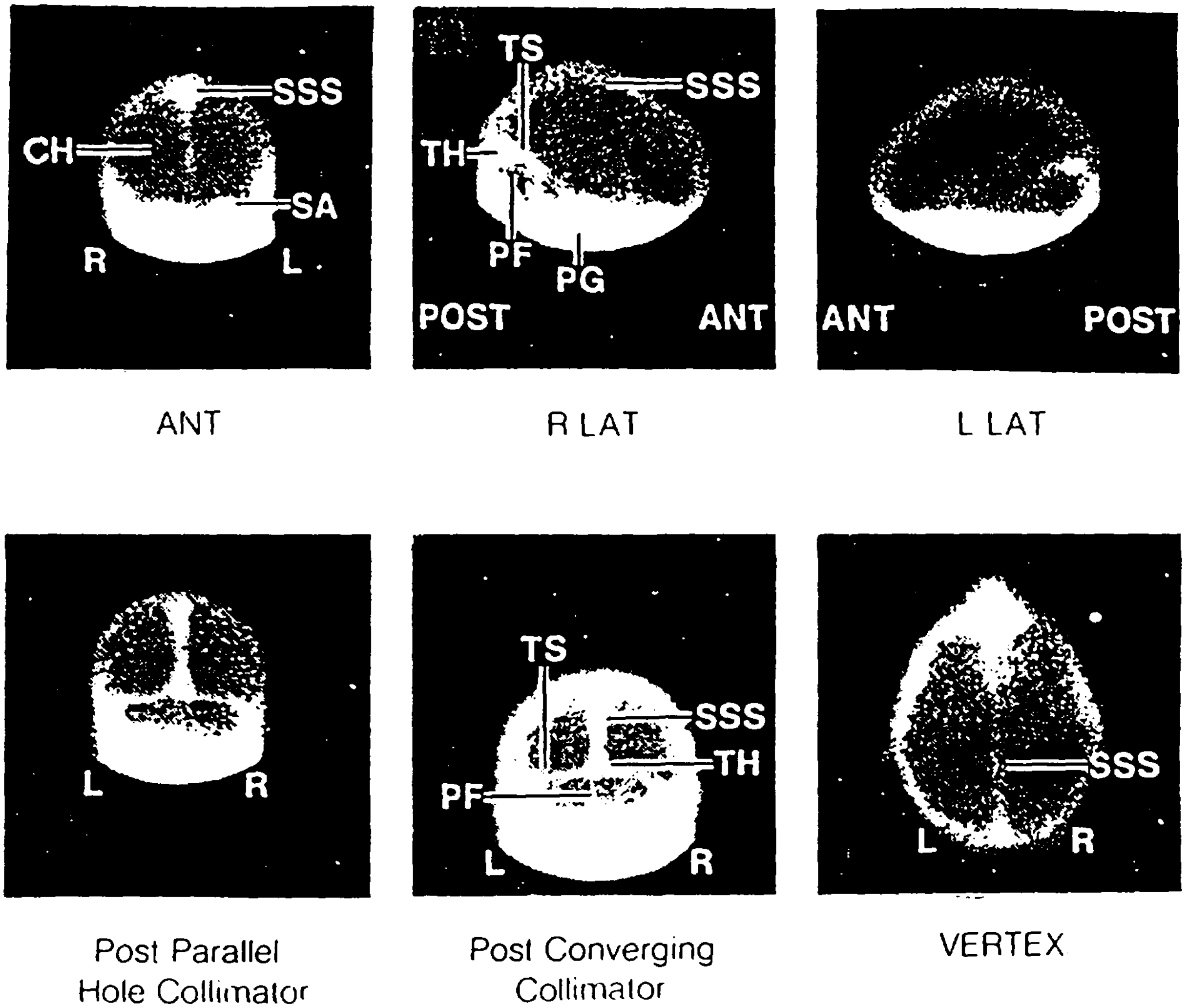

Fig. 20.2 Static Brain Scintigram. Normal. CH - cerebral hemispheres, SSS - superior sagital sinus, SA - supraciliary arch, TS - Transverse Sinus. TH - Tropic of Herophili, PF - posterior fossa, PG - parotid gland.

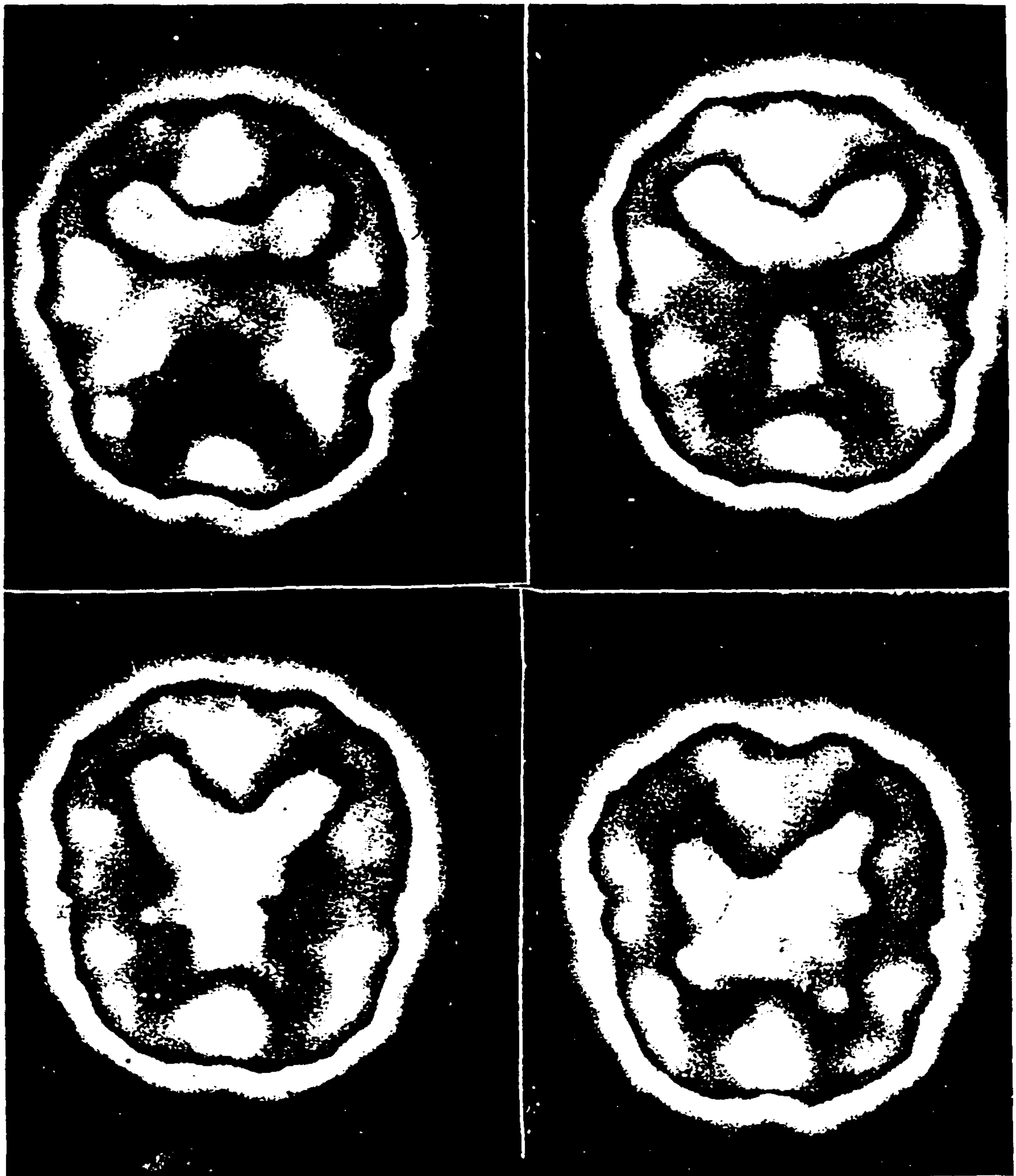

Fig. 20.3 A. shows normal HMPAO $^{99}Tc^{m}$ SPECT STUDY in Transaxial, sections. Note homogenous distribution of $^{99}Tc^{m}$ HMPAO, and separation of grey matter and white matter perfusion.

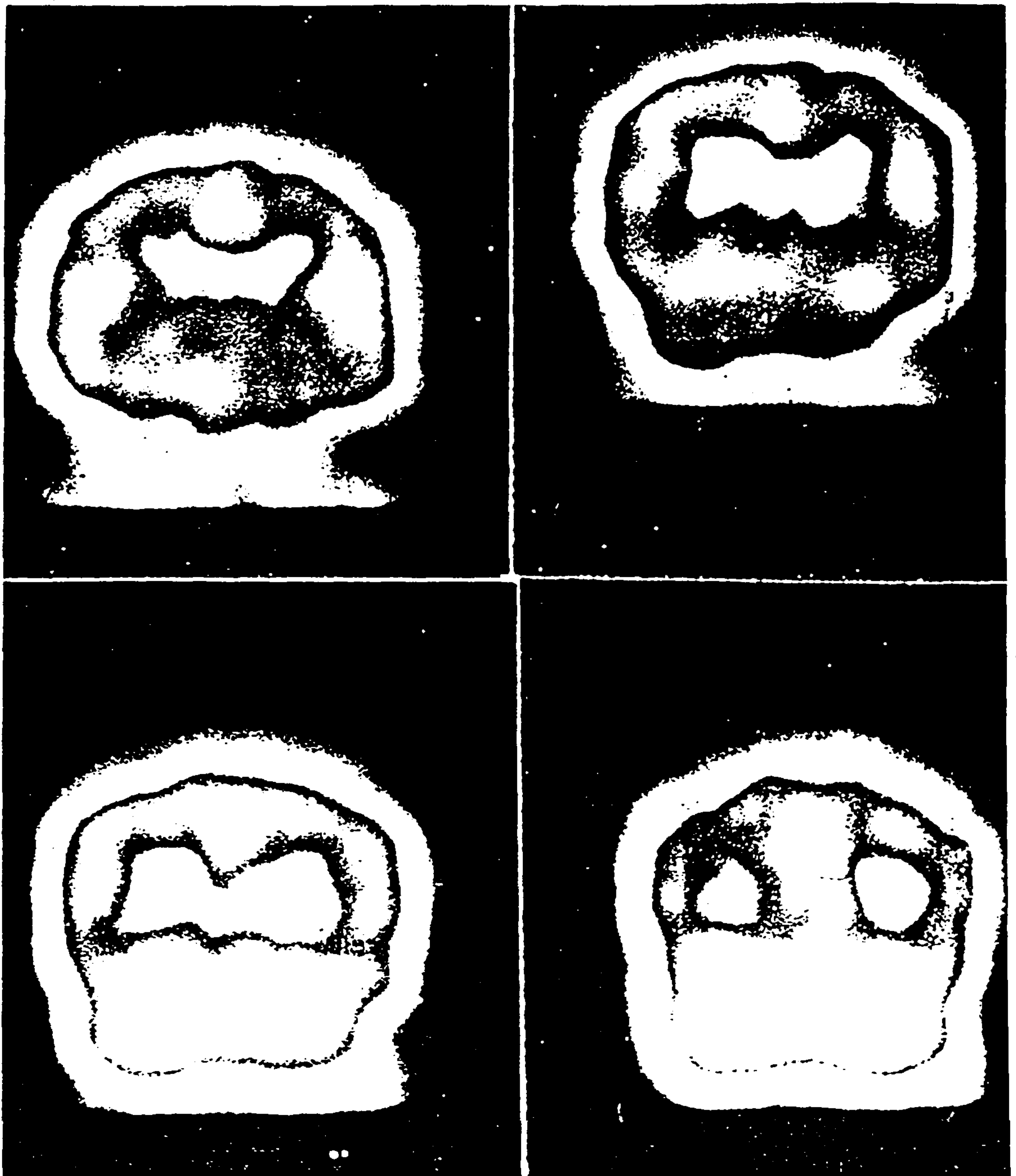

Fig. 20.3 B. shows normal HMPAO $^{99}Tc^m$ SPECT STUDY in Coronal sections. Note homogenous distribution of $^{99}Tc^m$ HMPAO, and separation of grey matter and white matter perfusion.

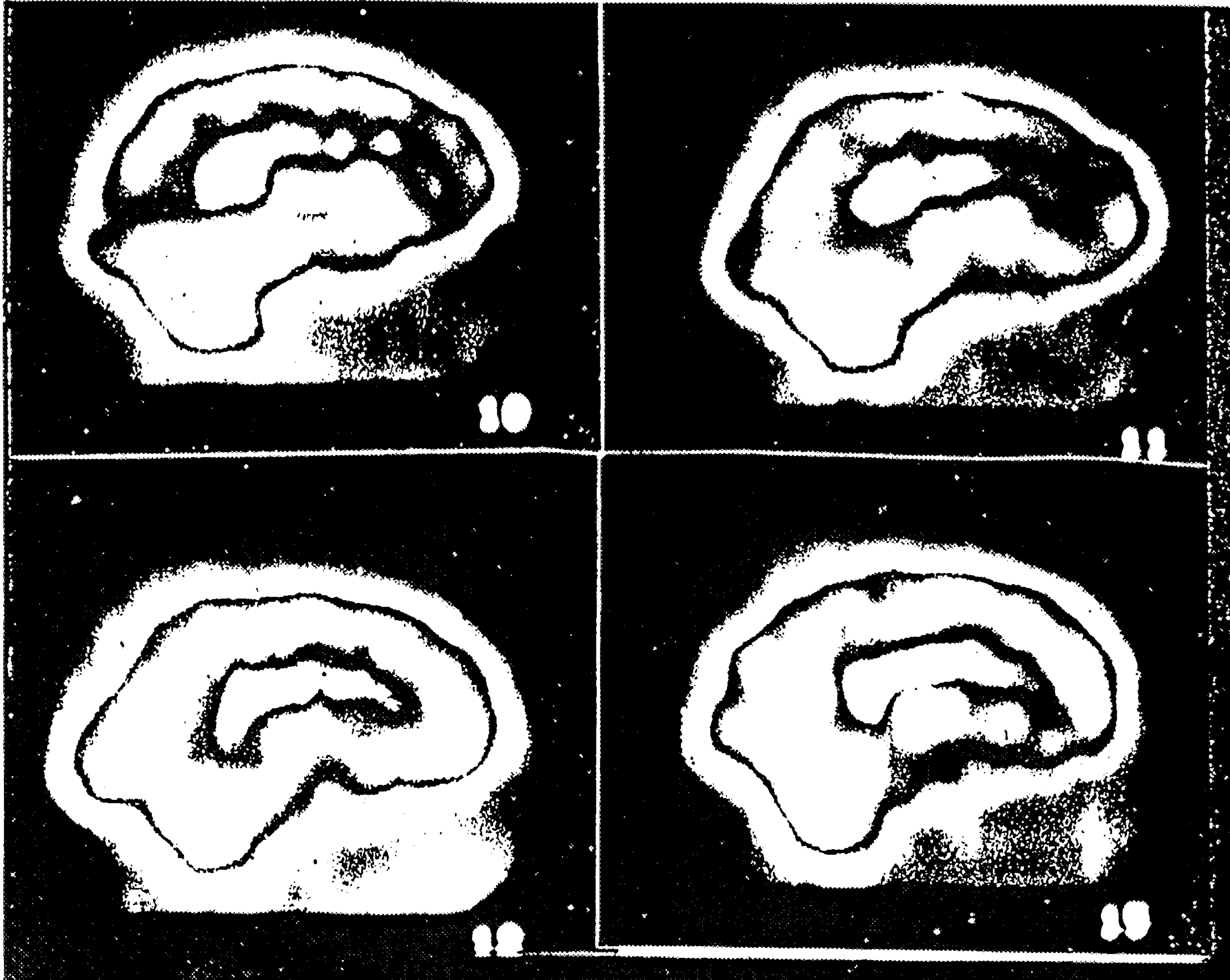

Fig. 20.3 C. shows normal HMPAO $^{99}Tc^{m}$ SPECT STUDY in Sagittal sections. Note homogenous distribution of $^{99}Tc^{m}$ HMPAO, and separation of grey matter and white matter perfusion.

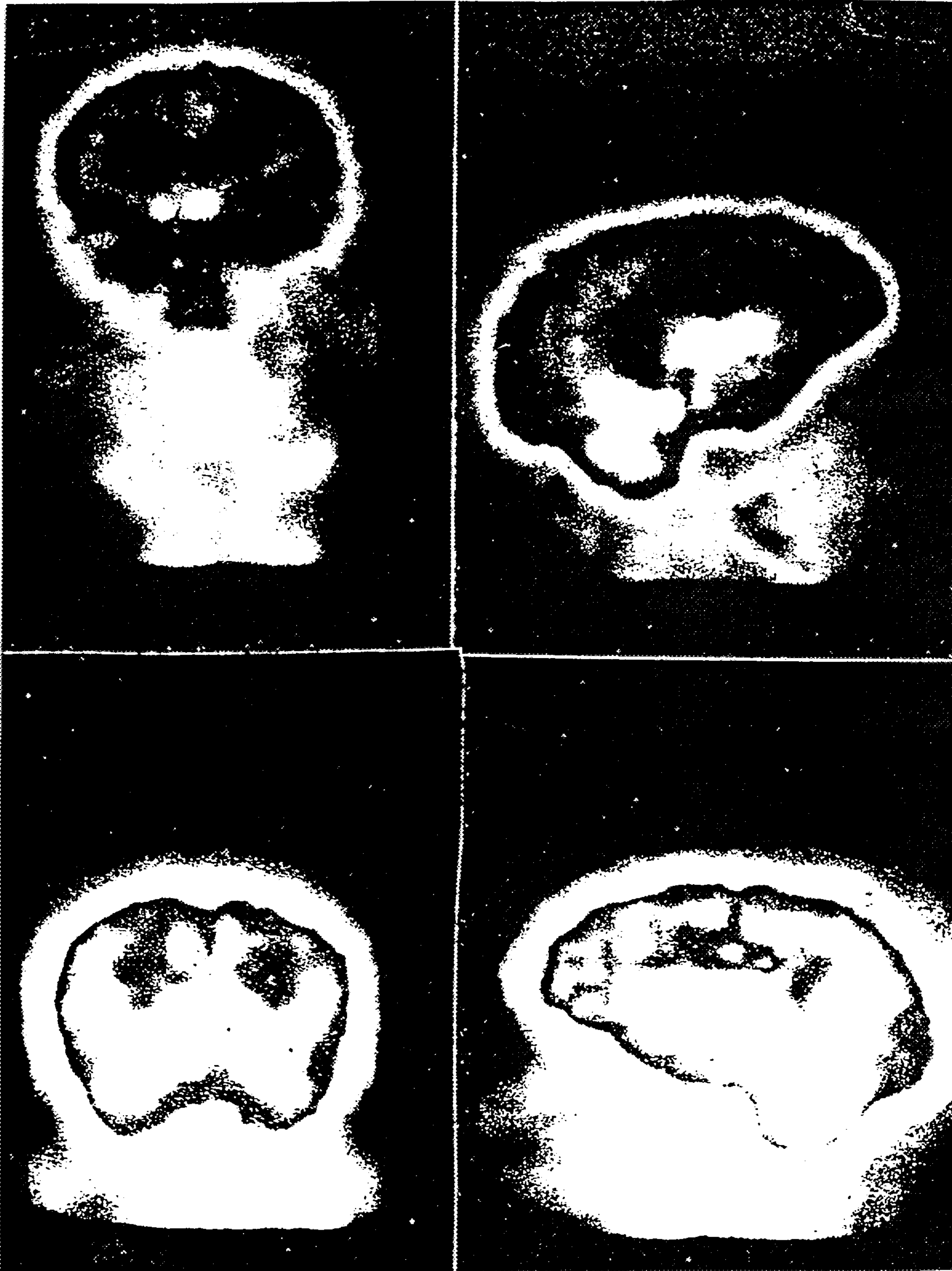

Fig. 20.3 D. shows normal $^{99}Tc^{m}$ HMPAO Planar study for comparison. Clockwise are Anterior, RL, LL and posterior views.

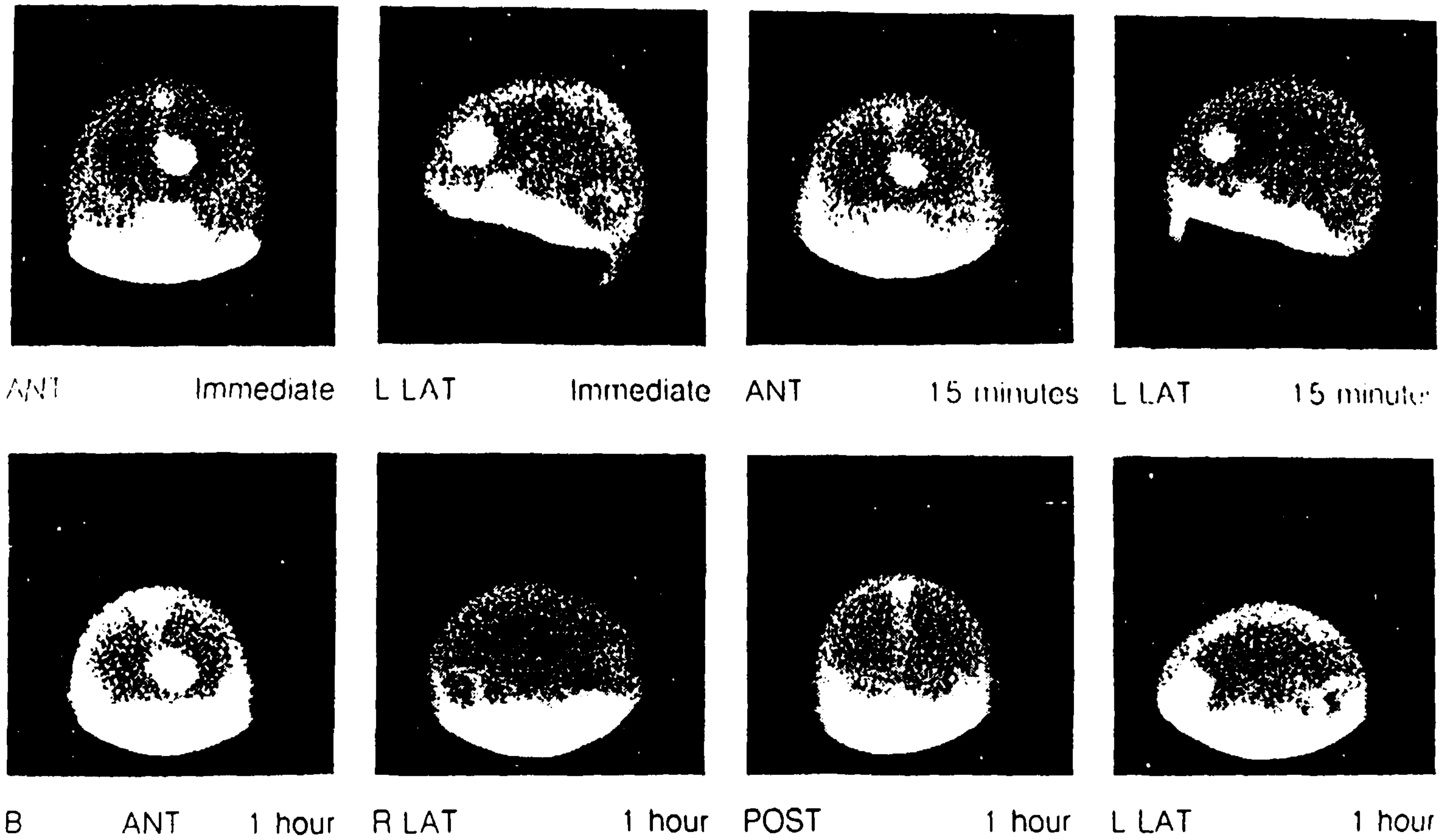

Fig. 20.4 Serial Scintigram: Para sagittal meningioma showing intense early radionuclide uptake which remains in delayed images also.

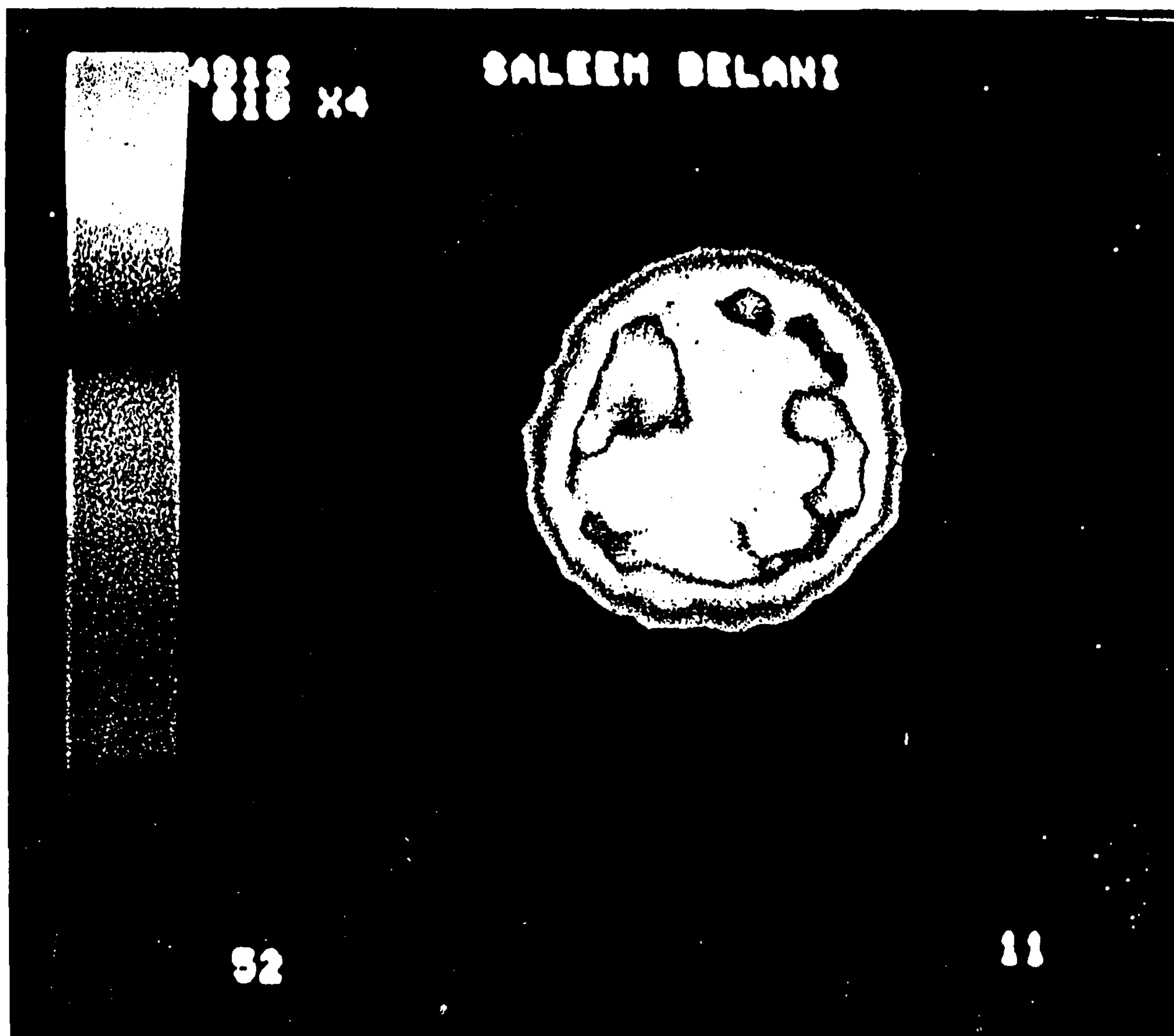

Fig. 20.5 $^{99}Tc^{m}$ HMPAO SPECT study showing diminished perfusion in the region of internal capsule on the left-side. CT scan was normal. Patient had sudden onset right sided hemiplegia.

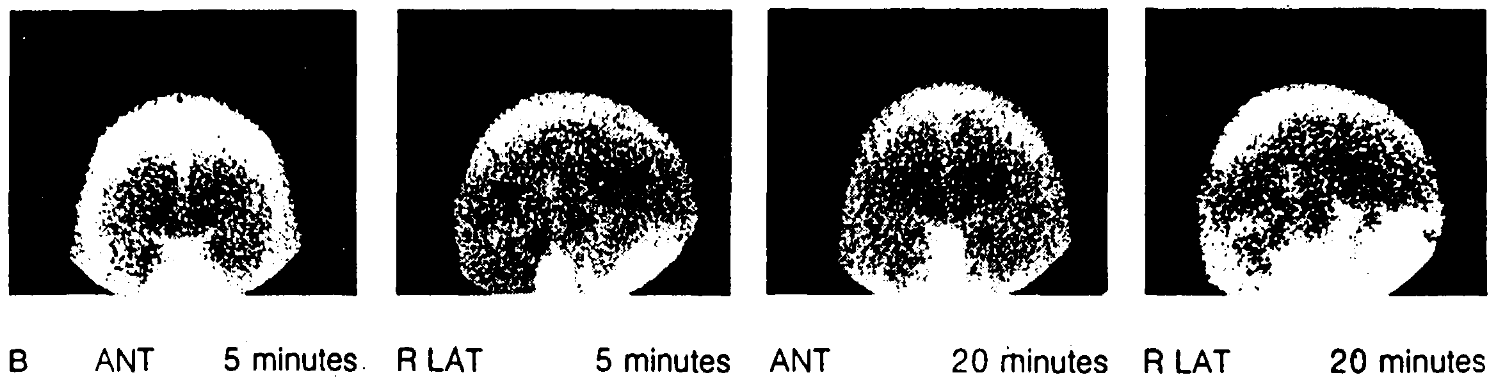

Fig. 20.6 Serial Scintigram. AV malformation Rightside, better seen in the early image (arrows) than in delayed images.

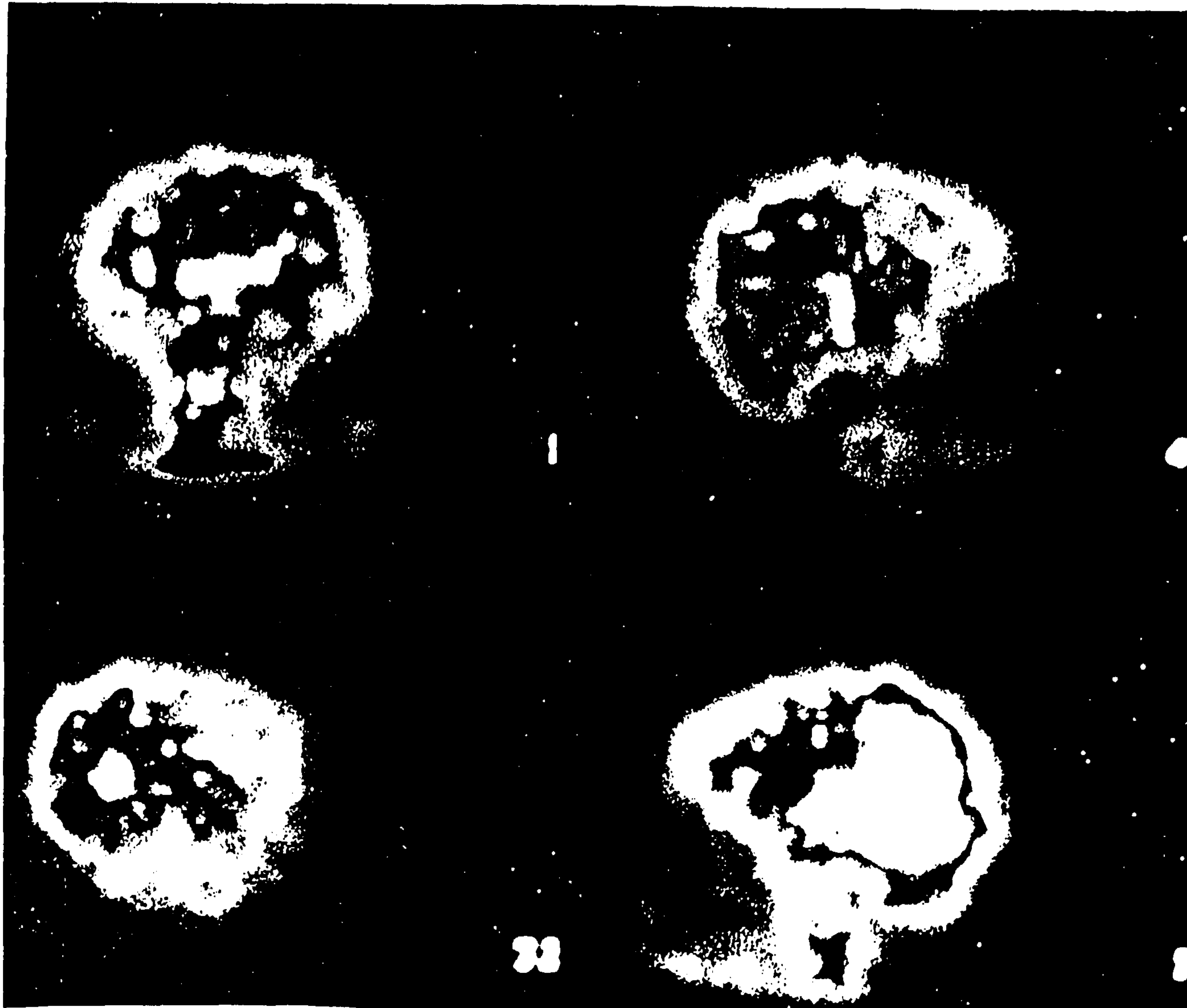

Fig. 20.7 $^{99}Tc^m$ HMPAO study in Herpes Encephalitis showing marked uptake in LT parieto-temporal region.

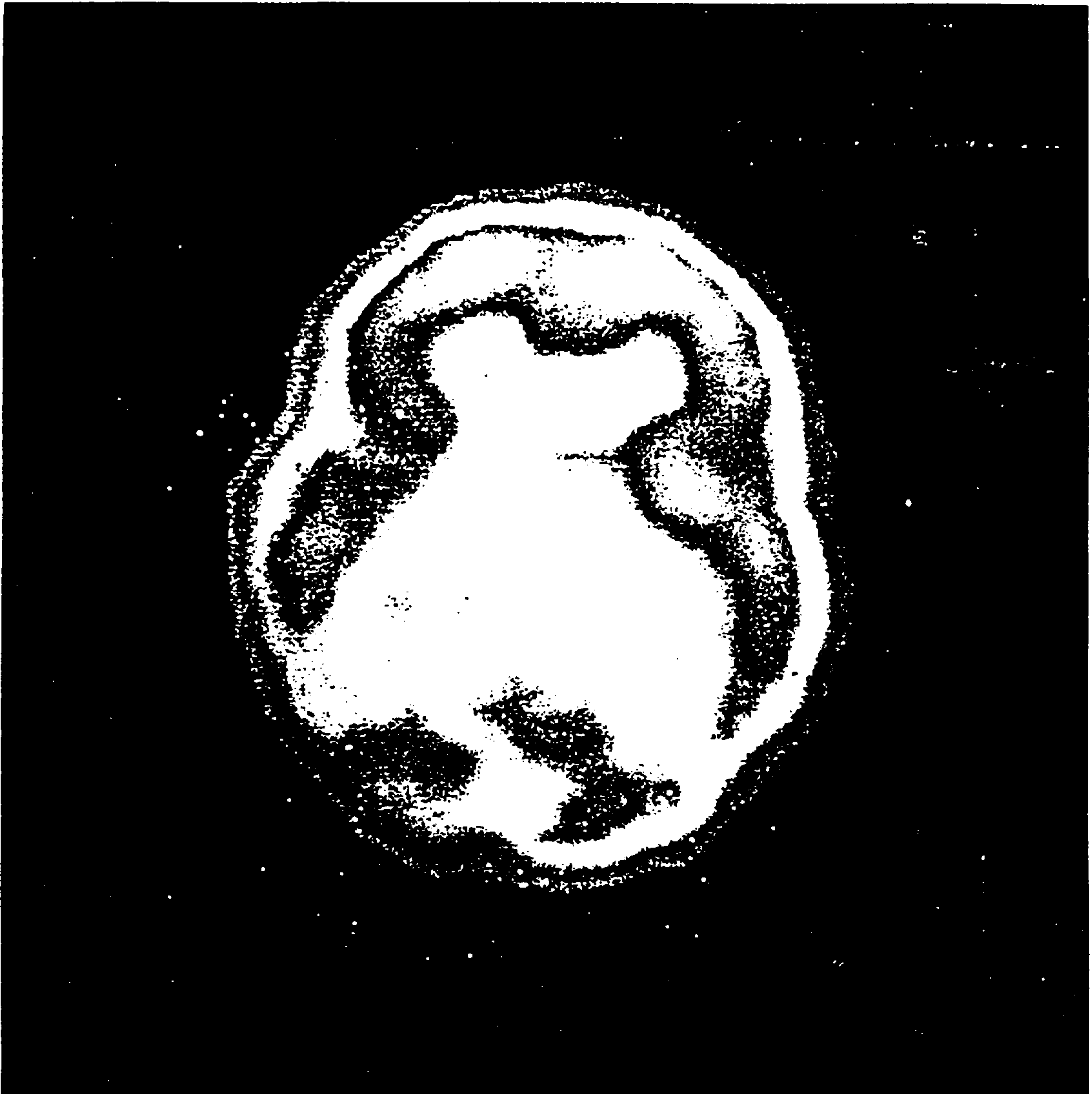

Fig. 20.8 $^{99}Tc^{m}$ HMPAO SPECT STUDY (Transaxial slice) in interictal phase showing decreased perfusion in the right parieto-occipital region.

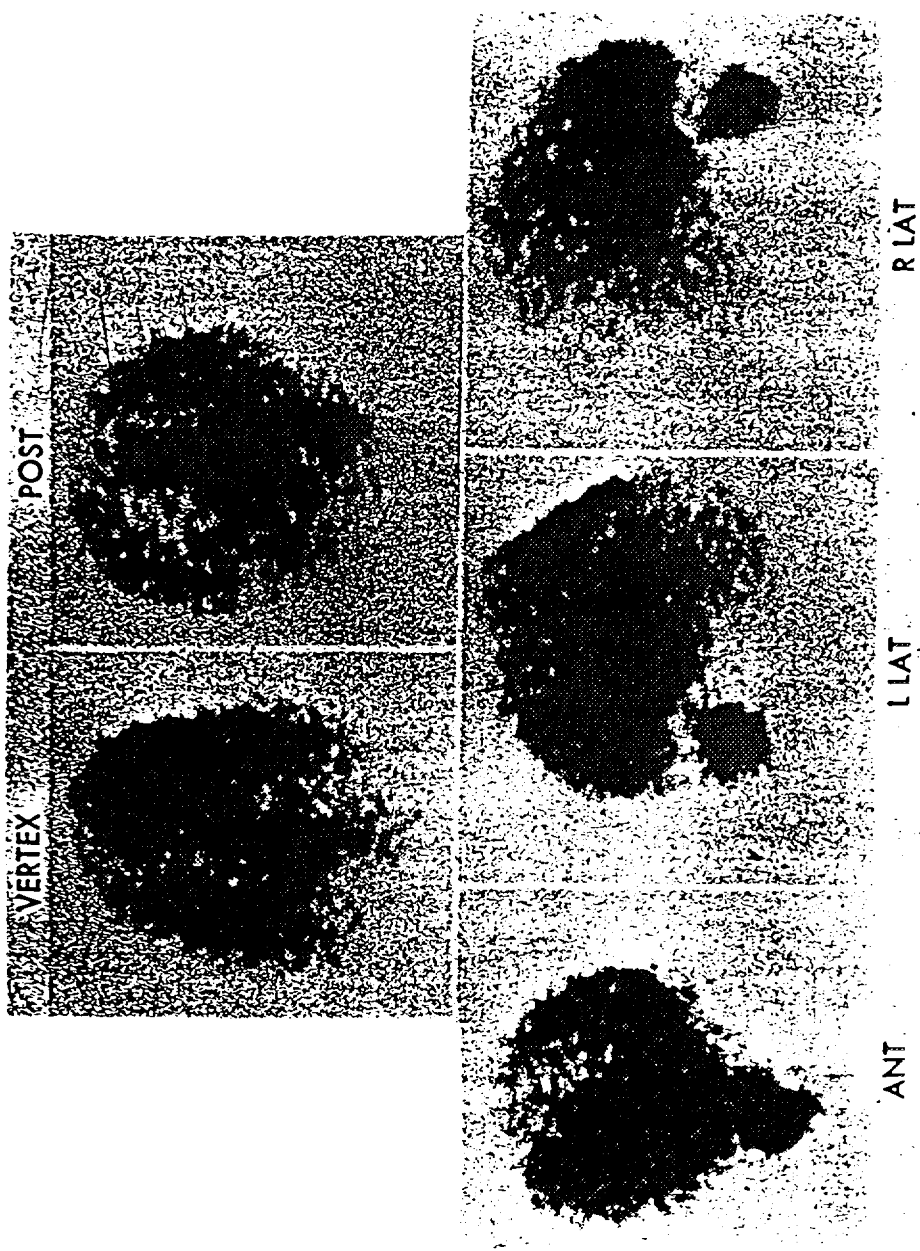

Fig. 20.9 Cisternogram showing CSF rhinorrhoea.

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Chapter 21

STRATEGY FOR THE DIAGNOSIS OF THYROID DISORDERS IN THE DEVELOPING COUNTRIES

Makumkrong Poshyachinda

Introduction

Thyroid disorders may manifest as abnormalities of anatomy or function of the gland. Most of them can be diagnosed after a careful clinical history and physical examination. Nevertheless, laboratory evaluation is important for an accurate diagnosis before embarking on treatment. There is also a small but a significant fraction of thyroid diseases that can be diagnosed only on the basis of laboratory findings. Often a diagnosis of thyroid disease may need more than a single test. Hence, it is essential to select appropriate tests for thyroid patients by their reliability, precision and overall cost.

Many thyroid function tests are nuclear techniques and this trend is not likely to change in near future. Radionuclides that are commonly used in the evaluation of thyroid disorders include iodine-131 (^{131}I), iodine-123 (^{123}I), iodine-125 (^{125}I) and technetium-99m ($^{99}Tc^{m}$-TcO4).

These tests may be divided into two different groups: "in vivo thyroid function tests" and "in vitro thyroid function tests".

THYROID HORMONE PHYSIOLOGY.

All chemical forms of iodine are converted to iodide before absorption. The uptake of iodide by the thyroid is dependent on the dietary intake of iodine. The uptake is low if the daily iodine intake is high and it is high when the intake is low. Other anions such as pertechnetate, perchlorate and isothiocyanate also use a similar transport mechanism for entry into the gland.

The iodine is trapped in the thyroid follicles and is converted to a labile form, which immediately iodinates the tyrosine molecules in the thyroglobulin matrix. Iodinated tyrosines are then "coupled" to produce thyroxine (T4) and triiodothyronine (T3). In the normal thyroid gland, T4 is formed about 10 times more abundantly than T3. Under stimulation by thyroid stimulating hormone (TSH) or in the presence of iodine deficiency, T3 becomes relatively more abundant.

Circulating T4 and T3 are bound to the transport proteins: thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin. The most important transport protein is TBG. Approximately 0.02% of T4 and 0.25% of T3 remain free. These free hormones are in a state of continuous dynamic exchange with the bound forms. The free hormones are biologically active and responsible for regulating the synthesis and secretion of TSH by feedback control. Usually the concentration of free hormones in serum correlates with the metabolic state of the patient.

Approximately one third of secreted T4 is normally converted to T3 peripherally, and this constitutes 80% of the circulating T3. Several drugs and conditions alter this conversion rate as shown in Table I. T3 has 10 to 20 times the metabolic potency of T4. In normal subjects, T3 accounts for over 80% of the metabolic activity of the thyroid hormones.

TABLE I. Drugs and Conditions associated with decreased peripheral conversion of T4 to T3

Pathophysiological conditions	Drugs
Fetal thyroid	Propylthiouracil
Systemic illness	Glucocorticoids
Kidney and liver disease	Iopaonic acid, ipodate
	Propanol, Amidarone

IN VITRO THYROID FUNCTION TESTS.

These tests measure the levels of thyroid and related hormones in circulation and the thyroid hormone binding ratio. The application of radioimmunoassay (RIA) methods for the diagnosis of thyroid disorders, over the last two decades, has lead to better understanding of thyroid physiology and significantly improved the management of thyroid diseases.

Radioimmunoassay method.

These methods have been described extensively in a separate Chapter but few of the salient factors as they apply to the thyroid hormone assays will be recapitulated here briefly. The principle of RIA test is based on the displacement of a radioactively labelled ligand of the substance to be measured (^{125}I-T4 or ^{125}I-T3) by that substance in the sample to be measured in the presence of a highly specific binding receptor such as monoclonal antibody. At equilibrium of a ligand (T4 or T3) and its binding antibody (T4Ab or T3Ab), separation of the bound(B) fraction from the free(F) fraction is done by various means like charcoal adsorption, precipitation by a second antibody, or by antibody coated to the walls of the reaction tube or to glass beads. A standard curve is constructed by adding increasingly larger known quantities of pure substance (T4 or T3) to a constant amount of radioactive ligand and the antibody mixture. Bound to free (B/F) ratio is plotted against concentration of the added substance. The quantity of the substance to be measured (T4 or T3) in serum sample can be estimated by fitting its B/F ratio on the standard curve.

Current RIAs for T4 and T3 are very sensitive, specific and relatively cheap. Because of a wide range of levels of thyroid related hormones in normal subjects, it is crucial that each laboratory establishes its own normal range.

Total serum T4.

The concentration of total T4 (TT4) in normal adults ranges from 6 to 12 μ/dL. The same normal range is valid for both sexes and all ages above 6 years. Values below or above this range, in the absence of thyroid dysfunction, are usually due to changes in the concentration of TBG (Table II). These changes may be congenital or acquired, less commonly due to a displacement of T4 from TBG as with phenytoin (Dilantin) or salicylate. The level of oestrogen in pregnant women especially in the last two trimesters raises the concentration of TBG, TT4 and TT3.

TABLE II. Factors causing changes in the concentration of TBG.

Increase	Decrease
Pregnancy	Nephrotic syndrome, Hypoproteinaemia
Cirrhosis, hepatitis	Severe chronic illness
Congenital	Congenital
Oestrogens	Androgens
Tamoxifen	Glucocorticoid

The level of TT4 may not always correspond to the free T4(FT4) concentration. The TT4 level can be elevated without thyrotoxicosis in patients with familial hyperthyroxinaemia due to abnormal albumin, hereditary excess of TBG, the presence of antibodies binding to T4, and the thyroid hormone resistance syndrome.

The TT4 level may be low or normal in thyrotoxic patients who have depressed serum levels of T4-binding protein because of severe illness or on a congenital basis. Such a situation of intercurrent illness may be quite common in patients of the developing countries. Thus thyrotoxicosis may exist when TT4 level is in the normal range; measurement of the FT4 or thyroid hormone binding ratio usually obviates this source of error.

Total serum T3.

The normal concentration of total T3 in adults is 80 to 180 ng/dL. There is a slight continuing decline of serum T3 with age from childhood onwards. Changes in serum TBG concentration also have an effect on the serum T3 level and many more factors influence levels of serum T3 than those affecting serum T4 levels.

In both thyrotoxicosis and hypothyroidism, the T3/T4 ratio is elevated as compared to euthyroid persons. This elevation is due to the disproportionate increase in serum T3 concentration in thyrotoxicosis and a lesser diminution in hypothyroidism relative to the T4 concentration. Accordingly, measurement of the serum T3 level is a more sensitive test for

the diagnosis of hyperthyroidism, and that of T4 more useful in the diagnosis of hypothyroidism. In patients with iodine deficiency, which is common in many parts of the third world, there is a relative increase in T3, as a part of body's attempt to conserve iodine. This fact should be borne in mind when interpreting results of these thyroid function tests in patients in the developing countries.

ESTIMATION OF FREE THYROID HORMONE CONCENTRATION.

Thyroid hormone binding ratio.

This test measures binding of the thyroid hormones to the transport proteins by the saturation analysis method. It used to be called T3 resin uptake test. It is very helpful in interpretation of the T4 and T3 values when concentrations of serum thyroid hormone binding proteins are abnormal.

The test is performed by adding known amount of ^{125}I-T3 and an anion-exchange resin or charcoal to the patients's serum. The resin or charcoal will compete with the serum TBG for the ^{125}I-T3. The resin is then separated from the serum and is counted for radioactivity. At present, an antibody-coated tube is commonly used instead of the resin. The percentage of bound T3 is related to a normal serum sample range, and the percent uptake then becomes an inverse ratio. This ratio is an inverse measure of deviation of TBG concentration in the sample from a mean normal plasma standard multiplied by total T4 or total T3. This parameter is termed free thyroxine index (FT4I) or free triiodothyronine index.

Free thyroxine index and free triiodothyronine index.

The FT4I can be calculated by the following formula:

FT4I = Total T4 x Thyroid hormone binding ratio

The FT4I and FT3I correlate closely with serum FT4 and FT3 levels although these values are actually different from the values for the concentrations of FT4 and FT3. The FT4I correlates well with the patient's metabolic state unless binding protein concentrations are grossly abnormal. Thus abnormal FT4I value may occur in euthyroid patients with deficiency of TBG or hereditary binding defect. Depressed FT3I value is also observed in some severe non-thyroidal illness. The other disadvantage of this indirect measurement of free thyroid hormone is the additional cost and probability of the error implicit in the conduct of two separate measurements. Nevertheless they offer considerably greater diagnostic accuracy than measurement of total hormone concentrations alone.

Free T4 and free T3.

The FT4 in normal subjects ranges from 1.0 to 2.2 ng/dL. The normal FT3 values are 0.25 to 0.65 ng/dL. A variety of commercial RIA kits for measurement of FT4 and FT3 concentration is available in the market but most of these tests do not measure the true free hormone concentration directly. These immunoassay techniques may be categorized on the basis of the method by which reaction of the tracer with serum protein is prohibited. "Analogue" methods employ a ^{125}I derivative of thyroxine as tracer that is chemically modified to inhibit its reaction with the protein. "Non-analogue" kits use ^{125}I-thyroxine as tracer and avoid reaction with serum proteins by physical means. The reliability of the current analogue methods has been questionable, and not sufficient for laboratories in the developing countries to embark on its routine use.

Occasionally in euthyroid sick patients, FT4 values may be falsely low or high due to the presence of an abnormal factor in the patient's plasma that binds or displaces T4 from normal TBG. FT3 is more frequently abnormal in this group of patients. Patients who have a generalized resistance to thyroid hormones at the receptor level may also present with high FT4 and FT3 concentrations.

Thyrotropin.

Thyroid stimulating hormone (TSH) increases markedly in the first few minutes after birth and falls by the third day to a normal adult level and remains so thereafter. RIA of TSH is an important and widely used investigation for detecting abnormalities of thyroid function. Its predominant use is in the diagnosis of hypothyroidism. A rise in TSH level is the earliest indicator of primary hypothyroidism. However, the method is not sufficiently sensitive to measure low levels of TSH accurately. TSH levels in normal subjects range from 0 to 8 mU/L depending on the assay method. TSH levels above 30 mU/L are usually associated with clinical hypothyroidism.

Recently, ultra-sensitive TSH (US/TSH) or super-sensitive TSH (SS/TSH) assays have been developed. These assays are immunoradiometric assays (IRMA) in which the antibody and not the antigen is labelled and usually the assay deploys two separate monoclonal antibodies of different specificities to react with separate loci on the TSH molecule. This method has a far greater sensitivity and a wider range of measurement than the RIA method for TSH. It can easily measure high, normal and low values in human serum. The SS/TSH value in normal subjects is 0.5 to 5.0 mU/L. A normal serum SS/TSH concentration, regardless of the T4 or T3 level, is strongly suggestive of euthyroid state. The SS/TSH assay is likely to have an extensive role in thyroid function testing. It may totally replace the conventional RIA for TSH and also eliminate the need for confirmatory TRH testing in patients with suspected hyperthyroidism.

CHAPTER 21

RADIOIMMUNOASSAY KITS VS BULK REAGENTS.

A large number of commercial RIA or IRMA kits for measurement of thyroid related hormones are available in the market. The high cost of the commercial kits limits its utilization in the developing countries. Moreover, the internal quality control of the assays may not be optimum due to the tendency to reduce quality control tubes as a cost-cutting measure. In order to reduce the costs and increase analytical reliability of the assays, the IAEA launched a Regional Project to support the developing countries by introducing the use of bulk reagents for assays of thyroid hormones and TSH. At the same time, the Project set up programs to educate the participating laboratories in the quality control measures for these assays. In few of the relatively advanced laboratories in the Region, it was possible to promote indigenous production of reagents. The project was exceptionally successful. Bulk reagents have been adopted for routine clinical services in many of the participating laboratories. The impact of this project has also improved the reliability of the assays and reduced the cost of the reagents, thereby helping these laboratories to expand their clinical services.

IN VIVO THYROID FUNCTION TESTS

Radioactive iodine uptake

This test was employed extensively in the diagnosis of both hyperthyroidism and hypothyroidism but its use has considerably declined after the development of RIA for the measurement of thyroid related hormones. Currently, it is used mainly in patients being evaluated for radioiodine therapy. It is also used, albeit infrequently, for the diagnosis of thyroid disorders like congenital hypothyroidism due to peroxidase enzyme deficiency (Pendred's syndrome), hyperthyroidism caused by latent thyroiditis and exogenous thyroid hormone ingestion. The uptake of radioiodine will be low or absent in this type of hyperthyroidism.

The test is performed by determining the uptake of the radioiodine by the thyroid gland during the first two to four hours and then at 24 hours after the administration of 5 to 10 μCi of ^{131}I orally. The 48-hour uptake is done in patients with rapid turnover of radioiodine in the thyroid gland. This kind of uptake profile is helpful in adjusting ^{131}I dose for the treatment, which may have to be postponed in patients with low uptake of ^{131}I or rapid clearance of ^{131}I. The uptake is expressed as percentage of the administered dose, which is determined by comparing the counting rate over the neck with a counting rate of a standard in a neck phantom. Further details are given separately in this Handbook in another Chapter.

The disadvantages of the radioiodine uptake include

(a) the variation of uptake values due to alterations in dietary iodine intake, iodine-containing drugs, iodine containing radio-contrast media, etc.

(b) considerable radiation dose to the thyroid gland and

(c) several days that are required to complete the test.

Thyroid suppression test

The thyroid suppression test is based on the principle of feedback regulation of thyroid function. When the circulating thyroid hormone level increases, it suppresses the pituitary TSH release. This, in turn, will decrease the thyroid function. This test is frequently used for differentiating between iodine deficiency disorder and mild thyrotoxicosis since the former is widely prevalent in many developing countries. Although the "TRH stimulation test" has largely replaced "T3 suppression test" in the developed world, the high cost of this newer test limits its use in the developing countries. Thus T3 suppression test is still frequently used whenever necessary.

The thyroid suppression test is usually done by determination of thyroid uptake of radioiodine before and after the administration of 75 μg T3 daily in three divided doses for 7 days (T4 may be used by giving 300 μ T4 daily for 3 weeks). Normally, the thyroidal radioiodine uptake falls by 50 percent or more after administration of T3 (or T4). Failure to suppress uptake to this level indicates thyroid autonomy, which is a hallmark of hyperthyroidism. Non-suppressibility also occurs in some euthyroid patients with Graves' disease or nodular goitre. This test should NOT be done in patients with heart disease, severely ill patients and elderly patients because they may not tolerate the administration of thyroid hormones.

Thyrotropin-releasing hormone (TRH) stimulation test

This test is designed to measure the ability of the pituitary to produce TSH after stimulation with TRH. It is likely that this test may not be necessary when the ultra-sensitive TSH assay becomes common.

The TRH stimulation test is performed by determination of serum TSH before and after giving a bolus intravenous injection of TRH in the amount of 400 μ/ 1.73 sq. meter of the body surface area. Serum samples are collected at 0, 15 and 30 minutes. Normally, the peak TSH level should be between 4 and 20 mU/ml above the basal level at 30 minutes.

In primary hypothyroidism, the basal TSH level is raised and the response to TRH is usually greater than 20 mU/mL above the basal level. In hyperthyroidism, the basal TSH level is suppressed and shows no response after the injection of TRH. The suppressed TSH response is also observed in various other conditions such as euthyroid patients with multinodular goitres, functioning adenoma, euthyroid Graves' disease, etc.

The test is useful:

(a) For distinguishing primary hypothyroidism from hypothalamic disease,

(b) For diagnosing mild primary hypothyroidism when other investigations are equivocal,

(c) For the diagnosis of mild hyperthyroidism when other data is equivocal.

Discharge test

This test is used for demonstrating organification defect of iodide in the thyroid. It is done by frequently measuring thyroidal radioiodine uptake over 2 hours after the administration of ^{131}I followed by one gram of potassium perchlorate (or potassium thiocyanate) given orally. In a normal person, iodide accumulation ceases promptly and the radioactivity in the thyroid may be discharged up to 10% during the next two hours. In patients with a block in organification, a significant fraction, greater than 15%, of the accumulated radioiodine, is discharged from the gland within few minutes.

THYROID SCAN.

Radionuclide scanning is an important investigation in the management of patients with thyroid diseases. However, the development of other techniques such as ultrasonography and fine needle aspiration cytology have, in recent years, altered the traditional role of radionuclide thyroid scanning.

$^{99}Tc^{m}$ as pertechnetate is most suitable for thyroid imaging because of its physical properties. The image can be performed within 20 minutes after an intravenous injection of 2 mCi of $^{99}Tc^{m}$-pertechnetate. More important, the radiation dose is much lower than that with ^{131}I. The other suitable radionuclide for thyroid scanning is ^{123}I since it has also the desirable physical properties and being an isotope of iodine it is physiologically compatible with the thyroid gland. But it is not possible to use ^{123}I in the developing countries due to its high cost and difficulties in obtaining it. ^{131}I is still largely used for thyroid imaging in the developing countries when the available instrument for imaging is a rectilinear scan. ^{131}I has also the advantage that it permits delayed imaging of the gland at 24 hrs and even later if required. Thyroid scan with ^{131}I is usually acquired with uptake prior to radioiodine therapy. In addition, ^{131}I is more satisfactory in cases where retrosternal thyroid tissue is to be visualized because attenuation of the photons by bone is much less with ^{131}I than with $^{99}Tc^{m}$ or ^{123}I.

Thyroid imaging can be done by a gamma camera with a pin hole collimator or by a rectilinear scanner. However, rectilinear scanner is preferred when ^{131}I is employed since the

high energy of this radionuclide is not ideal for gamma camera imaging. By and large, in nuclear medicine departments of most of the developing countries, it is still common to use a rectilinear scanner and ^{131}I for thyroid imaging. This practice also spares the gamma camera for large organ imaging and dynamic function studies.

The indications and usefulness of thyroid scan are as follows:

(a) Evaluation of the function of thyroid nodule,

(b) Diagnosis of retrosternal goitre,

(c) Investigation of congenital hypothyroidism,

(d) Management of thyrotoxicosis,

(e) Management of thyroid cancer,

Evaluation of the function of thyroid nodule

Thyroid nodules are classified as non-functioning (cold), functioning (warm) and hyper-functioning (hot) nodules. A cold nodule is usually demonstrated in the scan as a focal area of reduced or absent tracer uptake while a hot nodule shows greater uptake than that in the extranodular tissue (**Fig. 21.1A and 21.1B**).

Most of the cold nodules are benign. It may represent adenoma, cyst, colloid nodule, or focal subacute thyroiditis. A hot nodule may represent autonomous functioning thyroid nodule (AFTN), compensatory hyperplasia, or agenesis of one lobe of thyroid.

Thyroid scan has been most widely used to distinguish a non-functioning from functioning nodule because probability of malignancy in a hot nodule or a multinodular goitre is low as compared to a cold nodule. Thyroid cancer occasionally can appear hot with a $^{99}Tc^{m}$-pertechnetate scan and cold with radioiodine scan because the pertechnetate scan is done soon after the administration of the radionuclide, and the radioiodine scan is done usually after 24 hours. The earlier scan shows the concentration because of the vascularity and trapping, while the later scan shows trapping and the later organification of the iodine. Such a mismatch between the scans obtained with the two radio-pharmaceuticals suggests the possibility of a malignancy which remains to be excluded by other means. It is, therefore, always advisable to confirm and establish the diagnosis of a functioning nodule with radioiodine scan. However many adenomas and carcinomas have a good blood supply and may occasionally show a warm nodule on a scan. The combined use of radionuclide

angiography and static scan would increase the diagnostic efficacy as it can demonstrate the vascularity of a nodule.

Ultrasound is very useful for discrimination between solid and cystic lesions but it cannot differentiate between a functioning and a non-functioning nodule. Development of fine needle aspiration (FNA) for cytology has resulted in significance has increased the detection rate of malignancy in the nodules pre-operatively and thereby reduced unnecessary surgery. All non-functioning solitary thyroid nodules should have FNA cytology since it is an efficient definitive method for detecting cancer in a cold nodule on the scan. Some investigators use FNA as their initial investigation but it could lead to an increase in surgical rate since functioning nodules often have suspicious cytology. Nevertheless many thyroidologists prefer a combination of ultrasound and FNA cytology for evaluation of solitary thyroid nodule. Accuracy of FNA largely depends on the experience of the cytologist, something which should not be difficult to cultivate in a developing country.

Focal subacute thyroiditis may present as a thyroid nodule and appears nonfunctioning on a scan. Follow up scan is useful since it will show recovery of function in a previously cold nodule.

The diagnosis of autonomous functioning thyroid nodule (AFTN) should be confirmed with radioiodine scan or functional analysis that it does possess autonomous function, unless the patient is obviously hyperthyroid. The degree of suppression of extranodular activity on the scan depends on secretory activity of the AFTN, if secretory activity increases, TSH will be progressively suppressed. There is little or no visualization of extranodular tissue in toxic AFTN. Most AFTN are nontoxic and spontaneous degeneration of the nodule is common. Therefore nontoxic AFTN are usually left untreated. Degeneration of AFTN appears on a scan as central area of reduced activity surrounded by functioning tissue.

Not all hot nodules on a scan are AFTN as already mentioned. Differential diagnosis is important for proper management of patients. Measurement of supersensitive TSH may differentiate compensatory hyperplasia from AFTN. TSH levels should be elevated or high normal in compensatory hyperplasia whereas a suppressed TSH would indicate an AFTN. TRH test can be used if SS/TSH assay is not available. An exaggerated TSH response to TRH would suggest compensatory hyperplasia. The first laboratory evidence of increased thyroid hormone secretion by an autonomous nodule is suppression of the SS/TSH or blunting of TSH response to TRH. This may occur when serum T4 and T3 levels are still within normal range.

Repeat scan after suppression with thyroid hormone can also be used to differentiate AFTN from compensatory hyperplasia but it should NOT be done in elderly patients and in patients with cardiac disease. A large toxic nodule with complete suppression of the other lobe can be differentiated from a diffuse toxic goitre with unilateral agenesis of the other lobe by repeating the scan after exogenous TSH administration. A toxic nodule will show suppressed tissue.

^{201}Tl can be used to demonstrate thyroid tissue not identified by ^{99}Tc^{m} or radioiodine scan including malignant thyroid lesions but its role in evaluating thyroid nodule is still controversial. Ultrasound is a simpler alternative to demonstrate a nonfunctioning, hypoplastic or aplastic lobe of thyroid.

Diagnosis of Retrosternal goitre

Radioiodine scan and chest X-ray are used as initial investigations of retrosternal or intrathoracic goitre. Most of the retrosternal goitres can be demonstrated on the scan with radioiodine **(Fig. 21.2)**.

Investigation of Congenital hypothyroidism

Athyreosis and ectopic thyroid are major causes of congenital hypothyroidism. Thyroid scan is the most valuable investigation for diagnosis of these anatomical defects in infants with congenital hypothyroidism. Athyreosis is suggested when there is no tracer uptake by the thyroid **(Fig. 21.3)**. However interpretation of scan findings as absence of thyroid gland should be in conjunction with other investigations since there are other thyroid disorders and some medications that will cause non-visualization of thyroid on a scan.

Ectopic thyroid is frequently demonstrated in non-goitrous sporadic cretin. The ectopic sites may be lingual, sublingual, submental, pretracheal and mediastinum. The thyroid scan usually demonstrates the aberrant thyroid tissue with absence of radioactivity in thyroid bed **(Fig. 21.4)**. Some patients may present with a midline cervical mass and may be misdiagnosed as a thyroglossal cyst. Apart from establishing the diagnosis of ectopic thyroid, the scan will assist in avoiding unnecessary operation for a mass at the base of tongue or a midline cervical mass.

Management of Thyrotoxicosis

Thyroid scan provides useful information in thyrotoxicosis. It is important to perform thyroid scan in all hyperthyroid patients with nodular goitre in order to differentiate toxic multinodular goitre (Plummer's disease) from Graves' disease with multinodular gland and a toxic AFTN from Graves' disease with a solitary non-functioning nodule. Line of treatment will be different for both these conditions. Thyroid scan should be obtained in thyrotoxic patients with impalpable gland especially elderly patients in whom toxic nodules may be present.

Measurement of thyroid mass is a major factor in calculating therapeutic dose of ^{131}I. Ultrasound is perhaps the best method for the measurement of thyroid volume. However thyroid scan is also used to estimate size of the thyroid especially in patients with impalpable thyroid and recurrent thyrotoxicosis following subtotal thyroidectomy.

Management of Thyroid cancer

Thyroid scan is routinely performed prior to thyroid ablation with ^{131}I in order to estimate residual functioning thyroid tissue after total thyroidectomy for well differentiated thyroid carcinoma. Total body scan following administration of 10 mCi of ^{131}I is routinely used in detecting metastatic thyroid cancer that may take up ^{131}I.

HYPERTHYROIDISM

The most common cause of hyperthyroidism is Graves' disease, which is quite frequent in many developing countries. At our Institute, approximately 300 to 400 patients with Graves' disease are referred every year for ^{131}I therapy. Confirmation of the diagnosis is usually based on thyroid hormone assays except in those patients who are considered suitable for radioiodine therapy, in whom the thyroidal uptake of radioiodine is also included in their investigations. If serum levels of the thyroid related hormones do not establish the diagnosis, TRH stimulation test or the thyroid suppression test is considered. Increasing use of TSH IRMA has reduced the need for these two tests.

Nearly all patients with hyperthyroidism have elevated serum T4 and T3 levels and the elevation in serum T3 is usually greater than that of serum T4. Therefore, serum T3 determination may help to establish or confirm the diagnosis of hyperthyroidism in patients whose serum T4 and free T4 values are only marginally elevated, while the T3 is significantly elevated (T3 toxicosis). The excessive rise in serum T3 is considered to be due to the stimulated thyroid gland and not because of increased conversion of T4 to T3. The estimated frequency of T3 toxicosis in hyperthyroid patients varies from 4 to 30 percent. The greater frequency of T3 toxicosis may result from low dietary iodide intake as in the iodine deficient areas. In our experience, approximately 10% of hyperthyroid patients have T3 toxicosis.

Normal serum total T4 and T3 concentrations are found in hyperthyroid patients with TBG deficiency. Measurement of free hormones is indicated in these patients.

HYPOTHYROIDISM

The common causes of hypothyroidism in the adult are destruction of the thyroid gland by disease, such as Hashimoto's thyroiditis, or as a consequence of therapy for thyrotoxicosis. Congenital hypothyroidism is caused by athyrosis, dysgenesis, or ectopy. Hypothyroidism may arise from pituitary or hypothalamic failure. A definitive etiologic diagnosis is desirable in each patient because patients with hypothyroidism require lifelong replacement therapy.

Subclinical or "compensated" hypothyroidism can be detected only by the raised TSH value. This state of hypothyroidism may last for months or years before the clinical manifestations appear. As the thyroid gland is stimulated, the conversion rate of T4 to T3

within the gland increases, and extra T3 is secreted to keep levels of T3 normal for a considerable period, even after T4 level has fallen below the normal range. As the disease advances, the T4 concentration finally falls below the normal range, and the signs and symptoms of hypothyroidism show up gradually. The T3 level remains normal until very late in the course of the development of hypothyroidism. Thus frequent determinations of serum T3 are of little value in the diagnosis of hypothyroidism. Usually low serum T4 concentrations and elevated serum TSH levels suffice to make the diagnosis of primary hypothyroidism.

Hypothyroidism is a common sequelae of radioiodine therapy of thyrotoxicosis. These patients should be carefully followed up for many years as the onset of hypothyroidism can be very insidious and the abnormal levels of serum T4 and TSH may be the only clue for a long time before the clinical manifestations become evident.

Hypothyroidism arising from a pituitary or a hypothalamic failure shows a low T4 and a low IRMA TSH value. A TRH test should be done in these cases. If there is no rise in the TSH concentration following TRH administration, lesion in the pituitary gland is indicated while a significant rise in TSH concentration would suggest a lesion in the hypothalamus.

Programs for screening newborns for congenital hypothyroidism have been established in various parts of the world. Early recognition of hypothyroid infants is based on the findings of reduced T4 or elevated TSH levels or both. A filter paper blood spot assay is widely used. It has been reported that T4 methodology is simpler and more precise than the TSH measurement in the filter paper samples. High incidence of neonatal hypothyroidism is reported from many iodine deficient areas. In such regions, the screening programs are cost-effective and successful in reducing an important cause of mental retardation. However, all such programs are worthwhile only if a vigorous attempt is made simultaneously to raise the iodine intake of the population.

STRATEGY FOR EVALUATING THYROID FUNCTION IN THE DEVELOPING COUNTRIES

Strategy for determining the status of thyroid function of a patient has been a constant topic for discussion, especially in the developing countries where doing all the tests in each and every patient is prohibitively expensive and often an unnecessary waste of scant resources. It is necessary to recognize the relative role of each test, its cost effectiveness and the justification for the clinical problem posed by each patient. IAEA had organized a Coordinated Research Program on "Optimization of Nuclear Medicine Procedures for the Diagnosis and Management of thyroid disorders in the Developing Countries". In the final report of this project a strategy for thyroid function testing was prescribed for the developing countries. The recommendations suggest T4 as the test of first choice, since T4 is a robust assay providing good discrimination between various abnormalities of the thyroid function.

IRMA TSH is considered as a second test in equivocal cases because it can discriminate between suppressed levels of TSH in hyperthyroids from the normal levels of TSH, thereby obviating the need for TRH test in borderline hyperthyroids. It is also equally effective in the diagnosis of borderline hypothyroids. It was also recommended that this strategy should be tried out in a clinical context on a broader scale in the Agency's Coordinated Research Program (RAS/6/011), linked with the use of bulk reagents for thyroid hormone assays.

Following these recommendations, a project on clinical trial of in vitro thyroid function testing strategy was initiated and interesting results were presented before a Meeting held in March 1990. Within the context of this project, studies carried out by us in 182 patients with Graves' disease are shown in **Fig. 21.5.** The sensitivities of total T4, T3, FT4 and IRMA TSH for the diagnosis of these patients was 88.5, 98.9, 94.5 and 100% respectively. Similar results were reported in a study carried out in another country of the same Region where 141 patients with hyperthyroidism were studied along with a large number of patients with other thyroid disorders and non-thyroidal illnesses. They also proposed that supersensitive TSH test should be the first investigation for patients suspected of having thyroid dysfunction and non-thyroidal illness, and that second line test should be T3 for patients suspected of hyperthyroidism and T4 for those suspected of hypothyroidism or of having non-thyroidal illness. For borderline hyperthyroid or hypothyroid, TRH stimulation test may be necessary.

We encountered a relatively high frequency of T3-toxicosis; therefore our approach to investigations of thyroid function is somewhat different from others (**Table III**). Following the development of supersensitive TSH assay, our strategy has been further changed as shown in **Table IV**.

TABLE III. STRATEGY FOR THE DIAGNOSIS OF THYROID DYSFUNCTION

	CHOICES		
	1st	2nd	3rd
Hyperthyroidism	T3	T4	FT4
Borderline hyperthyroidism	T3	T4	FT4
Euthyroidism	T4	T3	FT4
Borderline hypothyroidism	TSH	T4	TRH
Hypothyroidism	T4	TSH	FT4

TABLE IV. PROPOSED STRATEGY FOR THYROID FUNCTION TESTING

	CHOICES		
	1st	2nd	3rd
Hyperthyroidism	T3	SS-TSH	FT4
Borderline hyperthyroidism	T3	SS-TSH	FT4
Euthyroidism	T4	SS-TSH	FT4
Borderline hypothyroidism	SS-TSH	T4	TRH
Hypothyroidism	T4	SS-TSH	

Non-thyroidal illness.

Total T3 and FT3I are frequently found to be much below the normal level in patients with non-thyroidal illness. A normal FT3I in a severely ill patient may actually represent thyrotoxicosis. Total T4 and FT4I are below normal in most of the patients with non-thyroidal illness but the change is much less than that in T3 and FT3I. In general, the more severe an illness, the greater is the FT4I abnormality. Measurement of FT4 by the analogue methods has not demonstrated superiority over the FT4I measurement in non-thyroidal illness. On the other hand, the non-analogue methods appear to be sufficiently accurate for the measurement of free thyroxine in this condition.

Serum TSH is quite a reliable thyroid function test in non-thyroidal illness but under extreme circumstances, serum TSH tends to be somewhat lower than normal.

Pregnancy

In pregnant women, increased concentrations of TBG, total T4 and T3 especially in last two trimesters have been observed. Significant decreases in free T4 levels in 2nd and 3rd trimesters have been commonly reported. A decrease in TSH levels was also observed in the 1st trimester, although higher or unchanged TSH levels have been also reported.

Treated hyperthyroid and hypothyroid patients

Although SS-TSH is helpful in the diagnosis of hyperthyroidism, it should not be used in hyperthyroid patients during treatment because a lag period of hypothalamo-pituitary axis may lead to misinterpretation of the TSH results. Serum T3 level may be affected by medications that cause decreased peripheral conversion of T4 to T3. Thus serum total T4 may be the best single test for monitoring the treated hyperthyroid patients unless FT4 is indicated for special reasons.

In treated hypothyroid patients, serum T4 level is generally considered to be a reliable indicator of thyroid function. Occasionally serum TSH levels may be needed to assess adequacy of treatment. Recent studies in which TT4, FT4, TT3, FT3 and SS/TSH have all been employed to evaluate the adequacy of the replacement therapy in hypothyroid patients seem to suggest that no biochemical test is reliable for this purpose and it is best to be guided by purely clinical criteria.

CONCLUSIONS.

1. The best single test for screening thyroid dysfunction is serum T4 followed by SS/TSH.

2. For hyperthyroidism, the first line test is serum T4 or preferably T3 in borderline cases or in regions where T3-toxicosis is frequent. FT4I or FT4 is useful, if alteration of binding proteins is suspected. Serum SS/TSH should be the second line test.

3. For hypothyroidism, the first line test is serum T4 followed by serum TSH. In borderline cases, TSH should be the first test, followed by serum T4, if necessary.

In summary, nuclear techniques play a major role in the diagnosis of thyroid disorders. The role of each test should be well understood along with the advantages and the limitations. Choice of an investigation should be on the basis of its justification for each individual patient as well as on its cost-effectiveness. Reliable performance of each test is the most important factor in obtaining valid results. Interpretation of thyroid function test should be with caution and care since misinterpretation may lead to misdiagnosis and inappropriate treatment. Lastly, it should be realized that the most cost-effective procedure for evaluating a thyroid patient is a careful clinical examination. All investigations supplement it, nothing supplants it.

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Fig. 21.1 A: Non-functioning thyroid nodule B: Hyperfunctioning thyroid nodule

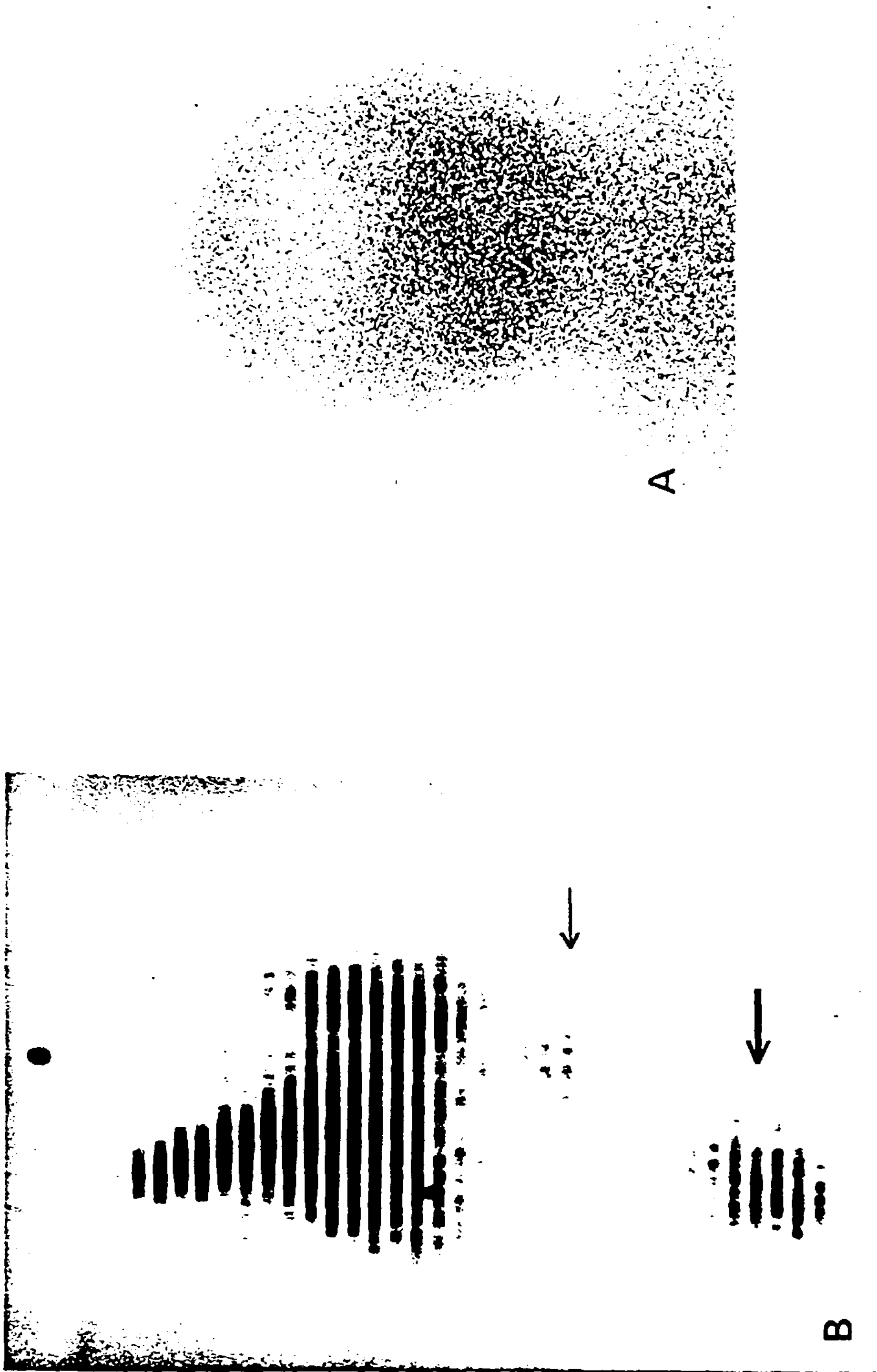
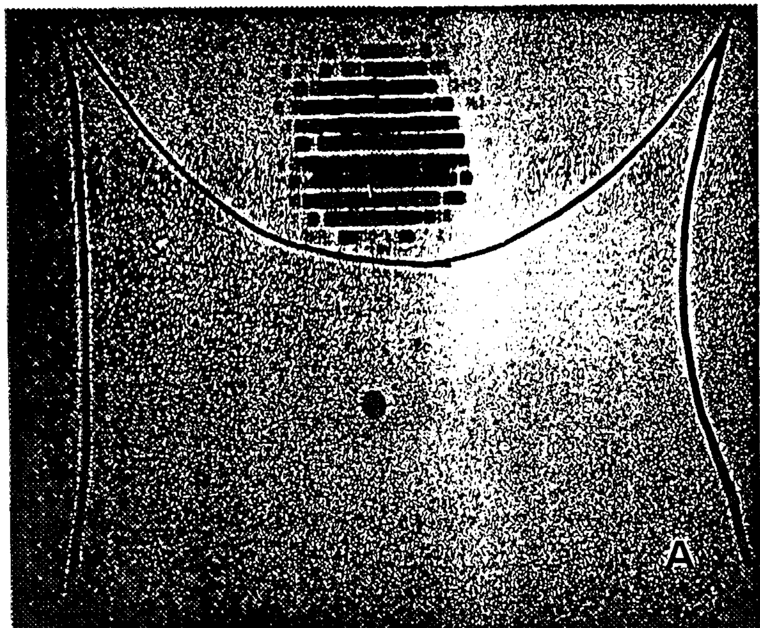


Fig. 21.3 Athyrosis

Fig. 21.2 Retrosternal goitre



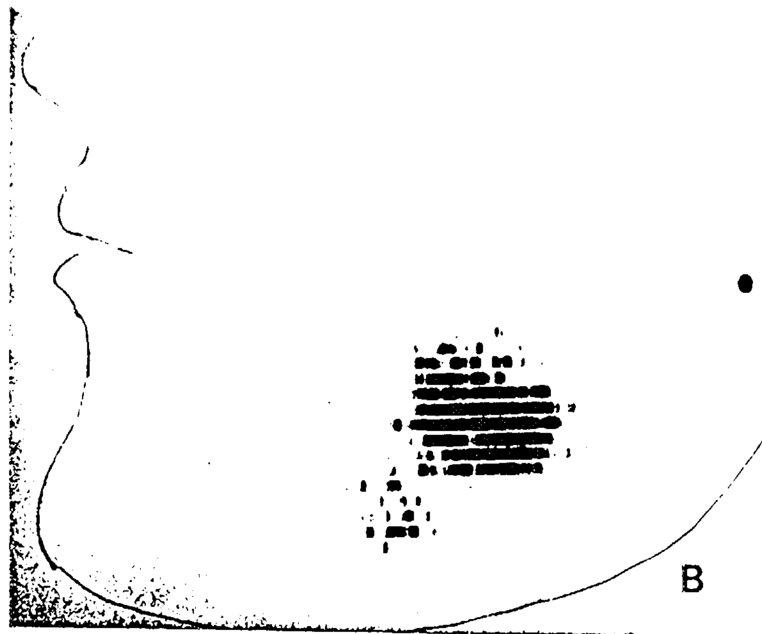


Fig. 21.4 Ectopic thyroid **A:** Anterior **B:** Lateral

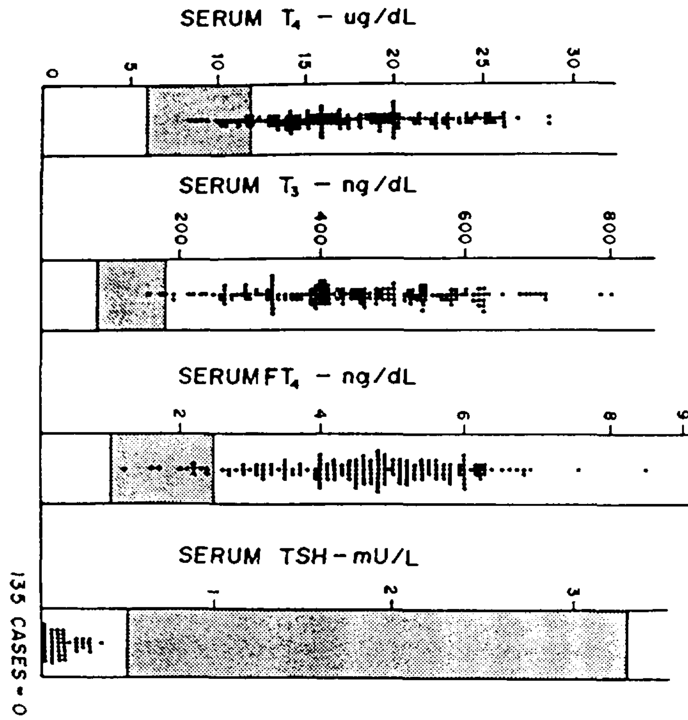


Fig. 21.5 Serum T4, T3, FT4 and SS/TSH concentrations in 182 patients with Graves' disease

CHAPTER 21

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Chapter 22

THYROID UPTAKE TEST

R.D. Ganatra

Measurement of the uptake of the radioiodine by the thyroid gland was one of the earliest applications of the radioisotopes in medicine but in advanced countries it is now seldom carried out. There, the test is considered, more or less, of historical interest only. However, the situation is quite different in the developing countries, where it is still the most commonly performed in vivo nuclear medicine investigation. Not only that, but in many of the laboratories, it is the only test that is being carried out. Reasons for this are quite simple: the radioiodine is easy to obtain, has a convenient shelf life, the requisite instrument is simple and commonly available in a radioisotope laboratory. Moreover, the thyroid is still the largest single referral to a nuclear medicine department from the hospital and the laboratory finds the thyroid uptake test as the simplest to offer.

As far as its clinical utility goes, the reputation of the test is undeserved. Its information content is poor and it gives quite a hefty radiation exposure to the thyroid gland, if done with ^{131}I. Most of the times it provides much less information than the radiation dose that it delivers to the gland, which is in the order of 1.5 rads per μc in the thyroid.

The test has other serious limitations also because it is based on a series of assumptions. It assumes that rapid and high uptake signifies increased hormone production. This is not always true because iodine deficiency, enzyme defect and several other causes can also give a similar uptake pattern. Increased import of the raw material does not necessarily mean increased production.

The uptake of radioiodine by the thyroid gland is also altered by the iodine content of diet or drugs. American diet has a high iodine content because each slice of the white bread contains nearly 150 μg of iodine due to the bleaching process employed in the production of the bread. This carrier content of iodine reduces the uptake so much, that the normal American uptakes are usually three to four times lower than the uptakes in the developing countries. The other drawback of the thyroid uptake test is that it is affected by the iodine containing drugs. Anti-diarrhoea medications are quite common in the developing countries and many of them contain iodine moiety. Without a reliable drug history, a low thyroid uptake value may lead to a misleading conclusion.

The test also produces variable results with methodological changes. Distance of probe from the neck, counting of standard in an appropriate phantom, body background, stability of spectrometer - these are some of the critical factors affecting results of the investigation. As early as 1962, IAEA published recommendation after an advisory group meeting where an ideal protocol was described for performing this test. Unless these recommendations are strictly adhered to, it is not possible to obtain reliable results.

The uptake study at one single time is not likely to lead to an accurate diagnosis. Early uptake, say at two hours, if rapid and high, indicates thyrotoxicosis. 24 hrs uptake as a

single parameter may miss some of the toxic patients because a very high uptake may return to normal by that time. 48 hrs uptake is helpful in finding the rate of turnover of the radioiodine in the thyroid gland. Thus, no single time period is pathognomonic for hyperthyroidism. Any single value is a resultant of trapping, hormonogenesis and washout of the radioiodine from the thyroid gland. No single value but a set of values obtained at different times is more helpful in the diagnosis.

Whenever the amount of stable iodine in the thyroid gland is low, the radioiodine enters a contracted pool of iodine in the gland, in which the turnover of the radioiodine is rapid, and as a result the uptakes are high, in spite of the fact that the thyroid function is basically normal. After surgery also the gland is physically reduced in size which has the same effect as the contracted pool of the radioiodine.

Some variations of the basic thyroid uptake tests are mentioned below but without details of the procedures because they are carried out very infrequently.

(a) **Neck - thigh ratio** is designed to eliminate the influence of high body background in the early stage.

(b) **Thyroid clearance test** measures the rate of clearance of radioiodine from the circulation after an i.v. injection of the radioisotope.

Thyroid clearance in ml/min = Thyroid uptake(150 min) - thyroid uptake(60 min) / 90 x plasma radioactivity (% dose/ml at 105 min)

(c) **urinary excretion of the radioiodine.** Assumption is that what is not picked up by the thyroid gland is eventually excreted in the urine. However, collection of the urine for 24 or 48 hrs is not likely to be reliable.

(d) $^{99}Tc^{m}$ thyroid uptake has not been very popular because this radioisotope is not a physiological counterpart of radioiodine, although it is avidly picked up by the thyroid gland. Early uptake after an i.v. injection shows the rate of trapping of the radioisotope by the gland and helpful in the diagnosis of hyperthyroidism.

Tests for estimating the level of the thyroid hormone in the blood.

(a) $PB^{131}I$ measures the protein bound iodine as % of the administered dose per litre of plasma. It is a crude measure of the circulating thyroid hormone and is affected by the size of the stable iodine pool in the thyroid.

(b) T3 RBC uptake, T3 Resin, T3 charcoal, T3 sephadex. They are all variants of the competitive protein binding assays but not as elaborate as RIA. Their greatest drawback is that they are all affected by the levels of the thyroxine binding globulin in blood and therefore not of any use in pregnant women or in patients taking oestrogens.

(c) RIA of the T3, T4 and TSH. They are discussed separately in another chapter. These assays provide the most direct measure of the circulating thyroid hormone. TSH is the single most important test for diagnosis of the hypothyroidism. Estimation of the T3 levels is useful for diagnosis of T3 toxicosis. This is a condition where T4 levels are normal and the hyperthyroid state is mainly due to high T3. Its incidence is expected to be high in the developing countries because of the endemic iodine deficiency, where the gland produces more of T3 than T4 to conserve iodine.

Test to detect autonomous function of the thyroid gland.

(a) T3 suppression. Involves giving 75 μg of T3 daily for 7 days. Thyroid uptake has to be done before and after this regime. The second thyroid uptake will not be suppressed if the gland is autonomous. In developing countries, it is difficult to obtain T3. T4 can be used instead of T3. 300 μg of T4 are required to be given daily for 3 weeks.

(b) Autonomous thyroid nodule is diagnosed on the basis of thyroid scan. In this condition, the palpable nodule will show good concentration of radioiodine. T3 or T4 suppression regime will not show suppression of the concentration in the thyroid nodule on the second scan.

Tests to detect the hypermetabolic state of the patient.

Except Basal Metabolic Rate, there is no other test which can be used for this purpose.

Rational use of thyroid function tests needs sound understanding of thyroid physiology. Each test studies only one step in iodine metabolism. There is no single test which evaluates the overall thyroid function. Laboratory diagnosis of the thyroid function is a highly developed art. A clinician should judiciously select few tests which would lead him to the diagnosis without much cost in time and money. When one does more than one test, paradoxical and discrepant results are not rare. That is the challenge and fun of being a thyroid expert. Above all, a good clinical examination of patient is most essential before deciding which tests to order for evaluation of the thyroid function. All tests supplement clinical examination, nothing supplants it. That is why most nuclear medicine specialists end up being thyroid experts also.

CHAPTER 22

PROTOCOL FOR THYROID UPTAKE TEST.

Object:

To measure the percent uptake of a tracer dose of ^{131}I by the thyroid gland. This is the simplest and most widely used test to evaluate the thyroid function. After oral administration of radio-iodine, the 2, 24, 48 hour uptake measurements are done to see the rate of uptake, total build up and discharge of radioiodine by the thyroid gland.

Materials:

1. Spectrometer.
2. Flat Field Collimated scintillation crystal probe.
3. Standard Phantom.
4. Standard lead shield; 4" x 4" x ½".
5. Marker.
6. Carrier-free sodium iodide (^{131}I) capsules-25 μCi.

Calibration:

1. Switch on the main supply and power switches on the Spectrometer.
2. After 1-2 minutes, switch on the High Voltage.
3. Increase the H.V. to optimum value.
4. Set the amplifier Gain.
5. Let the instrument stabilize for at least ½ hr.
6. Put the Intg./Diff. switch on Differential and window on 1.0 V.
7. Keep the standard capsule in the phantom 30 cm away from the probe, and find out the photo peak for ^{131}I starting from base line 300 and increasing by intervals of 0.5 V (i.e. 5 divisions) and each time counting for 50 seconds, till the maximum counts are obtained. (Calibration procedures may vary from instrument to instrument). Note the Base line reading.

8. Set the window from 1.0 V to 5 V and decrease the base line setting by 20 divisions. (ie. at 1 V the Base line is 360, hence at 5 V the Base line is 360 - 20 = 340).

9. Count all the capsules, and discard those which show gross discrepancy in counts.

10. Keep one capsule as STANDARD; the remaining capsules are used in patients to study their respective uptakes.

Uptake Measurement:

1. Count the Standard capsule by keeping it in a phantom 30 cm away from the probe by means of a marker. Take 2 readings of 2 minutes each. Calculate cpm. (S1).

2. Place the standard shield near the capsule and count the standard again for 2 minutes. Calculate cpm. (S2).

3. S1 - S2 = Net counts of the standard capsule.

4. Administer the radioiodine capsule to the patient after screening.

5. Two hours later, count the patient's neck, keeping a distance of 30 cm from the probe. Take 2 readings of 2 minutes each. Calculate cpm. (P1).

6. Ask the patient to hold the standard shield in front of the neck. Count for 2 minutes. Calculate cpm. (P2).

7. P1 - P2 = Net counts of the patient's thyroid.

8. $$\% \text{ uptake in the thyroid} = \frac{P1-P2}{S1-S2} \times 100$$

9. Repeat the counting at 24 hours and 48 hours, and calculate the % uptakes.

Limitation of the technique:

1. The test cannot be done in children and pregnant women.

2. Radiation hazard to the patient (?)

Pitfalls of the technique:

1. The prior administration of iodine containing drugs, thyroid hormones, antithyroid drugs, and several other compounds, may invalidate the test for a number of weeks to months.

2. Serial readings are necessary for 3 days for proper diagnosis.

Highlights of the technique:

1. Simple test.

2. Gives dynamic functional information.

SUGGESTED READING.

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Chapter 23

RADIOIODINE THERAPY

J.F. Torres, Jr. and H.B. Deliso

Introduction

For over 40 years now, radioiodine(^{131}I) has remained one of the most useful radionuclide for diagnosis and therapy in Nuclear Medicine. The wide application of radioiodine in the study of the thyroid gland and in the management of its disorders has been most rewarding. The medical literature is replete with reports of its efficacy, failures, and complications, but most of these studies have been conducted among Caucasian persons and in relatively affluent societies. Very few reports are available from the less developed and economically depressed areas of the world where thyroid disorders abound or and are even endemic. This chapter is an attempt to highlight the use of radioactive iodine therapy in the developing countries, particularly those in the Asian region.

Radioiodine therapy in thyrotoxicosis

Patient profile

Clinical assessment of patients suspected to have thyrotoxicosis in less developed areas of the world have been aided by simple scoring indices, the most useful of which, in our experience, is the cumulative scoring index formulated by Crooks and co-workers (**Table I**). A score of ten or less indicates euthyroidism while a score of 18 or more is highly suggestive of thyrotoxicosis. This kind of scoring index describes clinical findings in quantitative terms and helps the doctor in selecting patients for laboratory investigations. Confirmation of the clinical diagnosis by laboratory investigations is necessary before embarking upon definitive treatment like radioiodine therapy.

The thyroid function investigations have gone through a whole gamut of changes in last few decades. A large number of the investigations are nuclear techniques and these are the ones which have developed most in recent years. The emphasis has changed from in vivo to in vitro investigations but still it is not uncommon to come across many laboratories in the developing countries which solely rely on the thyroid uptake test for the diagnosis of hyperthyroidism!

In our setting, hyperthyroidism is said to be present when the clinical data show compatible signs and symptoms plus an abnormal change in or more of the following:

Elevated:

protein-bound iodine,
4-hour and 24-hour radioiodine uptake,
effective thyroxine ratio,
serum triiodothyronine or thyroxine,
free thyroxine index,
free thyroxine

Depressed:

Super-sensitive TSH.

The strategy for thyroid function investigations have been described in another Chapter where it is indicated when and how to deploy the above tests.

Although radioiodine is widely used in the treatment of thyrotoxicosis in the developing countries, the data is not documented and is not readily available for comparison with the Western literature. Whatever is reported is mostly in national and local journals. By and large, the clinical material from the developing countries has poor follow-up of patients. However, one can not be incredulous of the data from these countries and the clinical impression of a large number of nuclear medicine specialists from the developing countries. After all, the very foundation of the medicine has been the observations and perceptions of the practising clinicians. What we describe below is largely on the basis of our own observations and from the analysis of our patients. We can only add the reassuring note that our own conclusions have been largely endorsed by the colleagues from the neighbouring countries.

We have reviewed 1564 patients with thyrotoxicosis who were given radioactive iodine treatment from 1968 to 1985 in Santo Tomas University Hospital Nuclear Medicine Section, 1344 (86%) of whom were females. Age is not taken as an absolute limiting factor in the choice of treatment modality. The relative safety of a therapeutic dose in all age groups had been proven by long term studies by many investigators, some spanning 20 years or more. There has been a recent change in our choice of treatment and we increasingly favour radioiodine therapy in more clinical situations.

In the above Group, 1104 (70.6%) were below 40 years of age, and 7% were children and adolescents **(Table II)**. Radioiodine therapy was the first choice in 61.7 %. A great majority (84%) of patients had clinically diffuse goitres; only 10% had nodular goitres **(Table III)**. About 66% of the thyrotoxic patients were classified as having minimal thyromegaly while 4.6% had marked thyroid enlargement **(Table IV)**.

The high endemia of goitre in some parts of Asia is a known fact. It is not therefore surprising that thyroid surgery is frequently done in many provincial hospitals such that the local surgeons have acquired a very satisfactory level of competence. A Manila-based Medical/Surgical Organization does 20-30 thyroid surgeries in a week, forming 40% of their total surgical work. Hence, the choice of therapy is not influenced by lack of trained surgeons but rather by the clinicians' judgement and experience, together with the personal preference of the patient after the physician's explanation of the pros and cons. Knife is seldom a patient's first choice.

Radioiodine treatment

Initially it was believed that the optimum therapeutic dose of radioiodine for thyrotoxicosis is 160 μCi per gram of thyroid tissue. In an attempt to reduce the incidence of hypothyroidism resulting from this apparently "high" dose of radioiodine many investigators used lower doses ranging from 60 to 120 μCi of ^{131}-Iodine per gram of thyroid tissue. This change does reduce the incidence of early hypothyroidism after therapy but there is an increase in the relapse rate that may subsequently need re-treatment. Moreover, the overall incidence of late permanent hypothyroidism does not differ significantly in both the groups.

We administered a still lower dose of 60 to 80 μCi per gram of thyroid tissue. However, estimation of thyroid size being inexact, it is difficult to derive a precise dose for each patient on the basis of this criterion. Our experience of nearly twenty years indicates that about 5 mCi is an appropriate initial dose for most of the patients. The mean initial dose given in our series was 5.5 mCi and the mean total dose was 5.9 mCi. A total of 1418 (90.6%) patients were given radioiodine only once with a mean first dose of 5.5 $\pm$ 0.9 mCi. One hundred and forty patients had to be given a second dose to achieve euthyroidism.

All of our patients are admitted to the hospital overnight for their convenience, as well as for observation of any immediate reaction to the treatment likely to occur in the elderly and those with prominent cardiovascular and psychological symptoms. Moreover, hospitalization provides a large measure of reassurance to patients and their kin in communities and countries where "radioactivity" is associated with awe and apprehension, if not fear. Most of the patients have poor sanitary facilities at home and their brief hospitalisation is advisable from the point of view of radiation hygiene.

Patients are requested to report to the clinic for follow-up every month for the first 3 months and subsequently every 2-3 months depending on the clinical status of the patient. Once euthyroid state is established clinically and confirmed by laboratory tests, patients are advised to have a follow-up at least once or twice a year.

In our study, 76.9% of the patients were euthyroid after 1 - 3 months. At the time of their last follow-up, 81.2% of our patients were definitely euthyroid (**Table VI**) and none were hyperthyroid. Only 8.1% of patients were given either antithyroid medication or beta-blocker drugs to supplement the radioactive iodine treatment.

Hypothyroidism.

Overall incidence of definite post-therapy hypothyroidism in our series was 3.6% at the time of their last visit. Patients were classified as having definite hypothyroidism if they show clinical manifestations plus at least one of the following laboratory tests showing confirmatory evidence as follows:

= protein-bound iodine less than 3 μg%,

= thyroid uptake less than 10% at 24 hours (usually not very reliable after radioiodine therapy

= effective thyroxine ratio less than 0.87,

= decreased T3, T4 and free thyroxine index or free T4

= unequivocally elevated Thyroid Stimulating Hormone.

Probable hypothyroidism, defined as having equivocal clinical picture and/or laboratory findings, was present in 2.5%. Thus, combining the probable and definite hypothyroidism, overall incidence of post-therapy hypothyroidism amounts to about 7%. This incidence is lower than that observed in other similar trials of lower doses reported from the Western countries. At one year, we observed cumulative incidence rate (probable and definite) of 5.4% **(Table VI)**. Yearly increment of hypothyroidism is about 1.5%. The probable cumulative incidence of hypothyroidism at the end of 12 years follow-up utilizing the life table method was 21.7%; much lower compared to studies done in Western countries. A similar experience by another Philippine investigator was reported with an overall permanent hypothyroidism rate of 8.9%. [Bandong-Reyes E., Phil J Intern Med, 1988]. Sundram from Singapore [Singapore Med J, 1984] also reported a lower incidence of hypothyroidism which was 4% at the end of the first year. On the other hand, Ahmed and co-workers [Bangladesh Med. Res. Counc. Bull., 1988] reported 13.5% incidence of hypothyroidism in their series of 215 patients treated with radioiodine from 1980 to 1988. Sundram (Ann Academy of Medicine, 1986) reported that the prior administration of antithyroid drugs does not make a difference in the final incidence of hypothyroidism.

It is clear that there are other factors, besides the well-studied factors like dose, size of gland, presence of nodules, age, and previous surgery, which determine the occurrence of hypothyroidism. There are still controversies as regards the relationship of immunologic status and radioiodine treatment. It appears that ethnic variation is one of the factors determining the response to therapy and incidence of post-therapy hypothyroidism as well; a further attest to the long recognized difference in therapeutic response to a given dose

among blacks as compared to whites. Masjhur (Indonesia) also reports similar observations on ethnic differences in the response to radioiodine treatment (personal communication). Prior therapy such as iodine supplementation or antithyroid drugs were postulated to offer some protection against hypothyroidism. However, this had not been observed by DeGroot and Holm who reported resistance to radioiodine-induced hypothyroidism in areas with iodine deficiency rather than sufficiency (personal communication). This can possibly explain our low incidence of hypothyroidism as the Asian region has a high incidence of endemic goitre which is mainly attributed to iodine deficiency.

Generally in developing countries, the therapeutic option is greatly influenced by economic considerations rather than risks or benefits associated with the treatment modality. Thus, radioiodine treatment can be considered as a most rational choice as it offers a predictable and satisfactory response without the known high risks of surgery. Cost of a long term anti-thyroid drug therapy also works out to be quite high as the drugs themselves are expensive and the frequent follow-up visits to the doctor and their potential side-effects do not make them an attractive choice for the developing countries. It has been suggested that at least 2 years of anti-thyroid drug therapy is necessary to maximize benefits. In the Philippines, cost of thyroid surgery ranges from $ 200 - $ 1000, while antithyroid medication which may be given for a minimum of 1 year will cost about $ 156. Addition of beta-blockers (propranolol) in the medical management will cost about $200, bringing the total cost of medical drug management to at least $ 300. On the other hand, radioiodine (liquid form) costs $ 200, inclusive of one-day hospitalization. On the whole, radioiodine therapy appears as the least expensive treatment. The low cost of radioiodine, the paucity of side-effects and ease of administration as compared to the other treatment modalities were among the primary reasons for its overwhelming choice by 70% of thyroidologists surveyed in the United States.

Complications.

Transient post-treatment thyroiditis was observed in 10 patients. Patients were either reassured that it was self-limiting and therefore needed no more than observation or were given low dose of dexamethasone or acetylsalicylic acid for a week.

Nodules were palpated in 14 cases whose initial evaluation indicated a diffuse enlargement. Some underwent fine needle aspiration biopsy (FNAB) which showed no evidence of malignancy. The rest were simply observed or subsequently given a suppressive dose of thyroid hormone after the hyperthyroidism was controlled. It is however doubtful whether these nodules resulted from radioiodine treatment.

Radioiodine-induced thyroid storm or vocal cord paralysis were not observed in any of the patients. Transient hypothyroidism lasting for 2-4 months was noted in 24 patients. Cardiac complications attributable to acute exacerbations following radioiodine therapy were not observed in our study.

Radioiodine in nontoxic goitre

Radioactive iodine has been found to be valuable in the management of multinodular non-toxic goitres by some of the workers from the developed countries. We have used radioiodine therapy in 820 patients with large goitres and high 24-hour ^{131}I uptake and obtained good results. Thyroid size regression was notably significant and satisfactory. The incidence of post-therapy hypothyroidism was not very different from that observed in hyperthyroidism. This treatment is particularly suitable in patients with recurrent goitre following partial thyroidectomy. In patients who have contraindication to surgery or who refuse surgery, radioiodine therapy is also a rational alternative. However, before this therapy is widely used, the physician has to shed his fear and inhibition of using radiation in a non-toxic and a benign condition.

Radioiodine in thyroid cancer

While it is generally believed that iodine deficiency somehow increases the incidence of thyroid malignancy especially of the papillary type, there appears to be no convincing evidence so far that it is so. The chronic TSH stimulation of the thyroid gland was shown experimentally to induce adenoma formation. However, there is no known prospective and controlled study concerning the pathogenesis of thyroid malignancy in the setting of iodine deficiency. Statistically, the high endemia of iodine deficiency in Asian countries is not accompanied by a reported increase in the prevalence of thyroid malignancy. It is interesting to note that iodine supplementation in an area highly endemic to goitre in Chang Mai, Thailand, was followed by a significant increase in the incidence of thyroid adenoma as well as carcinoma. [Tantachamroon T, Proceedings of Asia and Oceania Thyroid Association Workshop on Endemic Goitre and Thyroid testing, Singapore, 1978]. In addition, the effect of other goitrogens, especially dietary, to cancer formation has not been adequately studied.

<u>Thyroid Cancer Diagnosis and Classification</u>

There is a paucity of clinical symptoms which can alert a patient to seek early medical consultation for a thyroid mass, though rapid increase in size and the onset of hoarseness or dysphagia can arouse concern. Early diagnosis rests on the clinician's high index of suspicion on the possibility of malignancy in a thyroid mass, particularly a nodular one. A thorough physical examination is essential; findings such as cervical lymphadenopathy, or a hard and fixed thyroid mass can be associated with malignancy. Solitary nodules have a higher incidence of malignancy, although what is appreciated as solitary on clinical examination may be shown to be multiple by high resolution ultrasonography. Furthermore, sonography can also show that an apparently diffuse thyromegaly may be actually nodular. Radionuclide scintigraphy showing a "cold" nodule is an indication to pursue the possibility of malignancy. About 9.5% of the solid cold nodules in 142 patients we studied turned out to be malignant. Scintiscanning is likewise useful in the diagnosis of metastatic disease; it can differentiate the sites as thyroidal or non-thyroidal in origin. The combination of ultrasonography, nuclear

perfusion studies as well as thermography was reported by Lakshmipathi and co-workers from India [Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985] to improve the diagnostic accuracy in the definitive diagnosis of thyroid malignancy. Hypervascularity by perfusion was shown to be highly sensitive (100%) and specific (94%) in their study. In our opinion, selective angiography and thermoscanning may not only be expensive but not very specific in the early diagnosis of malignancy.

Fine needle aspiration biopsy (FNAB) has recently become a widely popular technique in the diagnosis of thyroid malignancy owing mainly to its ease of performance, affordability, minimal discomfort to the patient as well as the high reliability in the hands of experienced cytopathologist. In a private thyroid clinic in Manila, 127 euthyroid patients with solitary nodules were studied from January to March 1990 and FNAB showed 100% accuracy in the 5 patients who subsequently underwent thyroid surgery. Other studies in India by Das, Shah and co-workers showed similarly gratifying results. (Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985) Large series of FNAB in Western countries have repeatedly showed its usefulness. Many investigators propose that FNAB should become routinely as the initial diagnostic investigation in the work-up of patients with solitary thyroid nodules. For the purposes of radiotherapy, it is most important to determine whether the thyroid cancer is well- differentiated or anaplastic and whether the malignancy is confined to the thyroid or has metastatic spread.

Another interesting development is the recent renewed interest in the study of the relationship between Graves' disease and thyroid cancer. This has an important bearing in our diagnostic approach in thyroid diseases; so far, it has been widely believed that thyrotoxicosis is somewhat an assurance that malignancy is unlikely. Thyroid nodules associated with thyrotoxicosis (Toxic nodules) will now have to be evaluated in terms of the likelihood of malignancy in addition to their disturbed metabolic state.

Management

There is no doubt that surgery is the primary treatment for thyroid cancer. However, surgery alone has remained inadequate to improve patient longevity or to ensure cure. Moreover, the extent of surgery in thyroid malignancy remains controversial. The multicentric nature of thyroid malignancies supports our position in suggesting 'near' total thyroidectomy not only to diagnose metastatic lesion but also to maximize therapeutic benefit. The pre-operative diagnosis and subsequent management of thyroid cancer has been enhanced by the increasingly wide acceptance of fine needle aspiration biopsy (FNAB). With improved early diagnostic accuracy, more definitive surgery can be performed. If at all possible, all solitary thyroid nodules should be aspirated for cytological evaluation. Likewise, suspicious multinodular thyroid lesions should undergo FNAB. After near total thyroidectomy is accomplished, post-operative management can become more successful.

The value of radioiodine for ablation of the residual thyroid gland after surgery is unquestionable. Ablation therapy is generally recognized as necessary for the complete

management of well-differentiated thyroid cancer. It is also known that ablation or cure of metastatic foci is possible even in the papillary variety. The data of Sharma in his series of 322 patients with differentiated thyroid malignancy treated with radioiodine-131 revealed a survival rate of 91% up to 15 years. [Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985] In the same series, patients with nodal metastasis showed 97% survival after 15 years following treatment. In the series of 259 differentiated thyroid cancers treated by Sharma and associates, metastatic sites concentrated radioiodine in 47% with follicular cancers showing, as expected, the greatest concentrating ability. Their data showed that 54.3% of papillary cancers took up radioiodine on routine body scan. More recently, Padhy and Gopinath from India (Proceedings of the International Thyroid Symposium, Tokyo 1988) reported very gratifying results with radioiodine ablation in 150 patients with lesions distributed in papillary, follicular, and mixed papillo-follicular groups. They reported seven-year survival rate of 90-100% with or without local or regional metastases, although papillary carcinoma had a slightly better outcome. In our study of 94 patients with differentiated thyroid cancers from 1970- 1990, five-year survival rate was 91%. Our longest surviving clinically stable patient is still being followed 19 years after treatment.

Dose

It is generally accepted that the ablative dose for normal thyroid is 10,000 to 15,000 rads. The formula favoured by us to estimate the treatment dose is:

$$\text{Rads delivered} = 74 \times \bar{E}_B \times \frac{\mu\text{Ci given}}{\text{ml distribution vol}}$$

$$\times \text{ fractional uptake } \times \text{ effective half-life}$$

Where, $\bar{E}_B$ is the average ß energy in meV/disintegration.

In this estimate, the distribution volume (wt. of the thyroid gland), variation in tissue sensitivity and tissue distribution of radioiodine are uncertain and difficult to determine. Accordingly, the administered dose calculated on the basis of this formula is in reality still arbitrary and heavily dependent on the clinician's instinct and experience.

The recommended ablation dose to the thyroid remnant is 80-100 mCi. A higher dose may be given in cases where metastases are likely to be present. The dose used by Sharma et al [Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985] for ablation of thyroid remnant was 50-80 mCi. He utilized a dose of 80 - 250 mCi for functioning metastases. Repeated radioiodine treatments were given if necessary but not exceeding a

cumulative dose of 1000 mCi given over a period of two or more years. It was his experience that lymph node metastases ablation was easy. Complete ablation of bone metastases was most difficult. In our own series, 82% (n=77) received a single dose of 100 mCi which was generally successful while 10.6% had to be given multiple doses. One patient with metastatic papillary cancer was given 1138 mCi over a period of 15 years!

Patient preparation

Prior to ablation therapy, whole body scan is performed using 0.5 mCi to 1 mCl of ^{131}I at least 4-6 weeks after surgery. During this interval, thyroid hormones are not administered. Other investigators give 1 to 5 mCi for the total body scan claiming better visualization of iodine concentrating foci. Further preparation of patients may include iodide pool depletion by dietary iodine restriction or diuretic administration. We give Furosemide for 3 days prior to radioiodine ablation therapy.

Patient isolation and personnel protection

Patients are hospitalized for 2-3 days. Specific orders are as follows:

1. Collect all excreta in sealable containers

2. Collect urine in used IV Fluid bottles. Fill each bottle up to 500 ml mark only. Label each bottle with the time started, ended and date of collection. Female patients may have to use bedpan; urine collected is then transferred to sealable bottles.

3. Have patient use only disposable napkin and tissues. Collect all in separate paper bag. Do not take anything out of the room.

4. Keep all eating utensils in the room at all times.

5. Keep all changed linens in the room until cleared by Nuclear Medicine Staff for laundry.

6. Do not remove any garbage from the patient's room until cleared by Nuclear Medicine staff for disposal.

7. Minimize personal contact with patients. Do not stay closer than one meter from the patient for more than 5 minutes at a time; no more than 30 minutes per shift.

8. Notify any Nuclear Medicine staff for any difficulty or in case of a spill of any kind.

The following are instructions to the paramedical staff and the visitors:

1. Radiation caution sign is placed at the door.

2. No visitors are allowed during isolation period.

3. Persons coming near to the patient should stay at least 5 meters away. This is also applicable to the patient companion who should stay on the other side of the room as much as possible.

4. The length of exposure or contact with the patient should be limited to 5-10 minutes each time or not more than a total of 30 minutes during a 8 hour shift.

5. All urine and excreta should be collected and placed in sealed containers. Used clean IV Fluid bottles are preferable for urine collection. These are collected by a technician everyday.

6. Linens and articles used by the patient will be isolated for at least one month after discharge or 10 physical half-lives if heavily contaminated.

Patients are discharged after careful isolation in the hospital for 2-3 days or until the body contains less than 30 mCi (about 7 mR/hr at 1 meter). In developing countries, it would be economical to set up a centralized isolation facility for patients undergoing radioiodine ablation therapy.

All excreta collected during the patient's stay in the hospital is stored in sealed containers in an isolated waste dump area and may be discarded in the main sewage system, in small aliquots, by a trained staff after 4-5 physical half-lives of ^{131}I.

At home, the patient is instructed to flush the toilet at least 3 times after use. If no suitable toilet facility is available, all excreta should be discarded in a covered pit.

In case of death during the isolation period, no special precautions are needed if the body caontains less than 30 mCi of ^{131}I. If an autopsy is to be performed, it should be under supervision of a Radiation Protection Officer. Burial is a preferred method of disposal of the body to prevent volatilization of radioiodine. If burial is not possible on religious grounds, cremation, preferably under the guidance of a Radiation Protection Officer, is allowed.

In all the above matters pertaining to radiation protection, the physician should be guided by the prevailing National rugulations and practices.

Patient follow up and monitoring

Immediate reactions that we have observed include thyroiditis which tends to be more severe in patients having a significant thyroid remnant after surgery. Reassurance, cold packs

and analgesics are usually adequate; corticosteroids may be administered in severe cases. Vocal cord paralysis from compression edema of thyroiditis is usually transient and does not need anything more than reassurance.

Patients are seen monthly for the first three months. They are checked every three months for the next five years, at least. They are subjected to a whole body scan 3 months after the treatment and then once a year for five years. If there is any doubt as to complete ablation, patients are advised to come more frequently for follow up. If there is a new growh in the neck region or suspicion of distant metastases (headaches, backaches, X-ray chest) a whole body scan is repeated.

Thyroid hormones are given a month after the ablation dose. T3, if available, is preferred in the initial three months. TSH, T3, T4 are regularly determined to monitor adequacy of replacement.

Thyroglobulin RIA provides a useful tool in monitoring cancer activity or recurrence. Shah [Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985] that Tg remains in the normal range when patients are free of the disease. Highly elevated values can indicate a metastatic disease which may be organ specific: Tg >1000 ng/ml can indicate bone metastases while minimal elevation would indicate lymph node involvement. Thyroglobulin assay, however, is not always available in many Asian medical centers except those with well established cancer therapy facilities. It is important to note that whole body scanning and serum thyroglobulin determination are complementary to each other in improving the diagnostic accuracy of thyroid cancer recurrence. One therefore cannot rely on monitoring either parameter alone, although thyroglobulin assay reduces the frequency of whole body scanning. Jeevanram et al [Proceedings of a National Seminar on Thyroid Cancer, Bombay, 1985] introduced the use of specific activity of Tg to predict recurrence of thyroid malignancy. Further clinical validation of this technique can fortify its position in cancer management.

External radiotherapy

Whenever possible surgical removal of thyroid cancer is preferably done prior to external irradiation especially when the lesion is anaplastic. Metastatic lesions can also benefit from external radiotherapy, particularly if proved to be radioiodine resistant. The value of external radiation therapy is largely supportive, particularly in the rare cases of rapid cancer growth.

Radioiodine treatment holds a very important role in the management of various thyroid disorders owing to its efficacy, ease of administration and affordability in the developing countries.

TABLE I. CLINICAL SCORING INDEX FOR THE DIAGNOSIS OF HYPERTHYROIDISM. *

	DIAGNOSTIC IMPORTANCE.		
SYMPTOMS	PRESENT	ABSENT	
Nervousness	+2	0	
Increased sweating	+3	0	
Heat Intolerance	+5	-5	
Palpitations	+2	0	
Dyspnea on effort	+1	0	
Fatigue & Weakness	+2	0	
Weight loss	+3	-3	(Gain)
Increased appetite	+3	-3	(Decreased appetite)
Eye symptoms			
Hyperdefecation			
SIGNS			
Thyroid enlargement	+3	-3	
Thyroid bruit	+2	-2	
Exophthalmos	+2	0	
Lid retraction	+2	0	
Lid lag	+1	0	
Hyperkinesis	+4	-2	
Tremor	+1	0	
Hands			
hot	+2	-2	
moist	+1	-1	
Tachycardia (>90/min)	+3	-3	(<80/min)
Atrial fibrillation	+4		

Note: A score of > +18 indicates hyperthyroidism
A score of < +11 indicates euthyroidism

*Source: Crooks et al.[Q J Med.1959; 28:211]

TABLE II. AGE DISTRIBUTION
(n = 1564)

Age Group (in years)	Number	(%)
< 20	111	(7.10)
20 < 30	496	(31.73)
30 < 40	497	(31.80)
40 < 50	284	(18.17)
50 < 60	130	(8.32)
60 < 70	35	(2.24)
70 < 80	9	(0.58)
80 < 90	1	(0.06)

TABLE III. FREQUENCY DISTRIBUTION ACCORDING TO THYROMEGALY.

Classification	Number	(%)
Normal	75	(4.82)
Diffuse	1315	(84.07)
Nodular		
Solitary	144	(9.25)
Multiple	30	(1.93)

TABLE IV. SIZE OF THYROMEGALY

Size	Number	(%)
Normal	75	(4.8)
Minimal	1032	(66.0)
Moderate	377	(24.6)
Marked	72	(4.6)

TABLE V. FREQUENCY DISTRIBUTION ACCORDING TO MEDICATIONS BEFORE TREATMENT.

Medications	Number	(%)
None	1015	(64.30)
Anti-thyroid drugs	330	(21.29)
Iodine	84	(5.37)
Beta-blockers	17	(1.09)

TABLE VI. CLASSIFICATION OF PATIENTS WHEN LAST SEEN.

Group	Number	(%)
Euthyroid	1270	(81.20)
Uncertain *	198	(12.65)
Probably hypothyroid	40	(2.55)
Definitely hypothyroid	56	(3.58)

* Patients who were followed up for too short a period to evaluate response; given exogenous thyroid hormone for ophthalmopathy; underwent thyroidectomy after ^{131}I treatment; became pregnant during follow-up.

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[1] As an exception, in this Chapter some references are allowed in the text only as they are of highly local or Regional nature, cited mainly to substantiate the statements of the author. References of more general nature are included in the list given below.

[10] RONGA, G., FIORENTINO, A., PASERIO, E., et al., Can iodide-131 whole-body scan be replaced by thyroglobulin measurement in post-surgical follow-up of differentiated thyroid carcinoma? J. Nucl. Med. 31 (1990) 1766-1771.

[11] BLAHD, W.H., Serum Thyroglobulin in the Management of Thyroid Cancer (editorial). J. Nucl. Med., 31 (1990) 1771-1773.

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Chapter 24

NUCLEAR CARDIOLOGY

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Introduction.

The assessment of cardiovascular performance with radionuclides dates back to 1927, when Blumgart and Weiss conducted the first clinical studies using a natural bismuth radioisotope ^{214}Bi, in that time known as "Radium C". They injected a solution of this radionuclide into the vein of one arm and detected with a cloud chamber the appearance of its highly penetrating gamma rays in the contralateral arm. Their aim was to study the "velocity of the blood". In these pioneering studies, the mean normal arm-to-arm circulation time proved to be 18 sec., but it was found to be prolonged in patients with heart disease. Subsequently, they were able to calculate the pulmonary circulation time and the pulmonary blood volume by using a forerunner of the Geiger counter with platinum needle electrodes over the right atrium and the left elbow, and to study the effects on them of various heart and lung lesions, thyroid disorders, anaemia, polycythaemia, and drugs. Such classical studies, while appearing crude by today's technology, illustrate that minds and methods were fully prepared to exploit the eventual appearance of the artificial radioisotopes of elements of a more physiological character than bismuth, and laid the foundation for the established techniques of present day nuclear medicine.

Although these studies on cardiovascular physiology were the first ever performed in humans with the aid of the radiotracer principle, the cardiologist had to wait for the accumulation of decades of research and development in the fields of radiochemistry, radiopharmacy and instrumentation before being able to capitalize this new approach for the non-invasive investigation of cardiac functions. Many other clinical specialties enjoyed long before the regular application of radionuclides in the study of regional physiology, but the small beating heart proved to be too elusive to the radiotracers and nuclear instruments prevailing in the different evolutive phases of nuclear medicine until the first half of the 1970s. Then, in a sudden spurt, progress in different technological fields converged all to create the ideal conditions for the birth and growth of nuclear cardiology (**Table I**).

TABLE I. TECHNOLOGICAL ADVANCES NEEDED FOR THE BIRTH AND GROWTH OF NUCLEAR CARDIOLOGY.
DEVELOPMENT OF NEW RADIOPHARMACEUTICALS.
DESIGN OF THE DIGITAL GAMMA CAMERA WITH MORE THAN 19 PHOTOMULTIPLIER TUBES AND THINNER SCINTILLATOR CRYSTAL.
INTRODUCTION OF DEDICATED DIGITAL SYSTEMS (COMPUTERS) FOR IMAGE DISPLAY, PROCESSING AND ANALYSIS.

Fifteen years ago, myocardial perfusion imaging or gated blood pool analysis was a methodology available only to those nuclear medicine centres having the most advanced computers and "state-of-the-art" instruments. At present, any nuclear medicine unit with a 12-year old gamma camera and a computer, both in good operating conditions, could perform any of the well established studies of nuclear cardiology, and even those without a computer could obtain important non-quantitative clinical information, unavailable by other non-invasive means.

Today, nuclear medicine techniques are routinely used in cardiological practice. They include procedures for the atraumatic investigation of different physiological processes in the various structures included in the central circulation: pericardium, myocardium, myocardial adrenergic innervation,cardiac chambers and valves, coronary microcirculation, and great vessels. Beside these **in-Vivo** procedures, they also comprise of **in-Vitro** methods for the detection and measurement in blood of various biological molecules of significance in the management of cardiac diseases. A common feature in this collection of **in-Vivo** and **in-Vitro** techniques is their ability to provide helpful clinical information for the diagnosis, prognosis and management of cardiac diseases. Their simplicity and safety for the patient allow their repeated use in the follow up of the progress of disease and in the assessment of the efficacy of the therapeutic measures.

Nuclear cardiology is then a very complex discipline, which should be moulded and guided with technical skill and clinical acumen. In fact, a nuclear cardiologist should be either a nuclear physician with specific clinical training in cardiology, or a cardiologist with **sui-generis** training in nuclear medicine. An acceptable compromise would be a close collaboration between a nuclear physician and a cardiologist. In this case, both should participate actively in the performance, processing and interpretation of the tests results. In any instance, the patient's ECG should be continuously monitored in the nuclear medicine unit during many of the in vivo nuclear cardiological studies, especially those involving some kind of stress. An emergency cart with all the drugs and devices for cardiological emergencies, cardiopulmonary resuscitation and electroversion must be always available in the nuclear medicine unit itself and be updated once a week (**Appendix I**). All the personnel of the nuclear medicine facility should receive periodical training in the management of such emergencies.

I. IN-VIVO NUCLEAR CARDIOLOGY.

In-Vivo nuclear cardiology comprises of several procedures to visualize the function and morphological features of the different structures in the central circulation. Their methods vary in instrumentation and in radiotracers, and possess a different clinical indication.

1. RADIOANGIOCARDIOGRAPHY (RAC).

Radioangiocardiography is designed to study the blood flow through the central circulation and to explore the morphology of the cardiac cavities and the great arteries. An

intravenous injection of a gamma emitter is given as a tracer of the circulating blood to delineate the boundaries of the intracavitary and intravascular blood pools.

A. Static radioangiocardiography (st-rac).

Static RAC is the oldest and simplest of all scintigraphic studies of the heart. It is performed with 5 - 10 mCi of $^{99}Tc^{m}$ as a label for human serum albumin or, preferably, for homologous red blood cells labelled either in-Vitro or in-Vivo. Persistence of these radiotracers in the circulating blood allows the acquisition of multiple static images of the chest blood pool from different views.

This procedure was originally intended for the differential diagnosis between pericardial effusion, cardiac hypertrophy and cardiac dilatation (Table II), but it could also be of value in the detection of intracavitary myxomas and thrombi, which produce filling defects in the affected cardiac chamber, in the localization of aneurysms and stenosis in the great arteries, and of haemangiomata in the peripheral circulation including the liver (Fig. 24.1).

TABLE II. CARDIAC BLOOD POOL IMAGING IN THE DIFFERENTIAL DIAGNOSIS OF PERICARDIAL EFFUSION, CARDIAC HYPERTROPHY AND CARDIAC DILATATION

Scintigraphic signs.	Clinical diagnosis.
Nearly no separation between cardiac, hepatic and pulmonary blood pools.	Normal.
Photopenic halo surrounding the cardiac chambers, separating them from the hepatic and pulmonary blood pools.	Pericardial effusion.
Small cardiac cavities without photopenic halo.	Cardiac hypertrophy.
Enlarged cardiac cavities without photopenic halo.	Cardiac dilatation.

B. Qualitative first-pass radioangiocardiography (qual-fp-rac).

This procedure could be also performed with a plain gamma camera with at least 37 photomultiplier tubes. Its purpose is to document sequential images of the chest immediately after an intravenous injection of the radiotracer, in order to follow its transit during its first pass through the central circulation, adding the dimension of time to the static study.

The type of radiotracer to be used depends on the indications for the study. If the aim is to visualize the central circulation in full, from the superior cava vein (SCV) to the aorta

(Ao), it is better to use $^{99}Tc^m$-pertechnetate or, even better, $^{99}Tc^m$-DTPA because of its rapid excretion from the body. If the interest is in studying a right-to-left cardiac shunt, then it is preferable to use $^{99}Tc^m$-macroaggregated albumin or albumin microspheres. The volume of either tracer to be injected should be less than 0.5 ml, with 8 - 20 mCi/sq.m., but never less than 2 mCi in total. Its injection into the superficial jugular vein is the best to avoid distortion of the radioactive bolus, but in case of injecting the tracer in the arm the choice is to use the right basilic vein. In any instance, the injection should be made in a single fast movement by using a No. 20 needle and a 3 ml venous catheter attached to a three-way valve. The radioactive dose should be immediately flushed with 20 - 30 ml saline solution to obtain a compact radioactive bolus.

The injection of the radiotracer should immediately be followed by the acquisition of sequential images at a rate of 1 sec./image during the dextrophase, and of 2 sec./image throughout the levophase to allow for the lengthening of the radioactive bolus with the traversed distance. During the injection the patient should be calm and any cause of Valsalva's artifice should be avoided since it can distort the bolus and even revert a central shunt. Position of the detector over the chest of the patient depends on the aims of the study and should be selected previous to the tracer's injection since only one single view can be obtained during its first transit through the heart. The right atrium (RA) is best defined from the right ventricle in the **right anterior oblique (RAO) view**, but it overlaps with the left atrium (LA). In this view, the right ventricle (RV) covers the left ventricle (LV). In the **anterior view (A)**, the two right cardiac cavities are not well defined and there is a slight superposition between the inferior region of the RA and the upper part of RV, but it is the best for visualizing the main pulmonary artery (PA) and its branches. The best view to separate both ventricles is the **left anterior oblique (LAO)**, which clearly shows the interventricular septum, but in this view the RV covers part of the RA. The LA is seldom visualized and that only in the LAO view. Aorta is clearly seen in all views, but to determine its normal anterior position the best views are the LAO and the left lateral (LL). Pulmonary veins are not visualized on these images.

The resulting images show in a dynamic fashion the transit of the radioactive bolus through the central circulation, giving morphological information similar to that obtained through radiological angiography through a cardiac catheter. They can depict an obstruction at the SCV and the corresponding collateral circulation, or the immediate transit of the tracer from the right cardiac cavities to the left chambers through an abnormal intracardiac communication. The images taken during the dextrophase are able to detect signs of atrial blood regurgitation to the inferior cava vein (ICV), pulmonary hypertension, tricuspid valve insufficiency, pulmonary stenosis or dilatation, and the presence of an intracavitary myxoma. The levophase is of value in the visualization of aortic anatomical abnormalities, such as aneurysms and stenosis (**Figs. 24.2A, 24.2B and 24.2C**).

Qualitative FP-RAC is specially suited for the study of cardiac congenital malformations, either in children or in adults, since it gives a neat image of the RA, which it is the cardiac cavity that defines the "**situs**" or spatial position of the heart: the normal position, when this cavity is at right, is called "situs solitus" by the cardiologists, while the abnormal position,

when the RA is sited at left, it is known as "situs inversus". Accordingly, this procedure is the most simple means to identify the specific morphological features of corrected transposition of the great vessels either in "situs solitus" or in "situs inversus", by following the very same criteria used for the interpretation of radiological angiocardiography to define the visceral situs, the characteristic morphology of both ventricular cavities, and the spacial positions of the cardiac apex, both ventricles, the PA and its branches, and of Ao **(Fig. 24.3A and 24.3B)**.

Atresia of the tricuspid valve is easily detected due to the direct transit of the tracer from the RA to the corresponding left cavity and its immediate flow through the LV and Ao, leaving an empty space at the site of the RV. The intracardiac abnormal communications with right-to-left shunts, as those present in cases of Fallot's tetralogy, Eisenmenger complex and corrected transposition with septal defects, are easily depicted by the transit of the tracer from the right to the left cavities, with the corresponding proportional decrease of pulmonary radioactivity according to the magnitude of the shunt. In the presence of patency of an arterial duct the tracer appears in Ao but it is not evident at the LV.

The use of $^{99}Tc^{m}$ labelled macroaggregated albumin or albumin microspheres is recommended in the study of right-to-left cardiac shunts. In these cases, a fraction of the tracer is diverted through the abnormal communication from the right to the left cardiac cavities, to be immediately distributed into the systemic circulation through the Ao and be trapped by the peripheral capillary beds, which can be visualized by scintigraphy even with a simple rectilinear scanner **(Fig. 24.4A, 24.4B and 24.4C)**. The magnitude of the shunt could be estimated simply as the ratio of the differences between the whole body (WB) and pulmonary (P) radioactivities multiplied by 100 and divided by the whole body radioactivity:

$$\text{Shunt } \% = [WB-P] \times 100 \,/\, [WB]$$

The whole body radioactivity can be measured with the gamma camera detector without collimator, positioned at a proper distance from the patient. The pulmonary radioactivity can be roughly estimated by measuring the whole body radioactivity at the very same distance but covering the patient's chest with a radiological lead apron. The difference between the first and the second measurements approximates the radioactivity present at the lungs.

Left-to-right shunts are recognized by a prolonged persistence of the tracer in both lungs due to its early recircling from the left cavities through the abnormal communication, and by a proportional decrease of the radioactivity flowing through the left heart and Ao. These features, however, are only evident when the shunt is sufficiently pronounced.

According to all these, the more complex congenital cardiopathy could be identified by the radioangiocardiographic signs corresponding to their distinctive features. As it is, the most common findings in Fallot's tetralogy, are: marked decrease in the diameter of the PA and its main branches (a sign for pulmonary artery stenosis); left ventricular filling immediately after the right ventricular filling (a sign for right-to-left shunt), and direct transit of the radiotracer from the RV to the Ao (a sign for aortic riding over the septum). If the

PA stenosis is too severe, then there is a marked pulmonary oligohaemia and the tracer is shunted from the RV to the Ao (which is the cause for cyanosis).

C. Semiquantitative first-pass radioangiocardiography.

In this instance the data need to be stored during acquisition in a video system, in a multichannel magnetic tape, or in a magnetic disk memory. An electronic device to select several regions of interest (ROI) on the images is also needed to generate their respective time/activity curves. It is always convenient to use a ROI on the SCV to evaluate the quality of the radioactive bolus. This is acceptable if the corresponding time/activity curve shows a single peak with a mean transit time less than four seconds, but the study should be repeated if the peak is wider or if there are two or more peaks. It is important to note that certain intrinsic factors distort the bolus and invalidate the semiquantitative test. The more significant of these factors, are: right ventricular hypertrophy or dilatation, tricuspid valve regurgitations, pulmonary hypertension, and left-to-right cardiac shunts.

The other ROIs are positioned on each cardiac chamber, on both lungs and on the Ao, in order to know the sequence of the appearance of the tracer in these structures and to measure mean transit time from the SCV to each one of these.

In the presence of a **right-to-left shunt**, the tracer appears in Ao in less than 7 sec. The site of the abnormal communication can be deduced by the appearance of a premature peak in the curve corresponding to the affected cardiac cavity in the left side, followed by a second peak representing the tracer that pursued its normal transit through the pulmonary circulation. However, if the shunt is through a patent arterial duct with inverted flow, the premature peak is only evident in the aortic curve (**Fig. 24.5**).

Left-to-right shunts are recognized by a prolonged persistence of the tracer in both lungs due to its abnormally early recirculation from the left heart to the right. The site of the abnormal communication can be deduced by a second late peak in the curve corresponding to the affected right cavity. But, if the shunt is through a patent arterial duct, the only evident abnormality is the overpersistence of the tracer in the lungs (**Fig. 24.5**).

D. Quantitative first-pass radioangiocardiography (quant-fp-rac).

In this case a digital computer is needed for data acquisition, processing, display and analysis. The methods for data processing and analysis depend on the aims of the study and on the available software. Those herein included are described in a general fashion and were selected for their relative simplicity, since they were developed by the author for their use in a low cost digital system based on a personal computer.

In general, data are stored during acquisition in the memory of the computer by using a 64 x 64 matrix and a frame rate variable between 25 to 50 frames/sec, depending on wether the test is performed at rest or during exercise. The acquisition should be continued during the first 30 to 45 sec after the intravenous injection of the tracer (**Fig. 24.6**).

Studies performed at rest.

The major indications of quantitative first-pass RAC at rest are in the quantitation of cardiac shunts, in the evaluation and monitoring of cardiac failure by the periodical estimation of global ejection fraction (EF) in any or both ventricles, and in the study of cardiac valvular regurgitations.

a. Shunt quantitation.

Data analysis is achieved by generating on the screen a series of sequential cardiac images, each representing 0.5 sec. The visual quality of these images could be improved by interpolation into finer matrixes (128 x 128, 256 x 256, or 512 x 512) and by smoothing with the nine-point binomial spread function. The needed ROIs are selected by using the cursor on summed up images of the dextrophase (right cardiac cavities and PA), the pulmonary phase (lungs), and the levophase (left cardiac cavities and Ao), after being interpolated into a 512 x 512 matrix and subjected to background substraction, carefully avoiding the inclusion of neighbouring structures inside each ROI.

Fig. 24.7 shows the analytical method devised for the quantitation of **right-to-left cardiac shunts** from the time/activity curve in the affected cavity in the left side of the heart, which shows a premature peak produced by the early inflow created by the shunt, followed by a second, normal peak, related to the fraction of the tracer which was not diverted and followed its normal transit through the lungs.

The best known method to quantitate **left-to-right cardiac shunts** makes use of the pulmonary time/activity curve to calculate the pulmonary flow/systemic flow ratio (Qp:Qs) by separating both components of the curve by fitting them to the gamma variate function. Results with this method show a good correlation (0.94) with those obtained through intracardiac catheterism when Qp:QS is between 1.2 and 3.0, but are some less accurate with Qp:Qs between 1.0 and 1.2, may be due to the bronchial circulation coming into view of the detector directly from Ao, acting as an early systemic recirculation which is added to the shunted radioactivity, rendering a falsely elevated Qp:Qs.

If this early bronchial circulation is obvious in the original curve it can be also subject to the gamma variate fitting to subtract the resulting peak from the curve representing the differences between the original curve and the gamma variate fit of its first component (A1) before performing the gamma variate fitting for area A2. When the early bronchial circulation is not clearly evident it is necessary to calculate the actual recirculation time to select the period in the curve that needs to be fitted to the gamma variate function. The recirculation time can be easily derived from the period earmarked between the two peaks, which approximates one half the pulmonary time, which, in turn, equals one half of the time of the total circulation (**Fig. 24.8**).

It is important to note that in cases of patency of the arterial duct the pulmonary transit curve reveals a higher recirculation through the left lung than through the contralateral, and

that surgical shunts of the Potts, Blalock-Taussigh, or Waterston types produce a higher recirculation through the lung receiving the shunted blood.

b. Quantitation of ventricular function and motility.

First-pass RAC is also of value in quantitating the EF in both ventricles and in depicting parietal motility alterations. In these cases, the tracer is injected as described, but the position of the detector over the patient's chest will vary with the ventricle under study. The most suitable for the study of both ventricles is the 30° RAO view, in which both ventricles appear physically superposed but separated in the images by their different filling times if the radioactive bolus is of good quality. The 45° LAO view could also be used, since in this view both ventricles are clearly separated by the septum. A biplanar collimator allows the simultaneous acquisition of the 20° RAO and the 40° LAO views with a single tracer dose.

For data processing, it is necessary to draw ROIs around the particular ventricle under study, carefully avoiding the inclusion of neighbouring structures. This is better accomplished with the aid of phase analysis using the first harmonic of the corresponding ventricular volumetric curve (described later in this chapter), which objectively depicts the position of the tricuspid and mitral valves separating the ventricles from the corresponding atria. A second ROI is drawn adjacent to the heart to measure the background produced in the vicinity.

The time/activity curves in these ROIs are generated at a rate of 40 msec/frame. The ventricular curves are made up of a series of oscillations with the greatest amplitude occurring at the highest of the principal curves (**Fig. 24.9**). As these oscillations in radioactivity correspond with the variations of ventricular blood volume with time, the count rates at the peaks are proportional to the greatest ventricular blood volume attained during each cardiac cycle at the end of diastole (ED), while those at the valleys are proportional to the lowest ventricular blood volume occurring at the end of systole (ES). These curves need to be corrected by subtracting from them the normalized time/activity curve generated from the corresponding background ROI. The frames included in three to five consecutive peaks at the greatest height of the ventricular curve are averaged to increase the statistical reliability by using the first derivative to align their descending phases.

The ventricular stroke volume (SV) can then be calculated as the relative difference in count rates between ED and ES:

$$SV = ED - ES$$

The ventricular EF is calculated by dividing SV by the ED:

$$EF = SV / ED$$

The averaged information is also used to generate a series of 16 images showing the size and shape of the ventricles at 16 consecutive equal periods covering the entire composite cardiac cycle. The regional wall motion can be analyzed either by superposing in a single

static image the isocount outlines of the ES and the ED images, the isocount outlines of the 16 frames or, preferably, by displaying in a single iterative fashion the 16 consecutive outlines, adding cinematographic movement to the heart's palpitations.

These displays allow a clear definition of the abnormalities in the regional ventricular wall motion: **hypokinesis** or decreased wall motility, which might be general and diffuse, as in cardiac failure, or localized in one or various regions, as in ischemic myocardial areas; **akinesis**, or regional abolition of wall motion, as in acute and old myocardial infarctions; and **dyskinesis**, or paradoxical regional expansion of the ventricular cavity during systole, as in aneurysmatic areas.

Normally, the EF is 62% ± 5 for the LV, and 50% ± 4 for the RV. These figures are decreased in proportion with the magnitude of cardiac failure as a result of the global or regional reduction in the ventricular wall motion. The results with this simple method correlates satisfactorily with those obtained through radiological ventriculography (r = 0.82), but the correlation varies with the position of the regional wall motion abnormality: anterobasal segment, r = 0.92; posteroapical segment, r = 0.89; posterobasal, r = 0.76, and anteroapical, r = 0.72.

c. Valvular regurgitation.

First-pass RAC is very sensitive to the quality of the radioactive bolus and it is useless in the estimation of mitral valve regurgitations, occurring in the left side of the heart, but promising attempts have been made to measure tricuspid valve regurgitation, occurring in the right side of the heart, from the slopes of the descending phase of the time/activity curves isolated from the right atrium and the right ventricle.

Studies performed during exercise.

Quantitative FP-RAC at rest has no role in the study of coronary artery disease (CAD) since the regional wall motion might be normal under resting conditions when the narrowing of the coronary artery is less than 80%. In this case, it is necessary to induce a higher myocardial demand through exercise to increase coronary blood flow and to produce transient ischemia in the region irrigated by an stenotic artery unable to dilate.

To perform the exercise stress test the patient needs to exercise on an ergometric bicycle in a semi-seated position. The detector head of the gamma camera is tightly strapped in position over the patients's chest, interposing a soft cushion for his comfort, in order to document the 30° RAO view of the heart with a gentle caudal tilt to the collimator. A No. 20 needle is inserted in the antecubital vein, preferably in the right arm, to start a slow infusion of 5% glucose solution before the test is initiated. Then, the tracer could be easily injected when pertinent through the already inserted and patent needle, immediately flushing the radioactive bolus with a fast infusion of 30 ml of 5% glucose solution. Under continuing ECG, blood pressure and cardiac frequency monitoring, the patient is made to exercise with

gradually increasing loads according to the Bruce's protocol. The tracer should be administered as soon as any of the following signs appear:

a) maximal theoretical cardiac frequency for the patient's age (220 - age in years);

b) ST segment depression greater than 2 mm in the ECG tracing;

c) acute chest pain, or

d) extreme fatigue.

The tracer injection should immediately be followed by the acquisition of data during the next 30 to 45 seconds while the patient continues exercising.

Normally, exercise induces diffuse hyperkinesis and a 5-10% increase in left ventricular EF, but in patients with CAD the left ventricular EF remains unchanged, may increase by less than 5% or even may decrease with exercise. These findings are usually associated with regional hypokinesis or akynesis in areas with transient ischemia. In patients with one vessel disease, the EF may increase 5% with exercise, while the wall motion study may show regional hypokinesis in the area irrigated by the affected coronary artery and general hyperkinesis in the normally irrigated regions. This single observation indicates a higher sensitivity of the wall motion study than of the global ventricular EF alone in the study of CAD. In fact, the left ventricular EF during exercise is 85% sensitive and 72% specific for CAD, while the wall motion study during exercise is 95% sensitive and 75% specific.

This procedure is especially recommended for the study of CAD in developing countries due to its relative simplicity and low cost, since technetium is always available in a modern nuclear medicine unit and is less costly than ^{201}Tl or ^{99}Tc^{m}-MIBI. However, it does not give any specific information regarding regional perfusion, only on its functional effects on myocardial contractibility. Another disadvantage could be the need of and ergometric bicycle.

E. Equilibrium radioangiocardiography (e-rac).

First pass dynamic studies are limited to the counts acquired during 3 to 5 cardiac cycles. To increase the total number of counts, and hence the resolution and accuracy of the cardiac images and of the time/activity curves, it is necessary to acquire the information included in a significantly bigger number of cardiac cycles. This can be achieved by using radiotracers such as ^{99}Tc^{m}-albumin or ^{99}Tc^{m}-erythrocytes, which a few minutes after being injected reach an equilibrium in the circulating blood, remaining in circulation for the time needed for data acquisition. In this case, the detector is positioned for the acquisition of the 30° LAO view, with a slight caudal tilt, which is the best to resolve both ventricles and to visualize the septum.

Data acquisition is gated to the R wave of the patient's ECG as a physiological marker for the cardiac cycle (**Fig. 24.10**). The duration of each cardiac cycle, enclosed between two successive R waves, is divided into many equal intervals (16, 32, 64 or more), each one with a corresponding matrix in the computer's memory. Data acquired during a particular interval of the cardiac cycle are always stored in the same matrix so, after collecting the information gathered during 200 to 300 cardiac beats, the results is a sequential series of 16, 32, 64 or more memory matrixes with enough information density to generate on the screen a corresponding series of sequential composite images, each one showing the size and shape of the cardiac cavities in a particular period of the composite cardiac cycle. The stored information can also be used to play these images in an endless loop cinematographic fashion allowing a clear qualitative assessment of global and regional wall motion. The left ventricular volumetric curve can be generated from a ROI drawn around that cardiac cavity in a similar fashion to that followed in quantitative FP-RAC, and should be corrected by the background radiation measured in another narrow ROI adjacent to the LV. The RV volumetric curve, however, can not be generated through this method since both ventricles are superimposed in the RAO view, while the RA is situated at the back of the LV in the LAO view.

The resulting volumetric curve shows a better temporal resolution than that constructed during the FP-RAC (**Fig. 24.11**), depicting in all detail its architectural features, allowing the measurement of valuable physiological information regarding the global efficiency of wall motion: pre-ejection period, ejection time, peak ejection rate, diatesis time, fast and slow filling times and rates, beside the most useful EF. As with FP-RAC, the isocount outlines of all the images in the series can be superimposed in a single image for a better analysis of regional wall motion. Some computer softwares are able to give quantitative data on regional wall motion and EF.

The major indications for E-RAC with the patient at rest are those conditions which are known to affect global or regional motility and, hence, ventricular function. It is a very sensitive method in the detection of ventricular aneurysms and in the differentiation of true localized aneurysms from pseudo-aneurysms or regional diffuse akinesis. It is also very useful in the post-myocardial infarction patient for assessing the immediate and late prognosis, in the evaluation of coronary bypass graft surgery, angioplasty and thrombolysis, and in the periodical monitoring of Adriamycin myocardiotoxicity and of hypertrophic and congestive cardiomyopathies.

This method is of special significance in the study of aortic and mitral regurgitation. In this case, the only variation in the procedure is in data acquisition which is prolonged to eight to ten minutes or up to the collection of eight million counts, and in the selection of ROIs: one around the RV and another surrounding the LV in order to generate their respective time/activity curves. The ejected radioactivity in each cardiac beat should be normally the same in both ventricles, so their ratio should be near 1.0, reflecting the equal blood volumes expelled by the two ventricles. Based on this principle, an index has been proposed to calculate a regurgitation fraction (RF) as the ratio between the difference in the ejected radioactivity by the left and the right ventricles multiplied by 100 and divided by the ejected

radioactivity by the LV:

$$RF = [ERLV - ERRV] \times 100 / ERLV$$

Normally, this index is 1.15 ± 0.15, but increases proportionally with the magnitude of regurgitation to 1.36 and even 5.30. There is a satisfactory correlation between the results with this method and those attained through radiological ventriculography. The critical step is, however, in the selection of both ventricular ROIs according to the method for phase analysis in order to locate the mitral and tricuspid valves with accuracy. Failure in doing so may decrease the operating characteristics of the method.

As with FP-RAC, the major indication for E-RAC during exercise is in the evaluation of myocardial ischemia, which it is evidenced by altered wall motion as described for FP-RAC. An abnormal test is characterized by one or several of the signs shown in **Table III.**

TABLE III. RADIOANGIOCARDIOGRAPHIC SIGNS OF MYOCARDIAL ISCHEMIA
- Abnormal resting regional wall motion.
- Global LV EF at rest lower than 50%.
- Failure of LV EF to rise on exercise by an absolute five points or up to a point 5% greater than that of the resting value.
- An actual drop in LV EF during exercise when compared to the resting value. More than a 10% drop is generally seen in triple vessel disease.
- An increased end-systolic volume on exercise instead of a decrease as normally seen.
- Development of new regional wall motion abnormalities on exercise, which are reversed by nitroglycerine administration.

In patients with a single obstructed coronary vessel, slightly increased global EF during exercise is frequently found, while the wall motion study may show a regional hypokinesis in the area irrigated by the obstructed artery and global hyperkinesis in the normally perfused territories. This observation is in accordance with a higher sensitivity of the wall motion analysis in this disease (95%) than the global EF (85%). The most frequent causes for false negative results are vicarious hyperkinesis in normally irrigated myocardium and insufficient exercise.

It must be clearly understood, however, that exercise E-RAC is a test of ventricular

function which is not specific for the detection of CAD, especially when a large unselected group of patients with various types of cardiac diseases are subjected to the procedure. Nevertheless, the ability to detect exercise induced changes in global and regional ventricular function, which can or can not be reversed by nitroglycerine administration, increases both sensitivity and specificity of the test for CAD. On the other hand, if a patient with chest pain or abnormal exercise ECG shows a normal global left ventricular response to exercise, then the probability of significant CAD is very low and can be reasonably excluded without further need of coronary angiography.

Equilibrium RAC have demonstrated a reduction in left ventricular filling parameters during acute episodes of infarction. Besides, peak diastolic filling rate determined at rest and during exercise provides a potentially more sensitive index of CAD than global EF.

Another significant indication for E-RAC is in the evaluation of LV and RV performances in critically ill patients, especially in those in septic shock, the most common cause of death in a Critical Care Unit. Survivors of septic shock most commonly show a profound decrease in left and right ventricular EF at the onset of shock due to sepsis, and serial scans demonstrate that surviving patients usually regain normal EF in both ventricles in seven to ten days.

F. Parametric images derived from e-rac.

Parametric images are designed to show the regional distribution and quality of a certain function. They are derived by special computer processing of a sequential series of images obtained through a dynamic study like e-rac. This processing is accomplished by applying the mathematical equation defining the particular function in a pixel by pixel fashion in the full image matrix.

This new concept is especially important in cardiology and reserves some previous explanations. The quantitative regional differences in the variations of radioactivity in the ventricular cavities during the cardiac cycle are not a reflection of the functional quality of basic units or cells in these cavities, as in the lungs or the liver where a regional functional change may represent the altered physiology in the set of alveoli or hepatocytes included in the region. Notwithstanding, a regional hypokinesis in the left ventricle could be evidenced by the local decrease of EF in the pixels conforming that particular region in the matrix of the ventricular image, since the volumetric changes during the cardiac cycle and the corresponding variations in radioactivity are less notorious in these pixels. In this new approach, the pixels are used as the unitary functional cells of the cavities, each one with a particular EF.

The distribution pattern of EF in the resulting parametric image renders an objective evidence of the quality of regional function of the different wall segments. These patterns condense more creditable functional data than those provided by the bidimensional radial shortening of the cavity measured by geometry dependent radiological angiography, because the nuclear parametric nuclear images are generated by using tridimensional information,

totally independent of geometrical factors, obtained from the regional changes in radioactivity produced in front of the detector by the volumetric variations during the cardiac cycle.

The most elementary parametric images of the heart are obtained by manipulating the images of the cardiac cavities at ES and ED after background substraction:

(a) **Stroke volume image.** This parametric image is obtained by subtracting the data on the pixels in the matrix of the ES image from the corresponding pixels in the matrix of the ED image (**SV = ED - ES**). The stroke volume is mainly distributed at the periphery of both ventricles since the volumetric changes at the centre of the cavities are minimal.

(b) **Ejection fraction image.** It is generated by dividing each pixel of the stroke volume image by the corresponding pixel in the matrix corresponding to ED (**EF = SV / ED**). The regional intensity in this image is proportional to the regional EF. Normally, this image shows a ring with homogeneous intensity and an amplitude equal or wider than one third of the transverse diameter of the left ventricle at the inferoposterior apical level. The intensity in the ring is proportional to blood ejection when this ejection is bigger than 50% of the ED volume. The amplitude of the ring narrows in proportion with the decrease in regional wall motion (hypokinesis) and is interrupted in regions with akinesis or absent wall motion.

(c) **Auricular filling or "paradoxical" image.** This image is obtained by subtracting the data on the pixels in the matrix of the ED image from the corresponding pixels in the matrix of the ES image (**AF = ES - ED**). This image is integrated only with those pixels showing ES - ED differences bigger than 0. Normally, it shows only the pixels corresponding to the atria, which are filled during ES. But, when radioactivity and blood volume are paradoxically increased in any ventricular region during ES the corresponding pixels are alight, indicating the presence of a regional dyskinesis. In order to know the magnitude of this dyskinetic region, the radioactivity in all involved pixels is added and the result is divided by the ventricular radioactivity during ED.

Since the generation of these images depends on tridimensional data regarding the volumetric changes, and they are not based on geometric considerations as the radiological methods do, their results are more accurate independently of the position of the regional abnormality in wall motion within the heart.

HARMONIC ANALYSIS OF CARDIAC CYCLE.

The volumetric curves in the heart cavities are similar in nature to a sinusoid, which is characterized by its **frequency**, or number of cycles occurring per unit time, and by its **amplitude**, or value of its maximal deflexion. The frequency of this sinusoid is the same in the entire heart, but its amplitude may vary from one cardiac region to the other according to the regional efficiency of the wall motion. The maximal amplitude of the sinusoid is not synchronous in all cardiac regions, since the contraction wave starts at the cardiac base and spreads through both ventricles to end at the apex. This means that the volumetric curves in both atria and in both ventricles have the very same frequency, but they are dephased by a 180° difference between atria and ventricles, since the ventricular filling occurs when the atria empty their blood into the ventricles.

The E-RAC information stored in the memory of the computer and corrected by background substraction can be transformed on the diverse components of the temporal frequency of the composite cardiac cycle. This is achieved by the harmonic analysis of the volumetric curve in each individual pixel of the matrix, through the application of the discrete transform originally used by Fourier for the analysis of wave movement. This mathematical tool functions in a similar to the logarithm and is used to represent a periodical function as the sum of sines and cosines of different frequencies, each one characterized by specific amplitudes and phases. The final result is a set of parametric images objectively representing the amplitude and phase of the volumetric curve in each pixel in the cardiac matrix **(Fig. 24.12)**.

AMPLITUDE IMAGES. The amplitude of a sinusoid refers to its maximal deflexion and in this case represent the maximal variation of radioactivity or blood volume in a single pixel, independent of the time in which it occurs during the cardiac cycle. The resulting image shows the magnitude of regional amplitude of the wall motion in the four cardiac cavities, and it is equivalent to the stroke volume image. However, the amplitude image contains more data density and possess a better visual quality than the stroke volume image since it is integrated with the data collected through the complete cardiac cycle, while the stroke volume image is obtained through the manipulation of the images attained during two isolated and very short periods of the cardiac cycle (ES and ED), including no more than 1/16 of the cardiac cycle, each.

We have also applied the Fourier's transform to generate other parametric images of the heart function based on data collected through E-RAC. These, are:

(a) **Ventricular amplitude.** The ventricular volumetric curve is similar to the cosine function, with an important positive real component and an a very small imaginary component approaching zero. This fact can be used to generate a ventricular amplitude image representing the variations of the positive cosine, where the atrial amplitude is not included but where hypokinetic and akinetic areas are readily depicted.

(b) **Atrial amplitude.** The atrial volumetric curve is similar to the sine function, with an important positive contribution of the imaginary component and almost no participation of the real component. This image can be constructed by representing the variations of the positive sine, isolating the image of amplitude in the atria, without the image of ventricular amplitude. This image is equivalent to the auricular filling or "paradoxical" image, but contains more data density and is of a better visual quality. It will show the paradoxical regional filling in the ventricles during systole in places with regional dyskinesis.

(c) **Ventricular ejection fraction.** This image can be generated by dividing the matrix of the ventricular amplitude image, representing stroke volume, by the ED image. However, the ED image needs to be multiplied for the number of images in the series to attain a proportional intensity to that of the ventricular amplitude image. It will also depict the hypokinetic and akinetic regions as areas with decreased or abolished intensity.

PHASE IMAGE. The intracavitary volumetric curve phase is that precise moment of the cardiac cycle when the blood volume and the radioactivity reach their maximal levels in a particular region, just previous to the start of the next contraction. This, never occurs in a synchronous fashion in all cardiac regions, since the contraction wave spreads progressively from the cardiac base towards the apex (**Fig. 24.13**). The first step in this method is to display the sequence of 16 images conforming the series of E-RAC properly corrected by background substraction. However, the computer is programmed to show in each frame only those pixels where the corresponding volumetric curves are in phase. As it is obvious, any particular pixel can only be in phase in only one of the 16 frames of the composite cardiac cycle. The second, is to assign a different colour or a different intensity of grey to each frame, and to add the 16 frames into a single image, where the temporal distribution of the contracting wave is depicted by the distribution of pixels with different colours, each colour representing a distinct period of the cardiac cycle. The recorded images can be also displayed in an endless loop cinematographic fashion allowing the dynamic analysis of the temporal distribution of the contracting wave.

This parametric image could be complemented by a histogram showing the number of pixels in phase at each different period of the cardiac cycle, which is divided in 360°. Each bar in the histogram is made to coincide with the chromatic code in the image. It normally shows a small peak, corresponding to the atrial phase, separated by 180° from a second bigger peak, representing the ventricular phase.

The value of these parametric images is obvious in many conditions. Clinically, they are especially useful in the study of wall motion and conductive alterations, in the objective demonstration of the consequences of regional myocardial ischemia, either at rest or during

exercise, in the evaluation of the functional damage produced by an acute or old myocardial infarction, and in the monitoring of the therapeutic effects of coronary surgery, angioplasty or thrombolysis. Besides, they could be of help in the optimal placement of cardiac pacemakers.

Technically, they are a valuable adjunct for the precise location of the ROIs on the images of FP-RAC and E-RAC.

This procedure is also highly recommended in developing countries, especially in the study of CAD due to its relative simplicity and low cost when compared with the use of thallium-201 or $^{99}Tc^{m}$-MIBI.

G. Other instruments used in nuclear cardiology

The nuclear cardiac probe

The introduction of the nuclear cardiac probe or nuclear "stethoscope" revives and improves the old technique known as quantitative angiocardiography used in the 50s by Donato in the study of cardiac function. It is a low cost, portable alternative for the non-invasive assessment of both systolic and diastolic LV function. This device consists of a small scintillation probe attached to a small microprocessor and a display screen. It has the advantages of compact size and increased portability, allowing its use at the patient's bed, as well as its low cost when compared to that of a gamma camera. It has the capability to record and analyze the first pass time/activity curve according to the method of quantitative angiocardiography, to build a composite volumetric curve with several cardiac beats in the fashion of E-RAC, and to do a beat-to-beat analysis as well.

However, as a non-imaging device, the cardiac probe may have some limitations in measuring EF, especially in patients with regional wall motion abnormalities. For example, if the probe is positioned over an area of regional hypokinesis the contribution of normal or hyperkinetic segments will be under represented and the study may underestimate the actual EF. Conversely, the deliberate positioning of the probe on the region of maximal periodicity (maximal ratio of stroke counts to average counts), as routinely done, may overestimate EF. The same problem holds for the selection of the area to measure background, which is critical for the accurate measurement of EF. Thus, it is not surprising that in patients with CAD and regional left ventricular dysfunction the EF measured by the cardiac probe has only a modest correlation with the results obtained through E-RAC or contrast angiography. The probe, however, could be practical in monitoring in a beat-by-beat fashion the **relative changes** in the ventricular volumetric curve and in global EF in critically ill patients, or during pharmacological studies.

The cardiac vest

This instrument is a miniature radiation detector attached to a vest to be worn by the patient, and to an electronic device for the continuous recording of the ventricular volumetric

curve. It is of some value for the cardiac monitoring during normal life as a "nuclear Holter", but its correct positioning requires the use of a gamma camera to locate the right place. In any case, up to now, the cost/benefit ratio of the use of these two relatively simple instruments has been the biggest enemy of a probably good idea.

The mobile gamma camera

The mobile gamma camera is an excellent choice for nuclear cardiology. It is provided with a magnetic memory to record all data during acquisition, it has a smaller detector head than the usual gamma camera, which allows by itself a magnification of the images of the heart on the monitor screen and on the photodisplay, decreases the size and weight of the collimators, and facilitates its proper positioning over the patient's chest. More important than all that is the possibility it gives to bring nuclear cardiological procedures to the bed side of the patient, to the coronary care unit, and to the operating room.

Unfortunately, it has also some draw backs. The detector head is not properly shielded and the collimators are made of low density alloys to decrease weight, which means that the mobile gamma camera is limited to imaging with gamma photons with less than 200 keV, excluding the of use of radionuclides like ^{131}I and ^{67}Ga. Besides, the author has seen many mobile gamma cameras used as stationary in the developing countries, because the temperature differences between the nuclear medicine service and the hospital corridors endangers the detector's crystal or because the architecture of the hospital is not suitable for the gamma camera: narrow doors and corridors, small rooms and lack of appropriate elevators.

2. MYOCARDIAL PERFUSION SCINTIGRAPHY

Myocardial regional perfusion can be analyzed by following two basically different scintigraphic procedures, each designed to study a different level of circulation: the permeability of coronary microcirculation and the myocardial tissular perfusion. These studies are based on the indicator fractionation principle described by Sapirstein, according to which the fractional uptake of the injected tracer by an organ equals the fraction of cardiac output perfusing that organ during the measurement. This is absolutely true for non-recirculating indicators such as radioactive microspheres or macroaggregated albumin, since virtually all the radioactivity that reaches the organ capillaries is trapped in the first circulation. This explains why imaging with these radioactive agents has been considered by many as the "gold standard" for myocardial perfusion.

A. Coronary capillary bed scintigraphy

This procedure was introduced in 1970. It is, in fact, the only procedure in medicine which studies regional myocardial perfusion, but its application is always as an important adjuvant to radiological coronary angiography since it requires an intracoronary injection of the radiotracer. Its fundamental principle is the same as that of lung perfusion scintigraphy

with radioactive particles: the transitory lodging of these particles in the first capillary encountered by the injected particles.

As in lung perfusion scintigraphy, the most common radiotracers are: albumin macroaggregates or microspheres labelled with $^{99}Tc^m$, ^{111}In, $^{113}In^m$, or radioiodine. However, the measures for the quality control of the tracer must be particularly stringent before its administration to the patient. The labelling efficiency should be higher than 95%, the particles should be from 30 to 50 μm in size with an average of 30 μm, and the total number of particles should never exceed 150 000 in the individual dose. By following these strict rules our experience at the National Institute of Cardiology in Mexico has been excellent.

Usually, the slow intracoronary injection of $^{99}Tc^m$- macroaggregated albumin (MAA) is made three minutes after finishing the procedure for coronary angiography and ventriculography to avoid the irritant effects of the radiological contrast agent. In this case, the test is considered as performed "at rest". But, if the intracoronary injection of the tracer is made immediately after, or simultaneously with the radiological contrast agent, the study is considered as a "stress study" since coronary blood flow increases four to five fold its normal value by the effect of these agents. The usual dose is three to six mCi for the left coronary artery, and one to two mCi for the right. Nuclear imaging can be performed immediately after and during the next hour. This is accomplished with the patient in supine position and by using a high resolution or a convergent collimator, accumulating at least 500 000 counts per image. The A, LL, RL, LAO and RAO views are documented. The use of a computer is not necessary, but it is convenient for improving the quality of the image and to extract quantitative information such as the radial histogram of the distribution of radioactivity in the ventricles, the measurement of the ventricular cavities and the myocardial thickness. If a computer is available it is advisable to acquire the information in a gated mode for the regional analysis of ventricular wall motion, in a way similar to that described for E-RAC.

The indications for this study are, of course, the very same as for coronary angiography: **myocardial ischemia.** Coronary angiography is the best procedure for studying the anatomical features of the coronary macrocirculation of vessels with a diameter wider than 100 μm, but it is useless in the study of the coronary microcirculation. The scintigraphic method, in the other hand, is the only procedure able to study the myocardial perfusion at the capillary level, although it is unable to render a direct information on the anatomic structure of the main coronary arteries. However, a significant stenosis in one or more arteries reduces blood flow and produces a noticeable reduction of radioactivity at the tributary capillary bed corresponding to the particular affected vessel. Since myocardial ischemia could be found in either one or both levels of coronary circulation, these procedures complement each other improving their respective diagnostic accuracies. This is of special significance in diabetic patients with typical angina and normal coronary angiography, in whom the scintigraphic method is able to demonstrate the myocardial perfusion defects caused by the capillary alterations seen in diabetic microangiopathy (**Fig. 24.14**).

In a study of 135 patients with typical angina performed at the National Institute of Cardiology of Mexico, coronary angiography showed a lower sensitivity (85.0) than the scintigraphic study "at rest" (90.3%), but the combination of the results with both procedures was more sensitive (96.3%). Furthermore, it has been observed that in resting conditions capillary bed scintigraphy with radioactive MAA was more sensitive (95%) than thallium-201 scintigraphy (80%) in detecting CAD.

A double-radionuclide technique can be used by injecting $^{99}Tc^{m}$-MAA in one coronary and ^{111}In-MAA in the other. In this fashion, it is possible to learn with accuracy the boundaries of each capillary territory in order to get a precise evaluation of the heterocoronary collateral circulation and data on the patency of surgical aortocoronary implants.

A similar double-radionuclide technique can be also applied to perform the "rest"/"stress" studies simultaneously by administering $^{99}Tc^{m}$-MAA immediately after the injection of the radiological contrast media ("stress" dose), followed after 3 min. by an injection of ^{111}In or $^{113}In^{m}$ labelled MAA or iron macrocoprecipitates ("rest" dose). The different gamma energies of these two radionuclides allows the acquisition of "rest" and "stress" images separately.

If both images show normal tracer distribution in the myocardium, the images are considered as enough evidence to exclude the possibility of coronary and myocardial diseases. But, if the "rest" image evidences a normal myocardial distribution of the tracer and the "stress" image shows a perfusion defect in the myocardium, the result indicates CAD with reactive myocardial ischemia. On the other hand, if both images show the same perfusion defect, the result indicates myocardial infarction without reactive ischemia, but if both images evidence abnormal myocardial distribution of the tracer but these alterations are more severe in the "stress" image, it is a manifestation of previous myocardial infarction and reactive ischemia.

This procedure, which seems to be too complicated and cumbersome,needs good collaboration between the nuclear medicine unit, the radiological department and/or the haemodynamics service. However, it is highly recommended as a adjuvant to contrast coronary angiography, especially for hospitals with good cardiological infrastructure in the developing countries because it is safe when properly performed, it is of a relatively low cost when compared with other myocardial perfusion studies, and offers very important clinical information regarding the coronary microcirculation not available by radiological angiography. Its major limitation is, however, the need of coronary catheterization for the administration of the tracer, which places this procedure outside conventional nuclear medicine as it is no longer non-invasive. In fact, the ideal would be to have a radiotracer which is extracted from the blood by myocardial tissue in order to perform the scintigraphic procedure as a screening test prior to coronary angiography.

B. Myocardial tissue perfusion scintigraphy

Sapirstein's principle can also apply to recirculating indicators as long as organ extraction and systemic extraction of the indicator are the same. Since potassium is essential for muscular contraction and myocardium is a muscle, the first attempts to measure myocardial blood flow were made with radioisotopes of potassium and other radionuclides which are isotopes of elements analog to potassium. The former assumption was soon verified in animals with ^{42}K and other radionuclides which are isotopes of elements analog to potassium, such as cesium and rubidium. These radionuclides, however, were impractical for nuclear medicine imaging due to their unfavourable decay schemes and gamma radiation energies.

Thallium-201 as a myocardial tracer

Thallium-201 (^{201}Tl), a cationic potassium analog, became widely accepted as the tracer of choice for myocardial perfusion scintigraphy in man since its first introduction into clinical nuclear medicine in 1975. Although the regional myocardial uptake of ^{201}Tl has been shown to be an accurate representation of regional perfusion, the myocardial extraction fraction for this tracer exceeds by 15% the systemic extraction fraction when myocardial blood flow is low, normal, or moderately elevated, and this relationship is not altered by ouabain, regional ischemia or infarction. Administration of dipyridamole, however, increases further the myocardial blood flow and decreases the myocardial extraction fraction, equalizing myocardial and systemic extraction fractions.

Nonetheless, ^{201}Tl-chloride suffers from a number of constraints and is far from being the ideal tracer for myocardial perfusion, which should have the nine properties included in **Table IV**. However, of these nine properties, only three are met by ^{201}Tl. Indeed, thallium has a reasonably rapid blood clearance, it is distributed in the myocardium according to, and in proportion to regional blood flow, and has no pharmacological effect at the usual dose, but the intracellular pool in the myocardium influences its concentration. Besides, it has a biological half-life in myocardium of about seven hours, which aside from altering the imaging statistics results in a re-distribution of myocardial ^{201}Tl. This, means that after a lapse of time the distribution of the tracer in myocardium no longer reflects the regional blood flow at the time of injection. It is for this reason that imaging with ^{201}Tl should be performed immediately after the intravenous injection of the tracer. Furthermore, the effective half-life of thallium-201 (9.4 days) is far to long for the needed period for imaging (four to five hours), resulting in an unnecessary radiation load to the patient.

The physical characteristics of the radionuclide and its radiations have a direct influence on image quality. Since spatial resolution of the imaging system is a function of photon energy and collimation, ^{201}Tl is less than optimal for routine use. The characteristic x-ray emitted by mercury, product of ^{201}Tl transmutation, have energies which are near the lower end in the scale of the gamma-camera resolution. Furthermore, this x-ray emission causes a wide spectrum stretching from 69 to 83 keV, yielding a less than satisfactory energy resolution. Consequently, the use of high resolution collimation is almost mandatory. This results in a loss of sensitivity, which is already affected by the low administered dose of thallium, and extends the imaging time, which increases the problems in evaluating the rapid redistribution of the tracer.

Of these nine properties, only the first three are met by ^{201}Tl. Indeed, thallium has a reasonably rapid blood clearance, it is distributed in the myocardium according to and in proportion to regional blood flow, and has no pharmacological effect at the usual dose, but the intracellular pool in the myocardium influences its concentration. Besides, it has a biological half-life in myocardium of about seven hours, which aside from altering the imaging statistics results in a redistribution of myocardial ^{201}Tl. This, means that after a lapse of time the distribution of ^{201}Tl in myocardium no longer reflects the regional blood flow at the time of injection. It is for this reason that imaging with ^{201}Tl should be performed immediately after the intravenous injection of the tracer. Furthermore, the effective half-life of thallium-201 (9.4 days) is far too long for the needed period for imaging (4 -5 hours), resulting in an unnecessary radiation load to the patient.

TABLE IV. CHARACTERISTICS OF THE OPTIMAL RADIOTRACER FOR MYOCARDIAL PERFUSION

Present in ^{201}Tl-chloride	Absent in ^{201}Tl-chloride
- No pharmacological effects at usual doses	- No myocardial clearance or redistribution
- Regional myocardial distribution proportional to regional blood flow over the physiological range.	- Gamma-ray energy suitable for imaging
- Rapid blood clearance.	- Low absorbed radiation dose by the tissues.
	- Short effective half life.
	- Ready availability.
	- Reasonable cost.

The physical characteristics of the radionuclide have a direct influence on image quality. Since spatial resolution of the imaging system is a function of photon energy and collimation, ^{201}Tl is less than optimal for routine use. The emitted mercury x-ray's energies are near the lower end in the scale of gamma camera resolution. Furthermore, the x-ray emission of the daughter mercury causes a wide spectrum stretching from 69 to 83 keV, yielding a less than satisfactory energy resolution. As a result, the use of high resolution collimation is almost mandatory. This results in a loss of sensitivity extending the imaging time, which increases problems of evaluating rapid redistribution of the tracer.

There are a number of options that one might consider to preserve spatial resolution and

increase sensitivity. The administered dose (1 to 2.8 mCi) could be increased, but at the expense of increasing the radiation dose to the patient and the cost of the tracer, both already high in the normal situation. A second option is using all the ^{201}Tl photopeaks, collecting counts from the mercury x-ray (95% abundance) and both the 135 keV (2%) and the 167 keV (8%), increasing sensitivity without an additional radiation burden to the patient. In fact, although ^{201}Tl imaging has been used in a variety of clinical settings, it has only been through advancements in instrumentation that the drawbacks of this radionuclide have been tolerable. Thinner NaI(Tl) crystals, increased numbers of photomultiplier tubes, and single photon emission computerized tomography (SPECT) have all contributed to improve ^{201}Tl imaging. These advances, however, still do not overcome the problems of limited dose administration, lengthy imaging times, and expense.

Readily availability is another real problem with ^{201}Tl. It is not only expensive, but also unavailable except for routine scheduled procedures and creates serious logistic problems. As a result, large metropolitan institutions with round the clock cardiac care may not always be able to use ^{201}Tl myocardial imaging for acute care.

^{99}Tc^{m}-methoxyisobutyl isonitrile as a myocardial tracer

The most reasonable solution to all these problems related to the use of ^{201}Tl in myocardial perfusion imaging would be the use of ^{99}Tc^{m} as a label. The last few years have seen the development of a number of ^{99}Tc^{m}-labelled derivatives of the isonitrile complexes that show useful uptake by the human heart.

The first of these complexes to show myocardial uptake in the human was the **t-butylisonitrile complex (TBI)**, but it also showed myocardial redistribution and high lung and liver uptake and retention. By engineering the basic hexakis-(alkylisonitrile) technetium (I) structure it was possible to produce **carbomethoxyisopropyl isonitrile (CPI)**, which shows low lung uptake, myocardial clearance with rest redistribution, as well as high liver uptake with substantial gallbladder concentration and elimination in the early phases of imaging.

The second analog of TBI to be developed was **methoxyisobutyl isonitrile (MIBI)**, which retained the myocardial uptake benefits of its sister compounds, while increasing the clearance characteristics in the blood, lung, and liver. The superior heart-to-lung and heart-to-liver ratios offer a marked improvement in image contrast, but its more important feature is that myocardial MIBI uptake remains unchanged for long periods of time with no redistribution. This allows a number of variations in myocardial perfusion imaging with exercise tolerance testing. Post-exercise recovery time need not be compromised because of redistribution, and the SPECT and gated imaging may be done without concern about alteration in biodistribution.

The advantages of the use of (99Tcm)-MIBI when compared with that of ^{201}Tl are obvious and more significant for the developing countries, where this should be the tracer of choice for myocardial imaging. ^{99}Tc^{m} is the least expensive, most widely and readily

available radionuclide in current use. Most scintillation cameras are designed around its favourable physical characteristics. Its short physical half-life (six hours) and its monoenergetic photon emission are also favourable to dosimetry and allow for higher administered doses (5 to 30 mCi, instead of 1 to 2.8 mCi for ^{201}Tl) with a resultant increase in photon flux. Since myocardial uptake of either of these tracers is never higher than 4% of the dose, the magnitude of the administered dose is especially critical. The high count rate, improved energy resolution, and lower photon attenuation, result in improved spatial resolution for planar and gated imaging and for SPECT. In addition to the ideal physical factors, the possibility of an easily prepared myocardial perfusion cold kit provides for round the clock scintigraphy of acute myocardial infarction. Commercial cold kits, although more expensive than expected, can be easily fractionated to be used in different patients on different days, decreasing the individual dose cost to levels below the cost for a ^{201}Tl dose.

The absence of myocardial redistribution and the fast hepatobiliary elimination of MIBI have major implications for its clinical applications. Rest and exercise studies need to be performed with separate tracer doses, either on different days or during the same day. In the former case, the doses could be the same, but in the later the first study is performed with 5 mCi of MIBI and the second, five hours later, with 30 - 35 mCi. The stable intramyocardial distribution of this complex and the higher photon flux allow for optimal SPECT acquisition or for any imaging delay after injection. The resulting images reflect the distribution of myocardial perfusion at the moment of injection and the compound could be administered during an acute event such as chest pain or asymptomatic ST depression at the bedside of the patient in the coronary care unit or at the emergency room, with imaging up to four hours later, when the patient is stable. This flexibility also allows for pre- and post-imaging in those patients undergoing some form of therapeutic intervention such as percutaneous coronary angioplasty or thrombolysis. An additional advantage of this ^{99}Tc^{m} complex is the possibility to perform a FP-RAC during its intravenous administration, in order to obtain information regarding regional wall motion and EF besides regional myocardial perfusion.

Both, ^{201}Tl chloride and ^{99}Tc^{m}-MIBI can be used to study myocardial perfusion, either at rest or after inducing an increased coronary blood flow by exercise or by pharmacological intervention. However, the protocol is different for each radiotracer.

Myocardial perfusion imaging at rest. It should be initiated within the first 10 min after the intravenous administration of thallium (1 - 2.8 mCi) or from ten minutes up to four hours after the intravenous injection of ^{99}Tc^{m}-MIBI (5 - 30 mCi). Planar imaging of the heart is documented in each of the three views, A, 50° and 70° LAO. Although very sensitive in detecting myocardial "perfusion" defects at rest, the information lacks specificity because the origin of these "perfusion" defects could be several in nature (**Table V**). In any case, this procedure has a very special role in the accurate follow up of the evolution of all these pathological processes and in the periodic evaluation of medical therapy or surgery. In the developing countries it is of special interest in the follow-up of congestive myocardiopathies, as those due to Chagas' disease.

TABLE V. CAUSES OF MYOCARDIAL "PERFUSION" DEFECTS AT REST.	
Possible causes.	**Clinical examples.**
Real perfusion defect	Coronary artery disease. Diabetic microangiopathy
Displacement of myocardial tissue	Lymphomatous infiltration Amyloid infiltration
Myocardial inflammation	Cardiac trauma Congestive myocardiopathy
Myocardial necrosis	Acute myocardial infarction Congestive myocardiopathy
Myocardial replacement	Myocardial fibrosis Myocardial tumours

Myocardial perfusion imaging during exercise. Exercise increases oxygen demands by myocardium. The normal response to this is an increase in coronary blood flow and in the myocardial uptake of ^{201}Tl or ^{99}Tc^{m}-MIBI. These effects could not occur in the territory tributary of a stenotic semi-obstructed coronary artery or where the coronary capillary bed is abnormal since they are unable to increase blood flow to the affected area, producing transient regional ischemia and a corresponding regional tracer concentration defect. This is the fundamental pathophysiological fact used to increase the specificity of myocardial imaging with these tracers for CAD, which is achieved through the comparison of the myocardial images obtained during exercise and at rest. This will be reflected as a regional concentration defect in the image obtained during exercise, but not apparent in the image taken at rest. The filling of an exercise induced concentration defect in resting conditions is a clear evidence of transient ischemia in viable myocardium in the area. All other pathologies may produce persistent myocardial concentration defects in both physical conditions, reflecting the absence of viable myocardial tissue.

The methodology follows a different protocol according to the used radiotracer, but, in any instance, the patient need to be told to withhold beta blocker or calcium antagonist medication for 24 to 48 hours and long acting nitrates for six hours prior to testing. The patient's ECG must be continuously monitored and a cardiologist well trained in cardiac emergencies should be always present while the patient is at the nuclear medicine department.

Protocols with ^{201}Tl-chloride

Single dose stress/redistribution approach

The patient should be in fasting condition for at least the four previous hours. A small venous catheter is inserted in one of the right elbow veins of the patient and is maintained patent by a slow infusion of saline solution. The patient is made to exercise in a treadmill or in an ergometric bicycle according to Bruce's protocol. At near peak exercise according to the patient's age, a dose with 1.5 to 2.8 mCi of ^{201}Tl is injected through the inserted catheter while the patient continues exercising for another minute. Other exercise end points are exhaustion, development of moderate to severe angina, depression of the S-ST segment in the ECG, serious arrhythmia, or exertional hypotension.

Imaging acquisition should be started during the first ten minutes after the tracer's administration, documenting multiple views to define the exact location of any abnormality. The view defines the LAD coronary artery territory best, whereas the various LAO projections (30°, 45°, 60°, 70°) provide the best information regarding the distribution territories of the LAD, RCA and the circumflex coronary arteries. The very same set of images is documented again four hours after the tracer's injection, to obtain information regarding thallium distribution in "resting" conditions.

A reversible abnormality between the initial and delayed images is considered ischemic, while a persistent defect is considered as scar or other myocardial pathology able to replace functioning myocardial cells.

With this stress/redistribution approach two different phenomena are studied: perfusion with the initial images, and the myocardial potassium pool as an indication of myocardial viability with the delayed images. This approach also simplified the original method which was done with two separate injections, the first at peak exercise and the second, at rest, after an interval of several days.

In general, interpretation of these images is straight forward, considering the features of the tracer distribution in myocardium and the morphological characteristics of the ventricular wall and cavity in both images **(Table VI)**.

It is important to note the number, the size and the location of any concentration defect, since they are clues regarding the extension of the affected territory, the magnitude of ischemia, and the particular affected artery or arteries.

TABLE VI. INTERPRETATION OF STRESS/REST THALLIUM IMAGES.	
Scintigraphic finding in myocardium	**Clinical interpretation**
No concentration defects	No myocardial ischemia No myocardial infarction
Concentration defect during exercise, not evident at rest	Transient myocardial ischemia induced by exercise
Concentration defect during exercise which is smaller at rest	Old scar surrounded by ischemic myocardial tissue
Similar sized concentration defect in both exercise and rest images	Absence of functional myocardial tissue

In addition to evaluating perfusion, a number of clinically relevant observations can be made from ^{201}Tl scintigrams concerning the left ventricular architecture, as, for instance, the finding of a dilated cavity or hypertrophied left ventricular myocardium. However, the evaluation of chamber size and wall thickness from planar thallium images must be done with caution, since these parameters are significantly dependent on the wall motion, and, to a lesser extent, on heart rate, as shown by ECG gating of ^{201}Tl images. This technique decreases blurring due to wall motion, and the apparent wall thickness is greater and the cavity is smaller on the composite views of planar images when compared with the gated end-diastolic views alone.

Nevertheless, the composite nature of planar images allows to deduce physiological regional wall abnormalities from the regional altered shape of myocardium. Hypokinetic areas are narrower than normal, while hyperkinetic regions are wider, and a ventricular aneurysm may change ventricular morphology with a regional convexity. All these factors are added to transform the normal smooth triangular morphology of the ventricle into an irregular, semiround square. In our experience with both ^{201}Tl and ^{99}Tc^{m}-MIBI, we have noticed a correlation between these morphological alterations and the magnitude of ventricular malfunction.

Another important physiological sign in the exercise images is the appearance of pulmonary ^{201}Tl uptake, which is related to induced pulmonary venocapillary hypertension. Normally, the pulmonary/myocardial activity ratio is less than 0.40, but in patients with single vessel disease this ratio increases to 0.45 - 0.50, while in cases with two or more affected arteries it is higher than 0.50.

In the case of ^{201}Tl it is necessary to consider the radiotracer's myocardial clearance and redistribution, which, on one side, allow the stress/redistribution approach with a single tracer dose, but, on the other, add some complications in the pathophysiological interpretation of some studies.

Cellular uptake of thallium depends on arterial tracer concentration, nutrient flow and transcapillary extraction. Thallium washout results from a net clearance of the radionuclide from the cellular pool which is greater than the rate of tracer uptake. Consequently, thallium washout is faster in myocardial zones having higher blood flow and is directly related to myocardial perfusion. In ischemic zones the initial uptake is very low, but redistribution gradually increases the uptake until reaching peak activity at relatively long times (40 - 90 min) and, consequently, the net thallium washout over two to three hours is relatively slow. It is also interesting to note that myocardial thallium clearance has also been directly related to peak exercise heart rate, which again suggests a strong relationship between coronary flow and thallium redistribution.

The single dose stress/redistribution approach has been preferred for its simplicity and relatively low cost when compared with the technique using separated tracer doses for the exercise and the rest studies. It has, however, several limitations which affect its sensitivity and specificity. When the single dose stress/redistribution images had been compared with those obtained in a different day, in the same patient, after the injection of a second dose at rest, an important fraction of the persistent concentration defects found on the redistribution images were larger than on the rest images, while another, less significant fraction "filled in" after the rest injection. Furthermore, it has been also reported that after by-pass surgery normal thallium was demonstrated in nearly half of the persistent defects on the previous delayed redistribution images, and that 75% of the fixed defects detected in patients before angioplasty of their single vessel LAD lesion, improved or became normal after PTCA. In both cases, the stress/distribution approach fails in detecting viable myocardium and discarding the presence of prior infarction. In fact, the extent or presence of prior myocardial infarction is often overestimated, and the presence of viable myocardium is underestimated by the redistribution technique, significantly reducing the specificity of the procedure.

Delayed imaging and reinjection approach with ^{201}Tl

It seems that the time to complete redistribution after stress injection is related to the persistence of ischemia at rest and to the severity of the stenosis in the coronary artery supplying the affected area.

Based on these observations, some investigators consider the 24-hour ^{201}Tl delayed imaging as a more accurate method to detect viable myocardium and, more recently, a method of ^{201}Tl re-injection has been described using a second, smaller dose of thallium injected at rest (re-injection) immediately after the redistribution images, instead of several days later as in the early 70s. In this fashion, instead of comparing flow-related images (initial) to redistribution images (delayed), two different flow-related images are compared reflecting different conditions of flow (at peak exercise and at rest). Other studies have been performed that confirmed the superiority of the re-injection technique compared with the single injection stress / redistribution / delayed imaging.

However, even the re-injection approach failed to identify ischemia in a patient whose myocardial concentration defect remained basically fixed following re-injection, and only the images taken 20 hr after rest re-injection identified a significant amount of viable myocardium in jeopardy. Three months after PTCA, thallium scintigraphy, performed with the re-injection protocol, did not show evidence of increased lung uptake nor of cavity dilatation on stress images. Although there were moderate reversible defects, these normalized completely after re-injection.

We need to be aware, however, that the studies reported to date with the re-injection approach have investigated patients with a high pre-test probability of significant CAD. It is therefore not clear that these data will be applicable to unselected patients presenting to the exercise lab for perfusion imaging. In this regard, it is important to emphasize that in most circumstances the indication for thallium imaging is CAD detection. For this indication, both clinical experience and the accumulated data suggest that conventional stress/redistribution approach is adequate. As such, the additional expense, radiation exposure, and logistic difficulties attendant to re-injection should not be trivialized. Re-injection should be performed only when no redistribution is observed on the delayed images, and it may hold promise for identifying severely ischemic but potentially viable myocardium.

The problem of reverse redistribution of ^{201}Tl in the myocardium

It is also important to comment on another complication in the use of ^{201}Tl as a myocardial scintigraphic agent, which refers to a rapid washout of the tracer in an area of myocardial infarction in patients post-reperfusion. This is associated with a visual pattern of "reverse redistribution", where initially normal or near normal ^{201}Tl segments involved with the infarction worsened on delayed views. This phenomenon has been also reported in the absence of CAD and in canine experimental AMI after reperfusion. These observations may have physiological and clinical significance but the only available hypothesis involves regional hyperaemia and regional disparities in cellular function and blood flow during thallium redistribution. More data need to be collected before this issue can truly be settled.

Protocol with ^{99}Tc^{m}-MIBI

The following protocol has been successfully used by the author during the last 5 years. The first steps are identical to those already described for the ^{201}Tl studies: patient in fasting conditions, vein canalization previous to exercise according to the Bruce's protocol, and intravenous injection of 5 mCi of ^{99}Tc^{m}-MIBI. Imaging starts one hour after the tracer injection. In the mean time, the patient is suggested to breakfast with a greasy meal to increase hepatobiliary elimination of the tracer, reducing background radiation in the chest and increasing the myocardial contrast in the images. After exercise imaging the patient may leave the premises, but should be back four hours after the tracer's administration to receive a second (resting) dose, seven-fold bigger than the previous (35 mCi). He is suggested to enjoy a greasy meal again, and to be back for imaging one hour later. The myocardial constancy of the tracer allows the documentation of the usual planar images and the acquisition of SPECT images in each session.

The images taken during exercise are duly corrected by the difference between the two injected doses as measured in a dose calibrator. Both sets are interpolated into bigger matrixes and smoothed. The circunferencial histograms of the distribution of MIBI in the myocardium are displayed, together with the histogram of the differences. Contrary to the histograms with ^{201}Tl, the histogram during exercise shows a higher profile than the one at rest, in relation to the increased myocardial blood flow induced by stress. The tomographic images of the vertical and horizontal slices along the long ventricular axis, as well as those of the short axis are displayed, and the polar map of the tracer distribution in myocardium and the tridimensional images of the heart are generated.

Clinical interpretation of the images

The interpretation of myocardial images with ^{201}Tl or ^{99}Tc^{m}-MIBI should always be made in collaboration between the nuclear physician and the cardiologist. First, the planar images are analyzed and the findings correlated with the circumferential histograms and confirmed on the SPECT images. Only those defects appearing in more than three consecutive slices and confirmed on at least in three images in other planes are considered seriously. The polar or "bull's eye" map and the tridimensional images are not considered during the interpretation. We have found that either with thallium or MIBI the polar map is too sensitive and usually overestimates minor regional variations in concentration, both in size and magnitude, unduly decreasing specificity.

The planar and SPECT images obtained with ^{99}Tc^{m}-MIBI following this protocol are by far of a better visual quality than those acquired with ^{201}Tl. This is easily explained by the physical characteristics of the tracers and by the different dosages. Although the effects of photon attenuation by well developed male pectorals, by female breasts, and by left diaphragm in both sexes are more evident with thallium, the 140 keV photons emitted by technetium are amenable to similar artifacts. The only limitation to the use of MIBI is in accurately detecting viable myocardium in areas with very low blood flow, since the images reflect blood flow at the moment of the tracer injection and this is rapidly cleared from blood by the liver. In these cases the use of the re-injection approach with ^{201}Tl would be indicated.

Operating characteristics and selection of patients

It seems surprising to note that in spite of the development of SPECT with technetium based radiopharmaceuticals, the operating characteristics for myocardial perfusion scintigraphy have not really been modified since the procedure was introduced with ^{201}Tl and planar scintigraphy.

Exercise myocardial planar scintigraphy with ^{201}Tl or ^{99}Tc^{m}-MIBI is indeed the most useful noninvasive widely used test to display abnormal myocardial perfusion due to CAD. Diagnostic sensitivity has ranged from 70% to 90% and specificity from 86% to 100%, compared with 60% sensitivity and 81% specificity for the ergometric ECG test in optimal conditions. However, there is an evident inverse relation between sensitivity and specificity. Those publications reporting high sensitivity are associated with a lower specificity, and vice

versa. These results are typical of receiver operating characteristics curves for an imperfect test, in which an observer who overinterprets borderline images as being positive will also thereby read as positive a number of studies that are negative, thereby obtaining a larger number of false positives, i.e., a lower specificity. Conversely, conservative interpretation will increase the specificity but decrease the sensitivity.

A sensitivity and specificity near 85% might suggest that the test could be adequate as a screening procedure for detecting CAD in asymptomatic patients. This sensitivity and specificity, however, do not account for the effect of disease prevalence on the diagnostic accuracy and utility of stress myocardial scintigraphy when it is applied to asymptomatic populations. A number of papers utilizing Bayesian analysis have indicated that the sensitivity and specificity of thallium stress testing is grossly inadequate for a screening test in populations with a low prevalence of CAD ranging from 5 to 10% of the population studied. An abnormal exercise thallium test in an asymptomatic subject in this population is associated with only a 10 - 20% probability of having CAD.

Clinical indications

Although the major aim of the procedure is in the diagnosis of CAD, its indications depend on the relative operating characteristics, invasive character, and costs of the different available diagnostic procedures, and on the patient's symptomatology (**Table VII**).

TABLE VII. CLINICAL INDICATIONS FOR MYOCARDIAL PERFUSION SCINTIGRAPHY
Abnormal rest ECG in asymptomatic and symptomatic patients.
Abnormal stress ECG test in asymptomatic patients.
Normal or non-diagnostic stress ECG test in symptomatic patients.
Abnormal stress ECG test in symptomatic patients.
During the recovery phase after an acute myocardial infarction.

In general, left bundle branch blockade, digitalis toxic effects and non-specific changes in the ST-T segment do not allow a clear interpretation of stress ECG, but myocardial scintigraphy may render information regarding myocardial perfusion at rest and during exercise.

In the case of an abnormal stress ECG test in an asymptomatic patient, scintigraphy is intended to corroborate the presence of asymptomatic CAD. A positive result might lead to coronary angiography, while a negative result may indicate a false positive for the ECG stress study, but the patient will merit further monitoring.

On the other hand, a normal result in a patient with angina whose stress ECG test

resulted normal or non-diagnostic, a normal perfusion study may discard angina but indicates the need of further monitoring of the patient, while a positive result will suggest the need of coronary angiography.

An abnormal stress ECG study in a patient with angina is usually considered as a clear indication for coronary angiography and the patient would be dispensed of the cost of myocardial scintigraphy. But, when performed prior to catheterization, this study may lead the angiographer to a better visualization of coronary abnormalities, may demonstrate the presence of viable myocardial tissue amenable to by pass surgery, or may demonstrate enough functional collateral circulation in the affected area as to make by-pass surgery unnecessary.

During the recovering phase of an AMI, a submaximal exercise myocardial perfusion study is useful to control rehabilitation. If the study shows only the fixed concentration defect corresponding to the infarction, without any ischemic response, it is indicative of single vessel disease. Then, the rehabilitation programme can be safely initiated without the need of coronary angiography or more aggressive therapy. If the study demonstrates ischemic response in one or several different areas, coronary angiography should be indicated and by-pass surgery should be considered. However, it should be remember that a submaximal exercise test may not be sensitive enough for ischemia. In this case it is preferable to use pharmacological stress with dipyridamole.

Experimentally, there must be perfusion ratios of 2 - 2.5 or more between normal and diseased regions before detection by nuclear imaging becomes practicable, a difference corresponding to a 70 - 80% diameter stenosis, which represents advanced clinical disease. Single photon emission tomography has not yet been shown to provide a solution, although it offers some borderline improvement with an increased sensitivity to 95%, but a significant decline of specificity to 56% and even 44%.

Scintigraphy or angiography, who judges whom?

The problem is further complicated by the lack of a "gold standard" for defining the severity of coronary artery stenosis. It is well known that coronary arteriography has several limitations. Its interpretation is complicated by marked interobserver and intraobserver variability. The universal use of a relative percent diameter narrowing as a measure of severity ignores other critical geometric characteristics of stenosis, such as length, absolute diameter, multiple lesions in series, eccentric lesions that looks worse in one view than in another, or lesions that vary in severity due to coronary artery spasm. Thus, determination of percent stenosis alone has limited theoretical and experimental validity for assessing the significance of a stenosis. Only quantitative digital angiography can provide accurate, objective basis from which the adequacy of an imaging technique can be measured. However, we should not forget that myocardial scintigraphy is also sensitive to obstructions at the coronary capillary bed, where even digital angiography has nothing to offer.

If scintigraphy is validated by angiographic results the outcome will be a lower specificity than the actual, as, on the other hand, angiography will result with a lower sensitivity than

the real one if it is validated by the scintigraphic results. The fact is that each procedure renders qualitatively different information and complement each other. Myocardial scintigraphy offers data regarding the effects of alterations in coronary arteries and capillary bed on the myocardial regional perfusion, while angiography only informs about the site of stenosis in the coronary macrocirculation. Myocardial scintigraphy, besides being noninvasive is, then, more sensitive than coronary angiography and should be used first. Furthermore, a positive scintigraphic result coincidental with a normal coronary angiography does not rule out, but confirms the existence of an abnormal capillary bed. This is especially important in diabetic patients.

The apparent decline in the specificity of myocardial scintigraphy as compared to early reports is likely to be due to the operation of post-test referral bias. As myocardial scintigraphy gains increasingly widespread acceptance for the assessment of CAD, positive test responders are preferentially selected for cardiac catheterization, falsely increasing sensitivity and reducing specificity of the test. Thus, when a test result is a principle determinant of the need for catheterization, the catheterized population with normal coronary angiograms constitutes an inadequate gold standard for the assessment of either the sensitivity or the specificity of the test. Since it is unlikely that a random sample of patients with suspected coronary disease will be catheterized regardless of myocardial scintigraphy results, the true specificity of this study will be difficult to determine.

Specificity and normalcy rate

As an alternative for specificity, it has been suggested that patients with low pre-test likelihood of CAD are more suitable for the assessment of myocardial scintigraphic results. The term "**normalcy rate**" has been used instead to describe the result for this group in an attempt to distinguish this assessment from conventional specificity derived from patients with normal coronary arteriograms. In contrast to normal volunteers, patients with a pre-test low likelihood of CAD represent a subgroup of the general test population referred clinically, who are closer in age and symptoms to the CAD population than typical young, healthy volunteers. As expected, the normalcy rates, as proxies of specificities, has been significantly higher than the overall specificities derived from the normal coronary angiogram population.

Since there are a greater number of potential sources of false-positive studies with SPECT than with planar imaging due to the increased technical complexity and processing of data, it is likely that the specificity of thallium SPECT will truly be lower than that of planar imaging. The normalcy rates for ^{201}Tl SPECT are 82% compared with 88% in comparable groups in a ^{201}Tl planar trial.

Maximal stress as the critical factor for accuracy

Notwithstanding all the above considerations, the sensitivity and specificity of the procedure depend upon three essential parts of the method (**Table VIII**). However, submaximal stress leads to diminished sensitivity and specificity, even when using the best radiopharmaceutical and the ideal instrument. On the other hand, a stronger stimulus for

increasing coronary blood flow will increase myocardial uptake relative to background, thereby yielding improved images and diagnostic accuracy even with suboptimal tracers and/or instrumentation. Consequently, the critical factor for increasing diagnostic accuracy is a maximal stress.

TABLE VIII. FACTORS AFFECTING THE OPERATING CHARACTERISTICS OF MYOCARDIAL PERFUSION SCINTIGRAPHY
Quality of the myocardial perfusion agent.
Power of the imaging instrument.
Adequacy of stress for increasing myocardial perfusion.

Myocardial scintigraphy after pharmacological stress

Not all patients with suspected CAD can be given a maximal exercise test. This problem is seen most often in patients with poor physical condition, obesity, smoking habits, lung disease, peripheral vascular disease, and in those patients taking beta blockers. Since submaximal exercise test is one of the major causes of decreased sensitivity in the detection of cardiac ischemia, pharmacological coronary vasodilatation using dipyridamole is an alternative approach which is particularly useful in this group of patients.

There are at least two more clear indications for this pharmacologic stress test: in evaluating patients recovering from an AMI, where the presence of thallium redistribution on the dipiyridamole-thallium scan could be the only significant predictor of death, reinfarction or readmission for unstable angina, and patients prior to peripheral vascular surgery. In some developing countries the use of this procedure should be considered when an adequate ergometric system for the exercise test is not available.

Dipyridamole administration could be either intravenous or oral.

Intravenous administration of dipyridamol

The standard regime is slow intravenous injection of the drug (0.142 mg/kg/min) during four minutes, followed three minutes after by the intravenous administration of the tracer, since the peak effect of dipyridamol on coronary blood flow occurs 2 - 2.5 min after its injection and then declines exponentially with a half-life of 33 min. ECG, heart rate and blood pressure should be monitored every minute for ten minutes and longer if necessary. There is a mildly reduced systemic blood pressure, increased heart rate and cardiac output and an increase in coronary blood flow up to five times the resting values. Injection in the upright or standing position or with isometric handgrip may potentiate the effect of the drug on the myocardial uptake of the tracer.

Oral administration of dipyridamol

Dipyridamole may also be given orally (300 mg) as crushed tablets mixed with corn syrup extract and diluted with carbonated orange drink, resuspending the residual drug in the cup to ensure the administration of the entire dose. ECG, heart rate and blood pressure should be monitored every five minutes for 45 minutes or longer if necessary. The tracer is administered 45 min following the ingestion of the drug or with the occurrence of angina if it occurs before this time.

Adverse effects of dipyridamole

Adverse effects for both intravenous and oral administration are similar and occur in 30 to 40% of patients. Usually, they are mild and short lived. The most frequent are included in Table IX.

TABLE IX. THE MOST COMMON ADVERSE EFFECTS OF DIPYRIDAMOL ADMINISTRATION	
Angina (25%)	Nausea (20%)
ST depression (14%)	Headache (13%)
Dizziness (10%)	Facial flush (8%)
Vomiting (6%)	Ventricular arrhythmia (2%)

Symptoms of angina pectoris can be alleviated by the intravenous injection of **aminophylline**, a direct antagonist to the effects of dipyridamole on the coronary vasculature. If a large dose of aminophylline (approx. 200 mg) does not relieve the symptoms within several minutes, **nitroglycerine** can be administered, relieving angina by its direct effect on coronary stenosis and through reduction of preload. Some authors favour the routine administration of 75 mg of aminophylline following initial imaging because of the frequency of perfusion defects, even in the absence of symptoms of myocardial ischemia.

Sensitivity and specificity of serial ^{201}Tl imaging after dipyridamole infusion are comparable with those reported for thallium exercise stress testing, but the oral administration should not be encouraged because its decreased sensitivity.

The major advantage of intravenous dipyridamole stress imaging is that it can be successfully performed in patients who are unable to undergo maximal exercise testing. Its major disadvantage is the lack of additional information provided by the ECG response to exercise.

Myocardial perfusion studies in paediatric patients. Thallium or MIBI scintigraphy is of value in neonatal patients when it is suspected that the left coronary artery has an anomalous origin in the pulmonary artery. In this case, the images will show a very small or nil concentration of the tracer in the affected myocardial region, making possible the differentiation with congestive cardiomyopathy, which shows ventricular dilatation and normal or homogeneously reduced tracer uptake by myocardial tissue. It is also useful to visualize neonatal right ventricular hypertrophy secondary to increased pulmonary vascular resistance since the magnitude of myocardial tracer concentration in the ventricle is directly related to ventricular hypertension.

3. MYOCARDIAL INFARCT SCINTIGRAPHY

There are three different approaches for myocardial infarction imaging, each based on a different concentrating mechanism for the tracer:

A. Myocardial tissue perfusion scintigraphy at rest

Two factors are essential for the myocardial uptake of $^{201}Tl^{m}$-chloride or $^{99}Tc^{m}$-MIBI: arterial blood flow to the region, and cellular integrity within the region. Both are totally disrupted in the region affected by an AMI, creating a concentration defect.

Images obtained early in the course of AMI (before six hours) are more frequently abnormal than those obtained after 24 hours. Patients studied both early and late, after separate injections, tend to show a reduction in defect size on follow-up images, since these defects represent an admixture of ischemic and infarcted myocardium. The decrease in size of these defects noted after 24 hours are related to a reduction in the size of the surrounding zone of ischemia. In any case, myocardial imaging with perfusion agents can detect an AMI before the occurrence of diagnostic enzyme changes or clear ECG alterations since the defect will be depicted immediately after coronary occlusion, with the added advantage of informing on the location of the infarction, from which it is possible to deduce which is the affected coronary artery. It is really unfortunate that perfusion imaging does not allow differentiation between recent and old infarction.

Serial myocardial scintigraphy will distinguish acutely ischemic reversibly damaged, from infarcted irreversible damaged myocardial tissue. Nearly one third of the concentration defects on initial images taken before 12 hours of the onset of chest pain, fill in later. Equilibrium RAC usually demonstrates hypokinesis in these transient defects, while regions with persistent defects show akinesis or dyskinesis. In general, those transient defects in regions remote from the zone of infarction are associated with additional significant CAD.

When using serial myocardial scintigraphy in the setting of AMI it is important to remember that the sensitivity of the test is greater in the first 6 - 12 hours after the onset of symptoms, especially in patients with relatively small infarcts, and that the defects in the apex or in the anterolateral wall can be secondary to left ventricular dilatation rather than ischemia or infarction. This fact should be considered if serial imaging is used to study the effects of

nitroglycerine or afterload reduction therapy. Disappearance of an apical or anterolateral defect might be totally or partially related to a reduction in left ventricular size rather than to resolution of ischemia. Conversely, enlargement of an apical defect may be related to left ventricular dilatation and not necessarily to an enlarging infarction. Thus, serial myocardial perfusion scintigraphy to determine the efficacy of therapy to reduce infarct size must be interpreted with caution.

B. Myocardial necrosis scintigraphy with $^{99}Tc^m$-Pyrophosphate (PYP)

The early observations regarding calcium deposition in irreversibly damaged myocardial cells, led to speculation regarding the use of $^{99}Tc^m$-PYP as a means to identify irreversibly damaged myocardial cells, reasoning that PYP might complex with these calcium deposits in them. Subsequent experimental and clinical studies have confirmed the validity of this assumption.

To perform this test, 15 mCi of $^{99}Tc^m$-PYP are injected intravenously. Two hours later,(an intermediate period between the maximal tracer concentration in blood and its maximal bone uptake), the images of the heart are acquired with a gamma camera in the usual A, LAO and LL views, accumulating from 500 000 to 1 million counts on each. No computer is necessary to process the images, although it can be used to improve the image quality taking special care in not introducing artefactual effects. Interpretation of the images depends on the relative PYP concentration in the myocardium, which is graded in five different levels (Table X).

TABLE X. DIAGNOSTIC CRITERIA BASED ON THE MAGNITUDE OF MYOCARDIAL CONCENTRATION OF $^{99}Tc^m$-PYROPHOSPHATE RELATED TO THAT IN STERNUM.

Gradation	Myocardial concentration	Interpretation
Grade 0	No obvious	Negative for AMI
Grade 1	Very low	Negative for AMI
Grade 2 (diffuse)	Low	Unstable angina
Grade 2 (focal)	Low	Positive for AMI
Grade 3	Similar	Positive for AMI
Grade 4	Greater	Positive for AMI

During the initial years of the clinical use of this procedure, the publications showed discordant and even contradictory results. Eventually, it was well recognized that the results depend on a wide spectrum of variable factors (Table XI), which are necessary to consider when interpreting the images, and that it is necessary to follow a rigid protocol.

Sensitivity of the procedure depends on the degree of PYP concentration in the irreversibly damaged myocardial cells. As these compete with bone for the PYP present in blood, the best results are obtained with a radioactive phosphate which is not efficiently incorporated into bone. Many reports of low sensitivity published during the initial years were due to the use of methylene diphosphonate or long-chained polyphosphate, which are among the best bone seeker radiopharmaceuticals. At present, the higher sensitivity is attained by the use of PYP and diphosphonate is preferred for bone scanning. But labelling of PYP should be efficient, since the presence of free pertechnetate in the cardiac blood pool may mimic myocardial PYP and decrease specificity.

TABLE XI. VARIABLES AFFECTING RADIOPHOSPHATE MYOCARDIAL SCINTIGRAPHY	
Type and quality of the tracer	Type and quality of instrument
Size and location of AMI	Time of evolution of the AMI
Age of the patient	Experience of the interpreter

The procedure can be performed with a rectilinear scanner, but at expenses of sensitivity. The gamma camera is quite efficient but its sensitivity decreases with distance. So, AMI occurring at the posterior wall of the LV can be missed. In our experience, the best results are obtained by the use of SPECT, which is able to explore every region of the LV walls and to differentiate radioactivity in ventricular blood pool from than in the myocardium, increasing both sensitivity and specificity.

Virtually all AMI equal or greater than three grams in weight can be visualized by PYP myocardial scintigraphy, especially if they are located at the anterior or anterolateral walls of the LV. Sensitivity of planar imaging is slightly lower in the detection of AMI at the posterior and inferior walls.

It is important to realize that infarction is a dynamic process. Calcium crystals are readily apparent in the mitochondria of irreversibly damaged myocardial cells 12-24 hours after acute coronary occlusion. Formation of these crystals grows steadily to reach a maximum at two days after arterial occlusion, when the damaged cells start to be replaced by granulation tissue with a consequent decrease in the amount of calcium deposits in the infarcted area, which usually are not apparent any more at the seventh day after occlusion. As these crystals are the cause of PYP concentration in myocardium, its behaviour in myocardium follows the very same pattern: PYP myocardial uptake begins approximately 10-12 hours after the onset of symptoms suggestive of AMI, becoming increasingly abnormal during the initial 48-72 hours after infarction. Thereafter, myocardial uptake starts to decrease until it is not longer apparent at the seventh day.

We have observed that PYP scintigraphy is 100% sensitive and specific in patients less than 40 years old. Both characteristics decrease with the patients age. This might indicate that myocardial tissue is more sensitive to anoxia and collateral blood flow is more difficult to establish as the patient gets older, thereby decreasing both PYP uptake and sensitivity. The decrease of specificity with age could be explained by the increase in prevalence of other pathological processes able to concentrate PYP in the cardiac areas, e.g., valvular calcification, ventricular aneurysms, old infarctions, etc.

As in many other imaging procedures, detection of the different shades of regional density and intensity depends on the experience and visual perception of the observer.

Clinical indications. It is an alternative non-invasive method to recognize, localize and size regions of acute myocardial necrosis. Sometimes it could be difficult to confirm the presence of AMI by using the more traditional techniques. In particular, recognition of subendocardial AMI is nearly impossible from ECG data alone. There are also temporal restrictions on enzyme elevations in blood that may limit their usefulness in individual patients, while in others, enzymes may be elevated because of injury to an organ other than heart. Clinical recognition of AMI using traditional techniques may be particularly difficult in patients with previous myocardial infarcts, with intraventricular conduction defects, particularly left bundle branch block, and in those who have received cardioversion for life threatening arrhitmias or which are living the perioperative periods of coronary or heart surgery.

Myocardial PYP scintigraphy in the prognosis of AMI. We regard serial myocardial imaging with PYP as being important in regard to AMI detection, but essential for the gathering of scintigraphic signs to build a clear short and long range prognosis (**Table XII**), which may indicate the need of special therapeutic measures.

TABLE XII. SIGNS OF PROGNOSTIC SIGNIFICANCE IN $^{99}Tc^{m}$-PYP MYOCARDIAL SCINTIGRAPHY.
Delayed concentration of PYP in myocardium
Persistent concentration of PYP in myocardium after the seventh day
The "doughnut" pattern of distribution of PYP in myocardium
The sign of the "inverted 3"
A negative result in a young patient with well recognized AMI

Occasional patients first develop an abnormal PYP scintigram four to five days after AMI. These patients are among those with the most severe intrinsic CAD. Presumably, the delayed development of collateral blood flow to the damaged region is responsible for the delayed appearance of the abnormal scan in such patients.

In 10 to 40% of patients with AMI the PYP myocardial image remains abnormal for at least three months after the acute episode. This finding appears to be correlated with a histological pattern featured by myocytolysis with regions of scar intermixed with areas of acute and chronic cellular injury, with recurrent hospital admissions due to chest pain and/or congestive heart failure, ongoing unstable angina, and a troubled clinical course in the few weeks to months following infarction.

Development of the "doughnut" sign seems to indicate an anterior or anterolateral AMI twice as large as those that do not show this sign, or a large AMI with extensive central necrosis or with congestive heart failure and ventricular dilatation.

The "inverted 3" sign (Fig. 24.15) is the reflection of a LV AMI extending towards the inferior and anterior walls of the RV. It shows the PYP concentration at the anterolateral, inferior and septal walls of the LF, as well as at the inferior wall of the RV. Appearance of this sign affects both prognosis and treatment, since involvement of the RV will cast shadows on prognosis and indicates special therapy to strengthen the contractile efficiency of the RV.

But the most sombre of all scintigraphic signs is a negative result in a relatively young patient during the third and fourth day after a well recognized AMI. This result is associated with a fatal prognosis in a few hours or days. It could be explained by severe infarction with extended necrosis without residual blood flow.

Other applications of myocardial PYP scintigraphy

It should be remembered that radioactive PYP is a marker for necrosis, so it can be used also in the study and follow-up of congestive myocardiopathies and other pathological processes capable of producing myocardial necrosis or sclerosis.

C. Myocardial immunoscintigraphy

Imaging with Indium-111 labelled monoclonal antimyosin antibody (AM MAb) has been introduced recently to detect, localize, and quantitate myocardial necrosis in experimental and clinical AMI. The detection and localization of irreversibly damaged myocardium with the MAb (Fab fragments) correlates well with histological and histochemical evidences of transmural and subendocardial necrosis.

The physiological mechanism for antimyosin imaging is well established. When myocyte membrane integrity is lost and necrosis ensues, as with AMI, the Fab fragments of antimurine AM MAb may diffuse across the membrane to be bound by cardiac myosin, thus permitting the scintigraphic visualization of necrotic myocardium.

Early reports revealed a high degree of agreement regarding the presence, extent, and location of experimental AMIs, with histological evidences of necrosis and ^{111}In-antimyosin uptake, and postmortem case reports have confirmed a similar close relationship in humans.

However, the AM MAb clears very slowly from blood and approximately 20% of the images obtained 24 hrs after the injection of 2.05 mCi of ^{111}In labelling 0.5 mg of Fab fragment of antimurine AM MAb might not be helpful diagnostically due to the high level of radioactivity circulating with blood. In this case, it is necessary to perform a SPECT to differentiate the concentration at the ventricular wall from the presence of the tracer in the ventricular blood pool, or to postpone diagnosis for another 24 hours, when also 5% of the images could not be of diagnostic value. Notwithstanding, this procedure is able to render earlier diagnostic data with higher sensitivity and specificity than by using radioactive PYP.

An additional advantage in the use of antimyosin is that it is sensitive to necrosis for a longer period than PYP, and could render positive results up to 15 days after the onset of the acute episode.

The high sensitivity of this technique has been demonstrated even in the setting of non-Q wave AMI (sensitivity = 84%), but specificity is a different matter since antimyosin scintigraphy has been reported to be positive in cases with abnormal conditions, other than AMI, accompanied with some degree of necrosis, such as acute myocarditis, amyloidosis, cardioversion, cardiac trauma, and doxorubicin cardiotoxicity.

By using a double-tracer technique with ^{111}In-antimyosin and ^{201}Tl it is possible to define three different clinical situations when the test is performed during the recovering period after AMI:

(a) Scintigraphic overlap, when antimyosin uptake is evident at regions with normal ^{201}Tl uptake. This pattern is characteristic of coronary recanalization with viable myocardium, which in later periods could be demonstrated by an improvement in regional wall motion.

(b) No scintigraphic overlap, when antimyosin uptake is evident in regions with defective ^{201}Tl uptake. This pattern is associated with temporal persistence of altered wall motion and it is considered as a feature of residual necrosis.

(c) Scintigraphic mismatch, when a region shows inability to concentrate antimyosin and a very poor ^{201}Tl uptake. This pattern is a feature of "myocardial jeopardy".

In any case, it is unfortunate that application of this potentially valuable technique has to be limited in the developing countries because of the high cost of both the commercial AM MAb and its label. It is hoped that in the near future this MAb could be labelled with ^{99}Tc^{m}, thereby decreasing part of its cost, and that the increased demand might reduce the cost of the AM MAb.

4. MYOCARDIAL INFLAMMATION SCINTIGRAPHY

Cardiac imaging with ^{67}Ga, performed 48 hours after the tracer injection, has been used with variable results in the diagnosis of active myocarditis. Although not very sensitive, this method seems to be of some value in following the progression or regression of the cardiac inflammatory disease. More research is needed in this regard and in the use of other inflammation markers, such as radiolabelled white blood cells.

5. MYOCARDIAL ADRENERGIC INNERVATION SCINTIGRAPHY WITH ^{123}I-MIBG

Metaiodobenzylguanidine (MIBG) is an analog to guanethidine, an adrenergic neuron-blocking agent. It shares with norepinephrine the same uptake, storage, and release mechanisms in the adrenergic nerve terminals, but it is not metabolized by catechol-o-methyl transferase and monoamine oxidase, so it can be viewed as a "non-metabolizable" norepinephrine. Labelled with ^{123}I or ^{131}I, it can be used for imaging neural crest's tumours, such as pheochromocytoma, neuroblastoma and paraganglioma. Promising results has been also reported on the use of the ^{131}I labelled form in the selective treatment of these chromaffin tissue tumours.

Aside from such clinical utility, this agent has the potential to be a new diagnostic tool in the in vivo assessment of various heart diseases from the standpoint of adrenergic nerve activity, such as autonomic denervation, cardiomegaly, congestive heart failure, AMI, congestive cardiomyopathy, and hyperthyroidism, all of which may alter myocardial catecholamine kinetics. However, the resulting images seem to express the sum of activity in various cardiac tissue components and more detailed compartmental analysis of this tracer are needed before predicting the real value of this procedure in clinical cardiology.

6. MISCELLANEOUS

This chapter deals with the procedures of nuclear cardiology. However, there are several procedures designed for the study of other organs and systems which are intimately associated with the heart and which when diseased may cause severe cardiac effects (**Table XIII**). The interested reader is referred to the specific chapters for further reading.

TABLE XIII. GENERAL NUCLEAR MEDICINE PROCEDURES OF VALUE IN CARDIOLOGY	
Studies	Indications
Thrombi detection	Cardiac intracavitary thrombi
Splenic scintigraphy	Infectious endocarditis
Renal function studies	Hypertension
Chromaffin tissue scintigraphy	Acute transient hypertension
Static and dynamic radiovenography	Suspected pulmonary embolism
Lung perfusion scintigraphy	Congenital cardiac diseases
Lung perfusion/ventilation studies	Suspected pulmonary embolism
Hepatic scintigraphy	Determination of visceral situs

II. IN VITRO NUCLEAR CARDIOLOGY

Nuclear cardiology also includes a series of **in vitro** procedures for the sensitive and specific detection and measurement, in blood and/or in other tissues, of substances which may play significant roles in the etiology of some cardiovascular disorders, which can be produced by certain lesions of the heart or other organs, or which are the product of iatrogenesis **(Table XIV)**.

TABLE XIV. INDICATIONS FOR RADIOIMMUNOASSAY IN CARDIOLOGY	
Analyte	Indications
Digoxin	Evaluation of degree of digitalization Evaluation of toxic effects of digoxin Evaluation of inadequate therapeutic response to digoxin
Myoglobin Creatinephosphotransferase Creatinephosphotransferase MB isoenzyme	Acute myocardial infarction markers
Angiotensin II Aldosterone Catecholamines	Systemic hypertension
Betathromboglobulin Platelet factor IV	Blood coagulation alterations
Thyroxine In vitro T_3 uptake Free thyroxine index T.S.H.	Cardiac frequency alterations.

1. RADIOIMMUNOASSAY OF DIGOXIN IN SERUM

Digoxin is a cardiotonic glycoside of great value in cardiology, but it is also an important source of toxic iatrogenic effects. After its administration, digoxin concentration in serum increases with a rate depending on the dose and the mode of administration.
A significant fraction is bound to serum albumin (23%), while the free fraction is bound by tissue receptors in myocardium and other tissues. After the initial phase, digoxin levels in blood remain more or less stable with a very slow gradual decrease in concentration. This occurs eight hours after oral administration or six hours after intravenous injection. During this period the serum/myocardium ratio is relatively constant, so its concentration in serum is a useful index to estimate its myocardial concentration. However, to get satisfactory information regarding digoxin concentration in serum it is necessary to wait at least 4 hr after its last administration before the blood sample is taken.

Unfortunately, there is a significant overlap between therapeutically effective and toxic serum levels of digoxin. The therapeutic range is very narrow - from 1.0 to 1.5 ng/ml - but to interpret that all lower values are sub-sub-therapeutic and all values higher than this range

are toxic may lead to clinical mistakes. On the other hand, to say that 0.3 ng/ml is a sub-sub-sub-sub-therapeutic concentration, and that a 5 ng/ml concentration is responsible for toxic signs or symptoms is not of much clinical sense, since in these extreme cases the clinical problem is obvious without the need of RIA results. Each patient should be considered individually and digoxin levels should be taken as relative to the individual patient. The physician should not rely on one single assay result but base his interpretation on a trend that is evident in serial determinations.

Digoxin RIA is especially useful in evaluating digitalization, mainly in patients with fluctuating renal function or when digoxin administration is irregular and when interaction with other drugs, as quinidine, is suspected. Another application is in evaluation the toxic effects of digoxin in patients with neurological or gastro-enterological symptoms, with cardiac arrhitmias or accidental, suicidal or criminal digoxin ingestion. Finally, it is essential in evaluating an inadequate therapeutic response when the patient is suspected of not taking the drug, when intestinal absorption is suspected to be abnormal because gastrointestinal disease, binding of digoxin to other drugs, use of digoxin preparations of low bioavailability, or when the patient is resistant to the drug, as in cases with thyrotoxicosis or mitral stenosis.

In any case, digoxin concentration measured by RIA is clinically useful to achieve an adequate therapeutic regime and to prevent digitalis intoxication. The symptoms and ECG signs of digitalis intoxication are evident when the drug is already toxic, but digitalis levels in blood could be used as an indicator of impending toxicity.

2. RADIOIMMUNOASSAY OF CERTAIN ACUTE MYOCARDIAL INFARCTION MARKERS

Myocardial ischemia leads to progressive cellular changes featured by the transit of certain cytoplasmic components to the extracellular space. Mitochondrial and lysosomic alterations begin with ischemia, which increases cellular permeability producing the outflow of nucleotides and enzymes towards the lymphatic pathways and later on to blood. These nucleotides and enzymes could be used as markers for AMI when they are detected in blood.

Myoglobin is a haemoprotein produced by striated muscles including myocardium. It is expelled from the myocyte whenever it is damaged and can be detected in blood by using RIA, even in concentrations lower than 0.5 ng/ml. Its normal concentration in serum ranges from 8 to 80 ng/ml, with significant differences related to sex, due to the different muscular mass:

Male: 50.3 ± 19.8 ng/ml
Female: 35.7 ± 10.4 ng/ml

Myoglobin concentration in serum starts rising three hours after the onset of acute chest pain in AMI, reaching a maximal level at eight or ten hours, progressively decreasing to normal at 48 hours. It is the first marker to be increased in blood after AMI.

However, as myoglobin is a normal component of any striated muscle it is not specific of myocardium and its concentration could be found elevated in several clinical situations unrelated to AMI, such as hypothyroidism, Duchenne's disease, myasthenia gravis, progressive muscular dystrophy, dermatomyositis, polymyositis, muscular trauma, even after an intramuscular injection or recent muscular exercise. Furthermore, hypermyoglobinemia could be found in a great variety of other conditions, as viral diseases (influenza and Legionnaire's disease), stress, infections, arterial thrombosis, regional enteritis and by the effects of several drugs (amphetamine, heroin, phenylclydine, succinylcholine, clofibrate, licorice, alcohol).

Creatinephosphotransferase (CK) is an enzyme found in myocardium, skeletal muscle, brain, digestive tract and bladder. Its concentration in skeletal muscle is five times of that in myocardium, and three-fold in myocardium than in brain and in digestive tract.

The peak of maximal concentration of CK occurs 23 hours after the onset of acute chest pain caused by AMI. But its concentration could be also found increased in muscular trauma, pericarditis, myocarditis, pulmonary embolism, cerebrovascular diseases, myxoedema, diabetic coma, hypothermia, and after exercise, surgery, cardioversion, radiotherapy, cardiac catheterization, and intramuscular injections. The peak of maximal concentration in serum occurs 23 hours $\pm$ 1.8 after the onset of acute chest pain caused by AMI.

Creatinephosphotransferase MB isoenzyme (CK-MB) is considered as cardiospecific, since only 4.2% is produced at the diaphragm, and only 0.2 - 1.5% is produced in other striated muscles. Its concentration is raised four to six hours after the onset of acute chest pain in AMI, reaches the maximum at 17 hours, and is normal again at 36 hours.

Since CK-MB represents 20% of total CK, the ratio CH-MB/CK is of special value in the diagnosis of AMI: it supports the diagnosis of AMI if it is greater than 5% and makes it certain if it is greater than 8%, but it excludes this diagnosis if it is lower than 4%.

3. RADIOIMMUNOASSAY IN THE STUDY OF HYPERTENSION

When investigating hypertension it is necessary to gather information regarding the renin-angiotensin system. Renin is a proteolytic enzyme produced, stored and secreted by the juxtaglomerular cells in the kidney. Once secreted into the blood, renin acts on angiotensinogen or renin substrate to produce angiotensin I, which act on the central nervous system and on adrenal glands stimulating catecholamine secretion, activating an increase of arterial tension through peripheral vasoconstriction and cortical renal blood flow redistribution. Angiotensin I is transformed into Angiotensin II by the action of convertase. This new component produces hypertension through peripheral, nervous and cellular actions. Some of the components of this system can be measured by using RIA techniques.

<u>Plasma renin activity.</u> It is the usual procedure to study the renin-angiotensin system. It is an indirect method based on the measurement of the amount of angiotensin I generated by

the action of renin on angiotensinogen during a known period of time. It includes two different measurements of angiotensin I, at 4°C and after incubation at 37°C. The difference between both measurements represents the activity of the plasma renin measured in ng/ml/h.

RIA of angiotensin II. Its normal plasma concentration varies between 10 and 100 pg/ml. It is very seldom performed since it relates to the activity of plasma renin and this is preferred for its simplicity.

RIA of aldosterone. Aldosterone is the most important mineralocorticoid hormone produced by the glomerular zone of the adrenal glands. Its secretion depends on the actions of the renin-angiotensin system, ACTH and plasma concentrations of Na and K. The lowest measurable concentration in plasma is 1 ng/dl. Its normal values in healthy subjects, in orthostatic position and in a sodium-free diet, range from 5 to 16 ng/dl. The lowest measurable concentration in urine is 1 μg, and the normal values in subjects in a sodium-free diet are 11.1 $\pm$ 4.6 μg/24 hours. Measurements in urine need to be performed after extracting the glucuronide with dichloromethane, followed by acidic hydrolysis of the extract to liberate aldosterone.

Radioassay of catecholamines. The most usual methods are based on the transference of a ^{14}C labelled methyl group to the amine fraction, either by the action of fenylethanoamine-N-methyltransferase or of catechol-o-methyltransferase. Similar methods are used to measure dopamine, adrenaline and noradrenaline. The lowest limits for sensitivity are 2 pg for dopamine, and 1 pg for adrenaline and noradrenaline.

4. RADIOIMMUNOASSAY OF BLOOD COAGULATION FACTORS

It is possible to measure two important factors involved in the process of blood coagulation:

Betathromboglobulin (btg). Its real function is unknown but it contributes to the stabilization of the different substances in the alpha-granules contained in platelets. It is liberated in great amounts during platelets reactions, which need to be avoided during sampling and RIA process. Its normal serum concentration is 10 - 65 ng/ml. It is increased in diabetic patients with vascular lesions, in AMI, and in pulmonary thromboembolism. Concentrations higher than 82 ng/ml are considered of high risk.

Platelet factor IV. It is a protein produced at the alpha-granules in the platelets and is liberated during platelet aggregation. The lowest limit of sensitivity is 2.5 ng/ml, and its normal concentration varies from 0 to 10.4 ng/ml. It is increased in CAD, diabetes, altered blood coagulation after the placement of artificial heart valves, coronary by-pass surgery, and AMI.

5. RADIOIMMUNOASSAY OF THYROID RELATED HORMONES

Cardiologists quite frequently face the problem of differentiating between tachycardia or bradycardia of cardiac origin and those produced by thyroid disfunction. This apparently simple issue often is made complex by the effects of thyroxine binding globulin (TBG) variations in serum, which affect both thyroid function and in vitro thyroid function testing. The interested reader is referred to the specific chapters in this book for a more detailed information.

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CHAPTER 24

Appendix I

EQUIPMENT NECESSARY IN A NUCLEAR CARDIOLOGY DEPARTMENT
Treadmill or ergometric bicycle
Sphygmomanometer and stethoscope
1 - 3 channel continuous ECG monitor and recorder
D.C. defibrillator
Cut down tray (sterile), with syringes and needles, intravenous sets and stand, adhesive tape, etc.
Airways (oral and tracheal)
Oxygenator and oxygen supply, with intermittent positive pressure capability
Bag valve mask hand respirator
Laryngoscope
Cardiopulmonary resuscitation facilities:
Defibrillator
Commonly used cardiac drugs
Establishment of intravenous route
Experienced staff in delivering cardiopulmonary resuscitation measures, including closed cardiac massage and mouth-to-mouth resuscitation

Appendix II

COMMONLY USED CARDIAC DRUGS FOR CARDIOPULMONARY RESUSCITATION

Antiarrhythmic agents:

Lidocaine (Xylocaine)
Procainamide (Pronestyl)
Propanolol (Inderal)
Quinidine
Diphenylhydantoine (Dilantin)
Disopyramide (Norpace)

Cardiac glycosides:

Digoxin
Ouabain
Deslanoxide

Other drugs and solutions:

Atropine
Catecholamines:
- Isoproterenol (Isuprel)
- Epinephrine (Adrenaline)
- Norepinephrine (Noradrenaline)

Nitroglycerine tablets
Amyl nitrate pearls
Morphine
Demerol
Methyl prednisolone sodium succinate (Solumedrol)
Calcium gluconate
Aminophylline
Furosemide
Diazepam
Dextrose solution
Sodium bicarbonate solution

Appendix III

COMMON EMERGENCIES AND MEASURES

Angina pectoris / Acute myocardial infarction:

Nitroglycerine tablets, sublingual
Oxygen
Reassurance
Morphine, 5 - 8 mg, intravenous, especially when pulmonary
oedema complicates acute myocardial infarction

If angina was produced by the administration of Dipyridamol:

Aminophylline, 200 mg, intravenously

Ventricular fibrillation:

Defibrillator shock, 300 J. Repeat if necessary.
Antiarrhythmic drugs:
Lidocaine, 1 - 2 mg/Kg, bolus in 30 sec, intravenously. Can be repeated every two min. Continuous infusion, 1 - 3 mg/min.
Procaineamide, 50 mg/m, intravenously.
Bertylium Tosylate, 5 mg/Kg, intravenously, followed by electrical shock. Dose may be increased to 10 mg/Kg, even to a maximum 30 mg/Kg. It can be repeated at 15-30 min intervals.
Quinidine gluconate, 0.8 mg in 10 ml ampoules. Dilute with 50-100 ml of 5% glucose solution. Given slowly, intravenously.
Hyperkaliaemia correction:
Calcium gluconate, 10% solution, 10-30 ml, intravenously over 1-5 min., under monitoring
Calcium chloride, 10% solution, 2.5-5.0 ml, given similarly as above.
Glucose-Insulin infusion.
Correct acidosis:
Sodium bicarbonate, 1 mEq/Kg initially, 0.5 mEq/Kg is repeated every 15 min.
Correct hypoxemia:
Oxygen, assists ventilation.

Heart block / asystolia (cardiac arrest):

Vigorous blow to precordium
Atropine, 0.5 mg, intravenously. Can be repeated at five min intervals.
Epinephrine, 5-10 ml of 1:10,000, intravenously. Can be repeated at five min intervals. Intracardiac route if intravenous route is not patent.

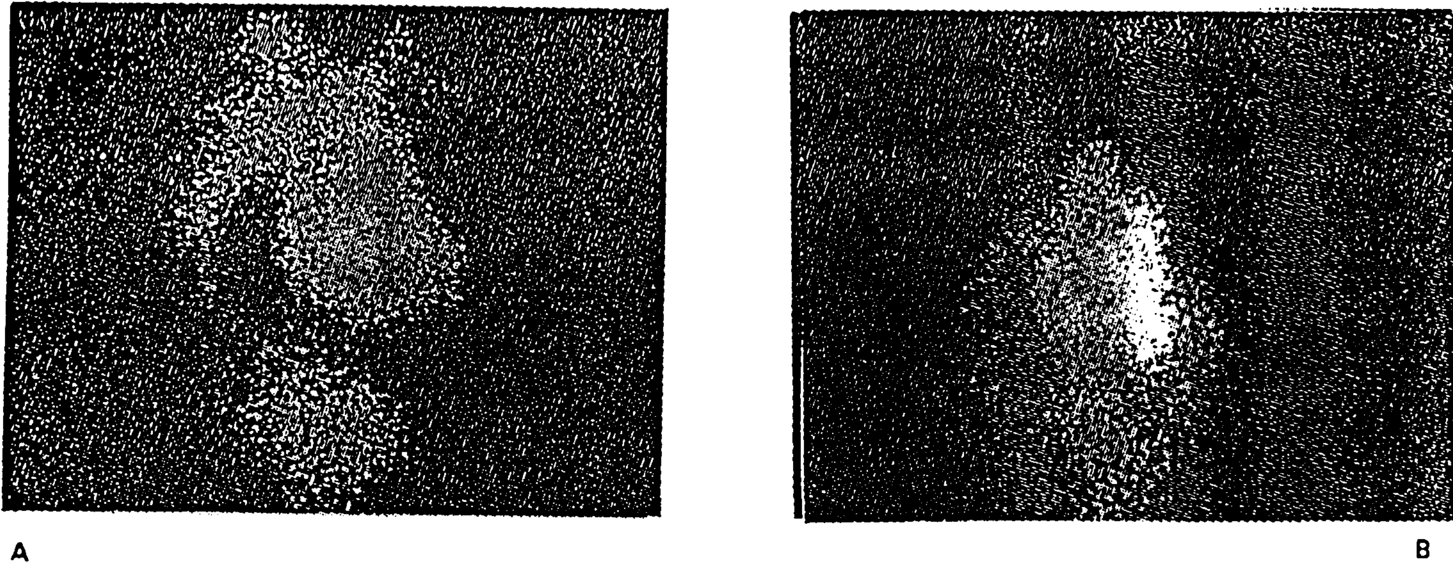


Fig. 24.1 Static radioangiocardiography in a patient with pericardial effusion, before (A) and after (B) pericardial puncture.

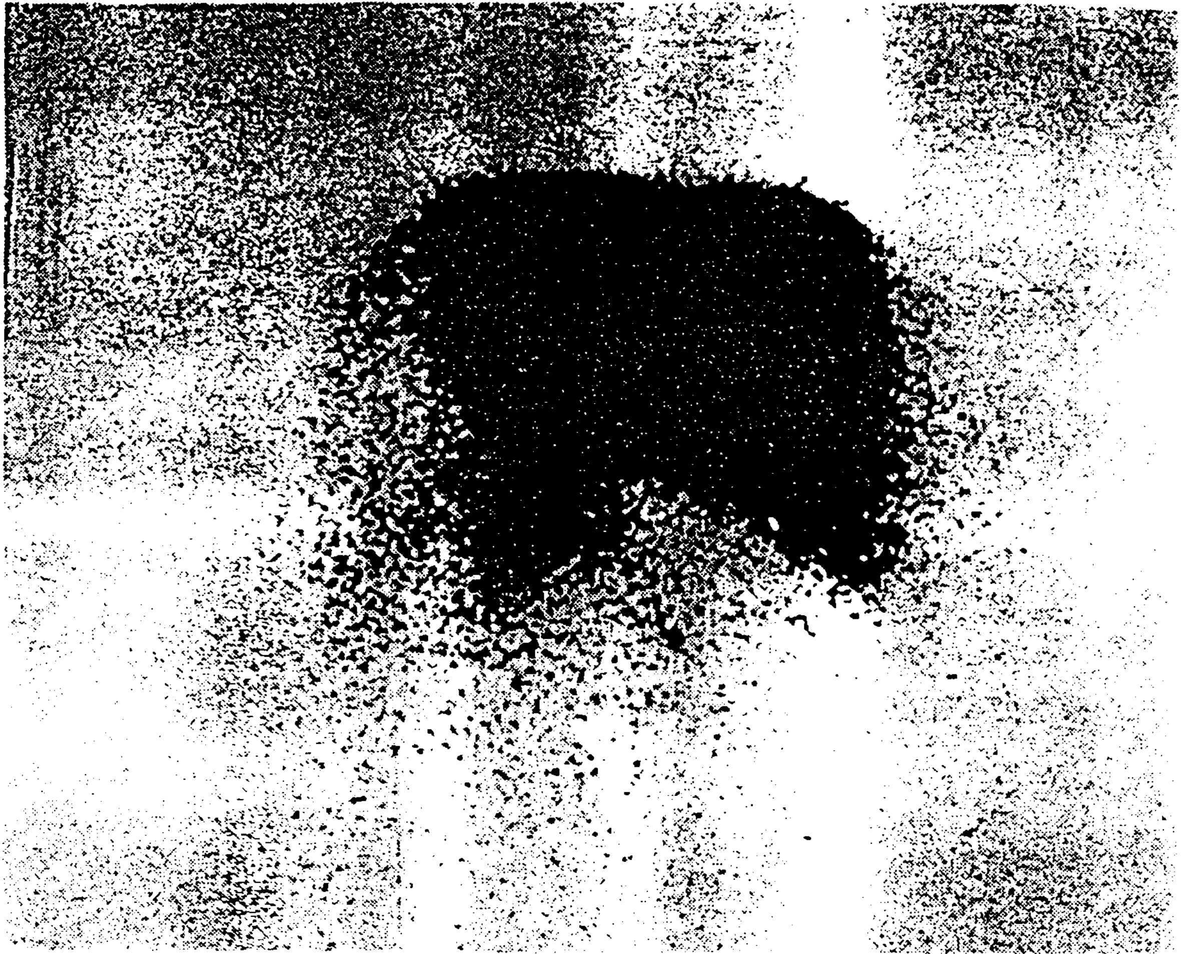


Fig. 24.2 A. First-pass radioangiocardiography in a patient with tricuspid valve insufficiency and severe pulmonary hypertension, showing right atrial dilatation and regurgitation of the tracer from the right atrium into the inferior cava vein.

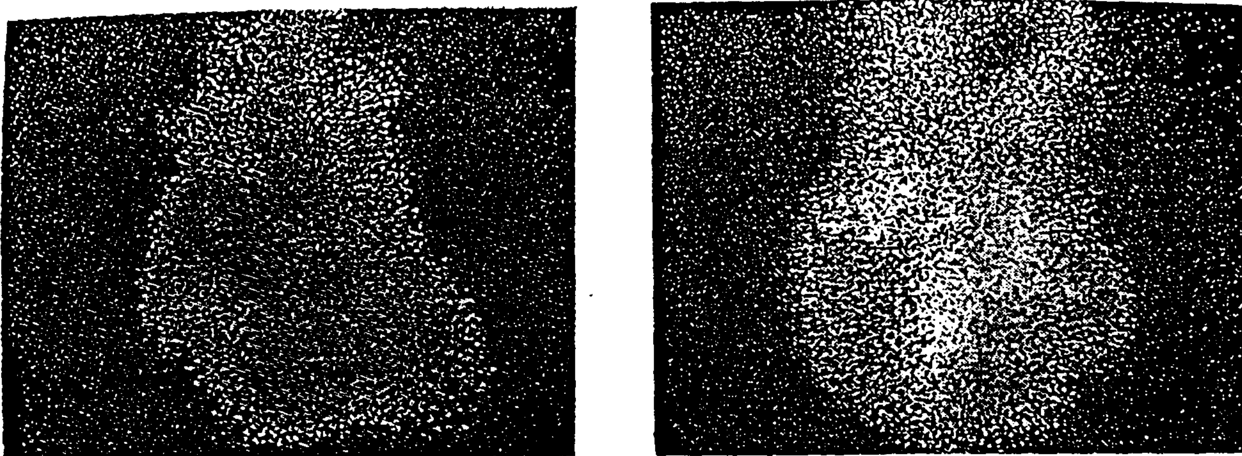


Fig. 24.2 B. Static radioangiography in a patient with a myxoma at the right atrium, clearly seen as a filling defect in the anterior view (at left).

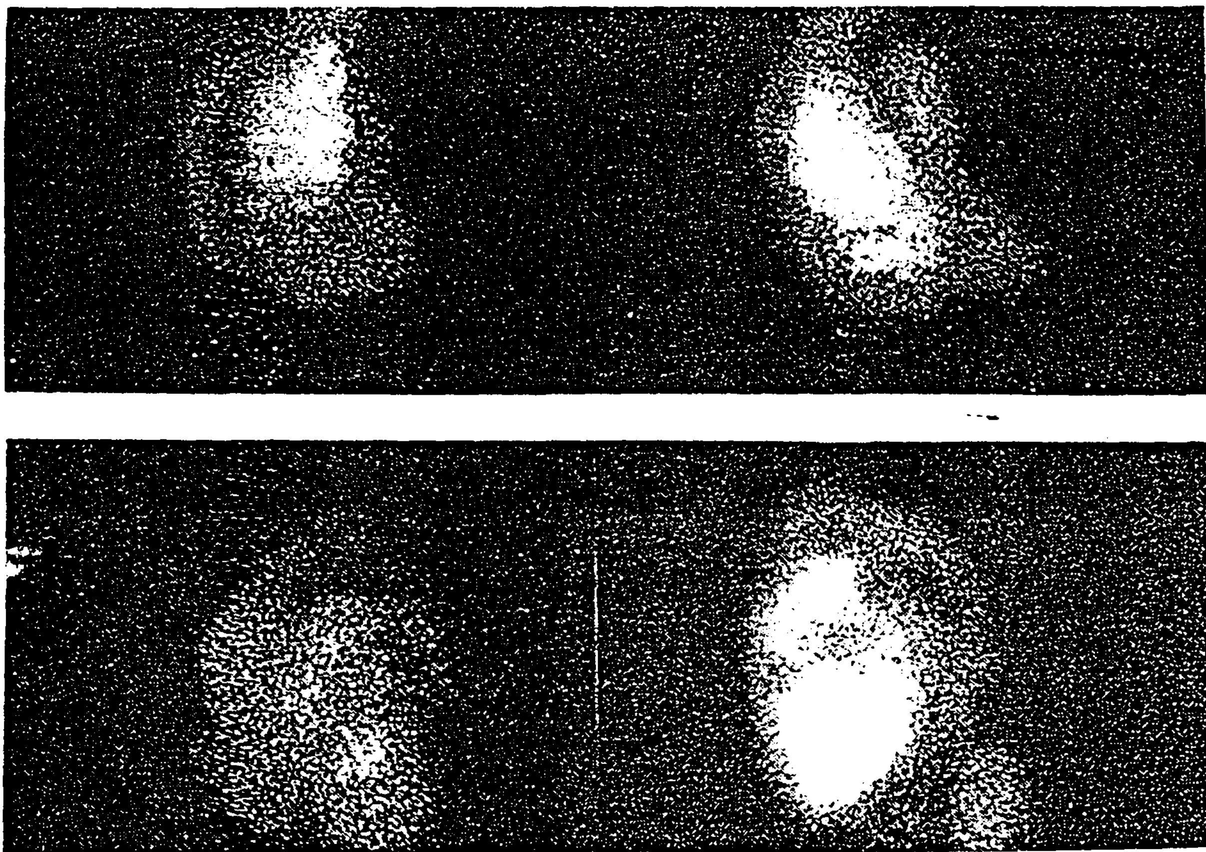


Fig. 24.2 C. Static radioangiocardiography in a patient with aneurysm at the ascending aorta. Upper left: right anterior oblique view; upper right: anterior view; lower left: left anterior oblique view; lower right: left lateral view.

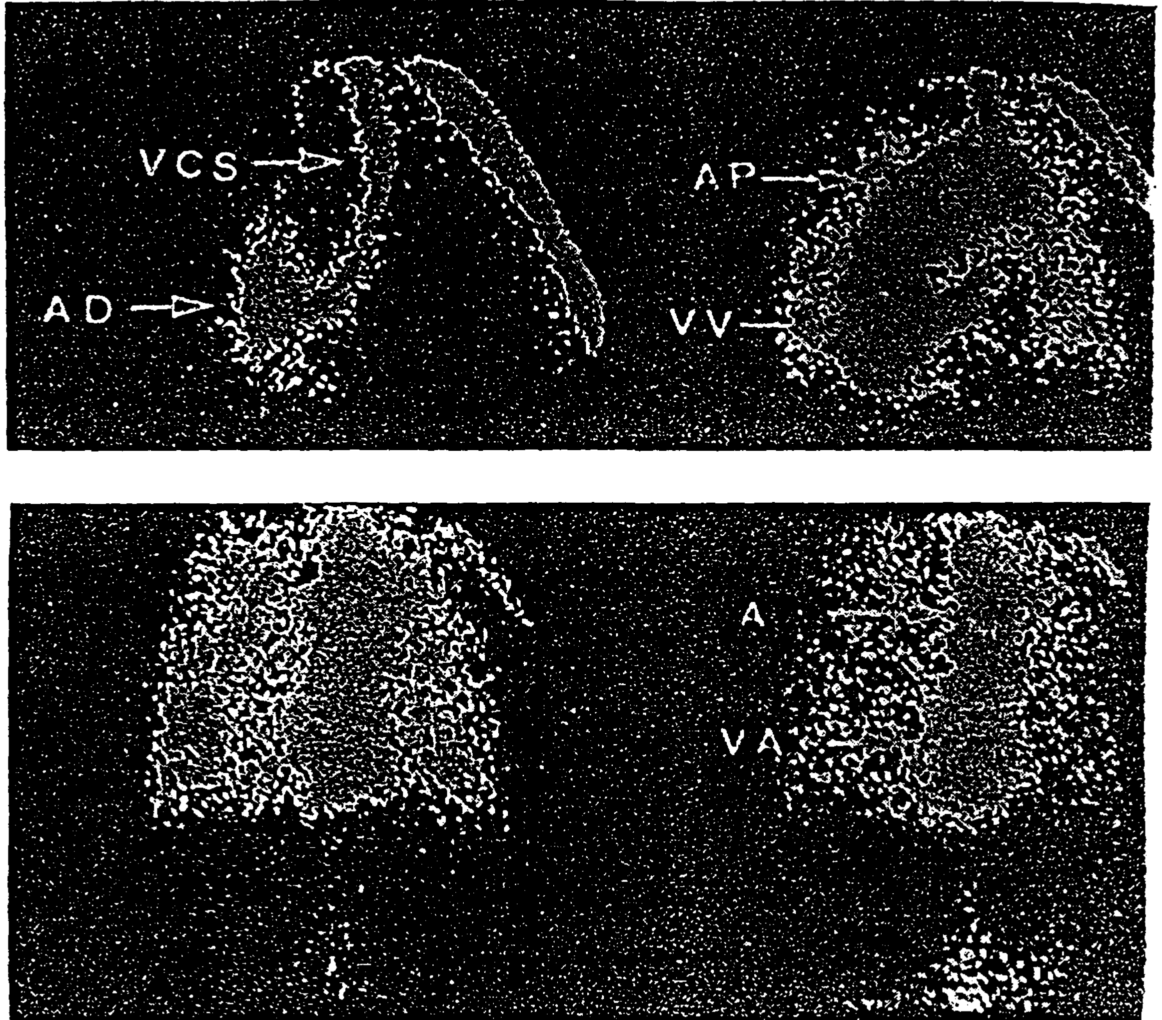


Fig. 24.3 First-pass radioangiocardiography. Anterior views.

A. In a patient with corrected transposition in situs sollitus with apex to the right. Superior cava vein (VCS) is centrally located and the right atrium is at the right. The morphologically left ventricle (VV) is ovoid and sited at the right side. Pulmonary artery (AP) runs from right to left. The morphologically right ventricle (VA) and aorta (A) are located at left.

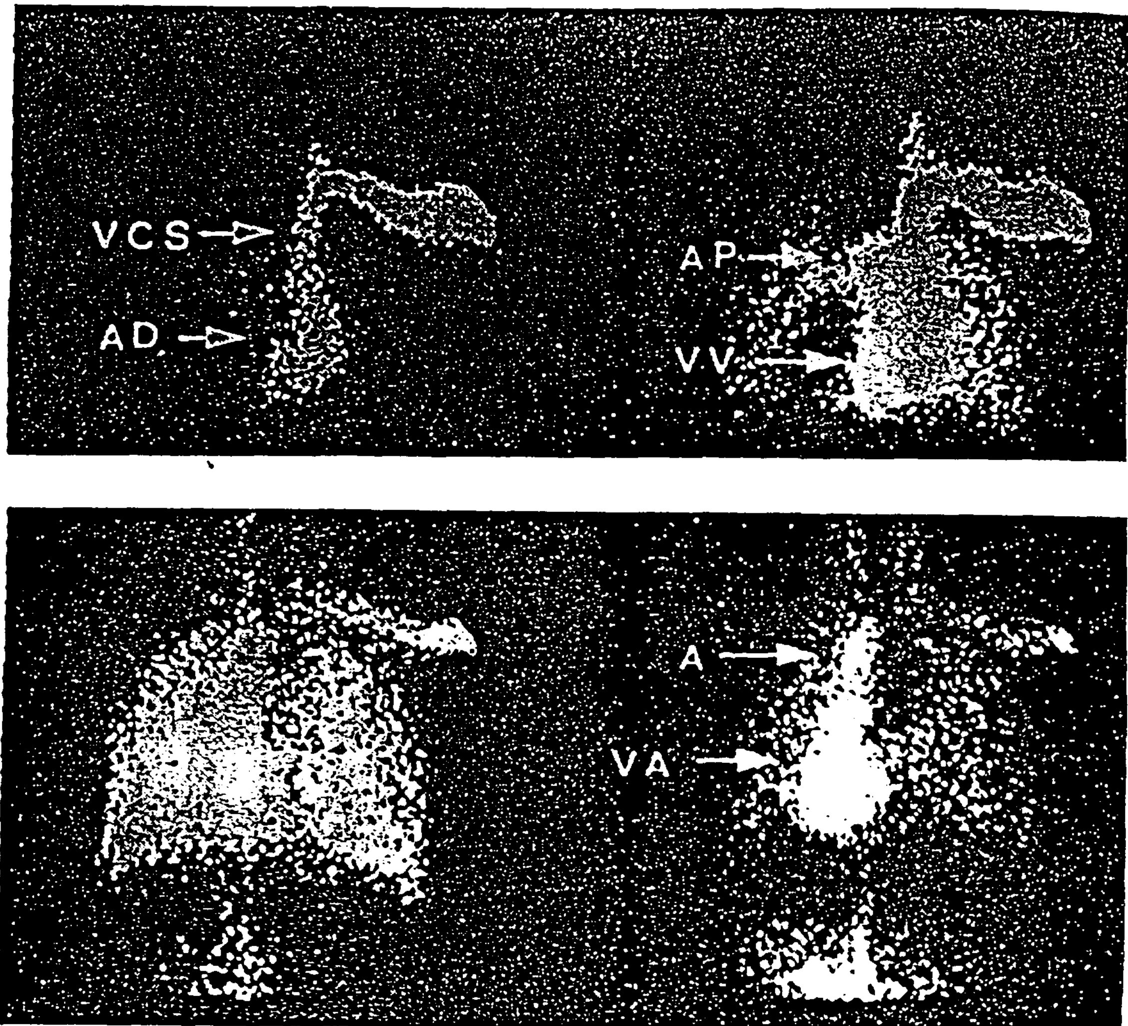


Fig. 24.3 B. In a patient with corrected transposition with situs inversus and apex to the left. Superior cava vein (VCS) and right atrium (AD) are sited at left. Venous ventricle (VV) is ovoid and sited at left with pulmonary artery (AP). The arterial ventricle (VA) has morphological characteristics of right ventricles, is located at right with aorta (A).

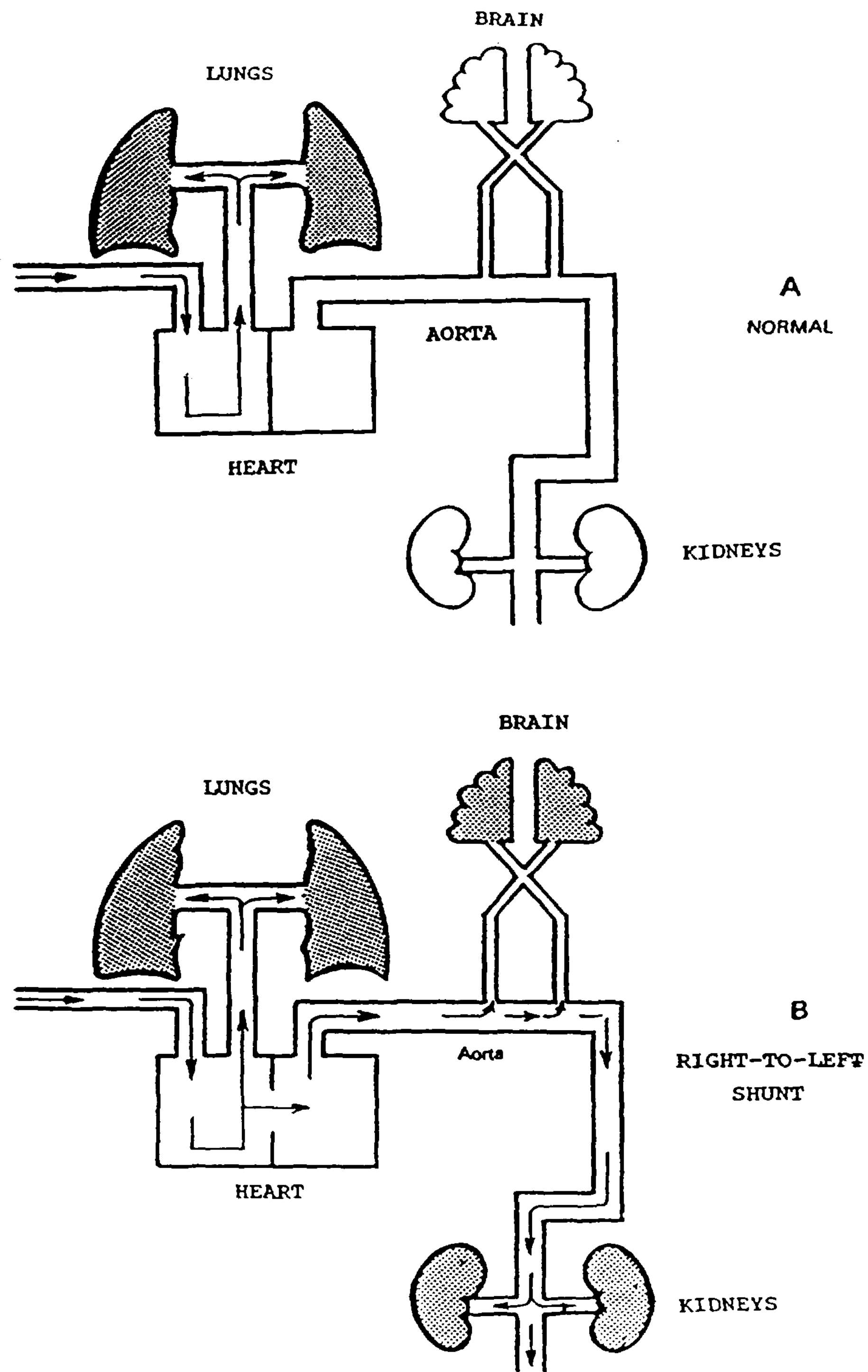


Fig. 24.4 First-pass angiocardiography with $^{99}Tc^{m}$-macroaggregated albumin in a patient with a right-to-left intracardiac shunt.

A. Diagrams of the transit of the tracer in normal subjects (A) and in patients with right-to-left shunts (B).

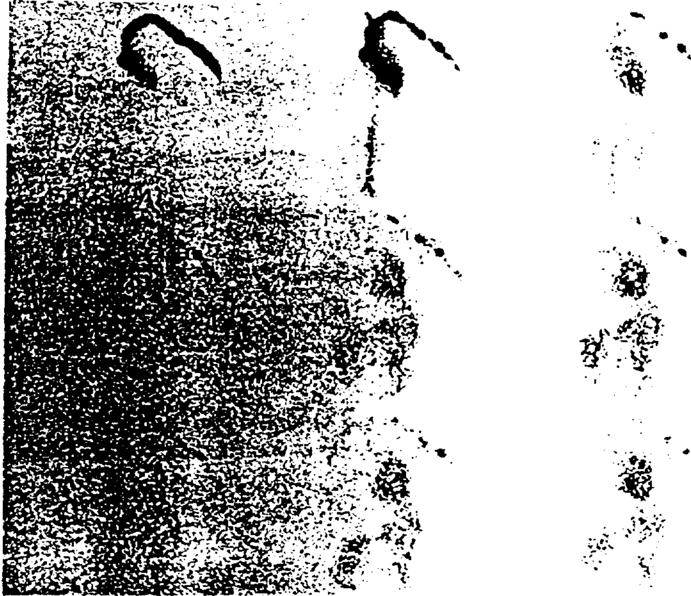


Fig. 24.4 First-pass angiocardiography with $^{99}Tc^{m}$-macroaggregated albumin in a patient with a right-to-left intracardiac shunt.

B. In this particular case, the tracer flows directly from the right atrium to the left cavities and aorta. Right ventricle, pulmonary artery and lungs do not show evidences of the tracer, but the radioactive particles are trapped by the coronary capillary bed.

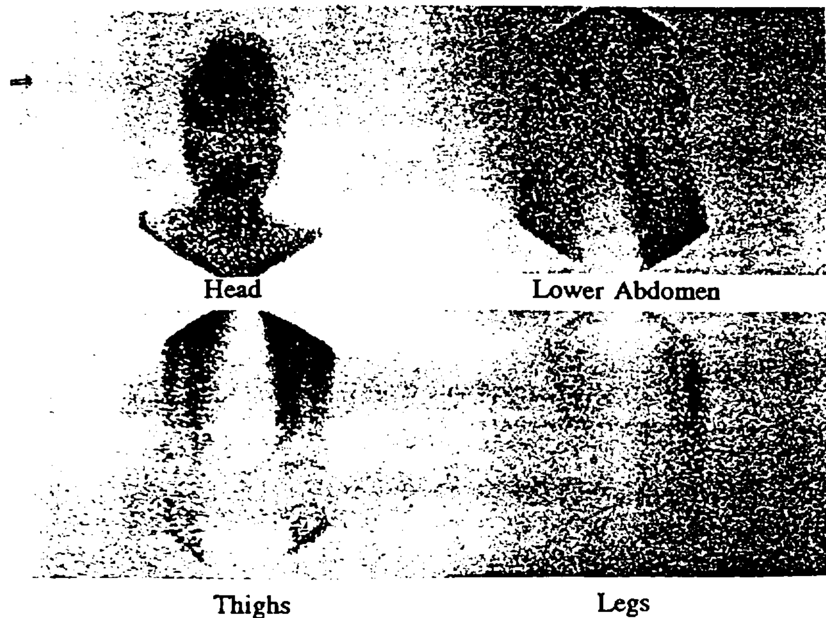


Fig. 24.4 First-pass angiocardiography with $^{99}Tc^{m}$-macroaggregated albumin in a patient with a right-to-left intracardiac shunt.

C. $^{99}Tc^{m}$-MAA at the peripheral capillary beds of the same patient. Note the perfusion defect at the right hemisphere of the brain and decreased perfusion at the contralateral lower limb both sequels of a previous arterial occlusion in the brain.

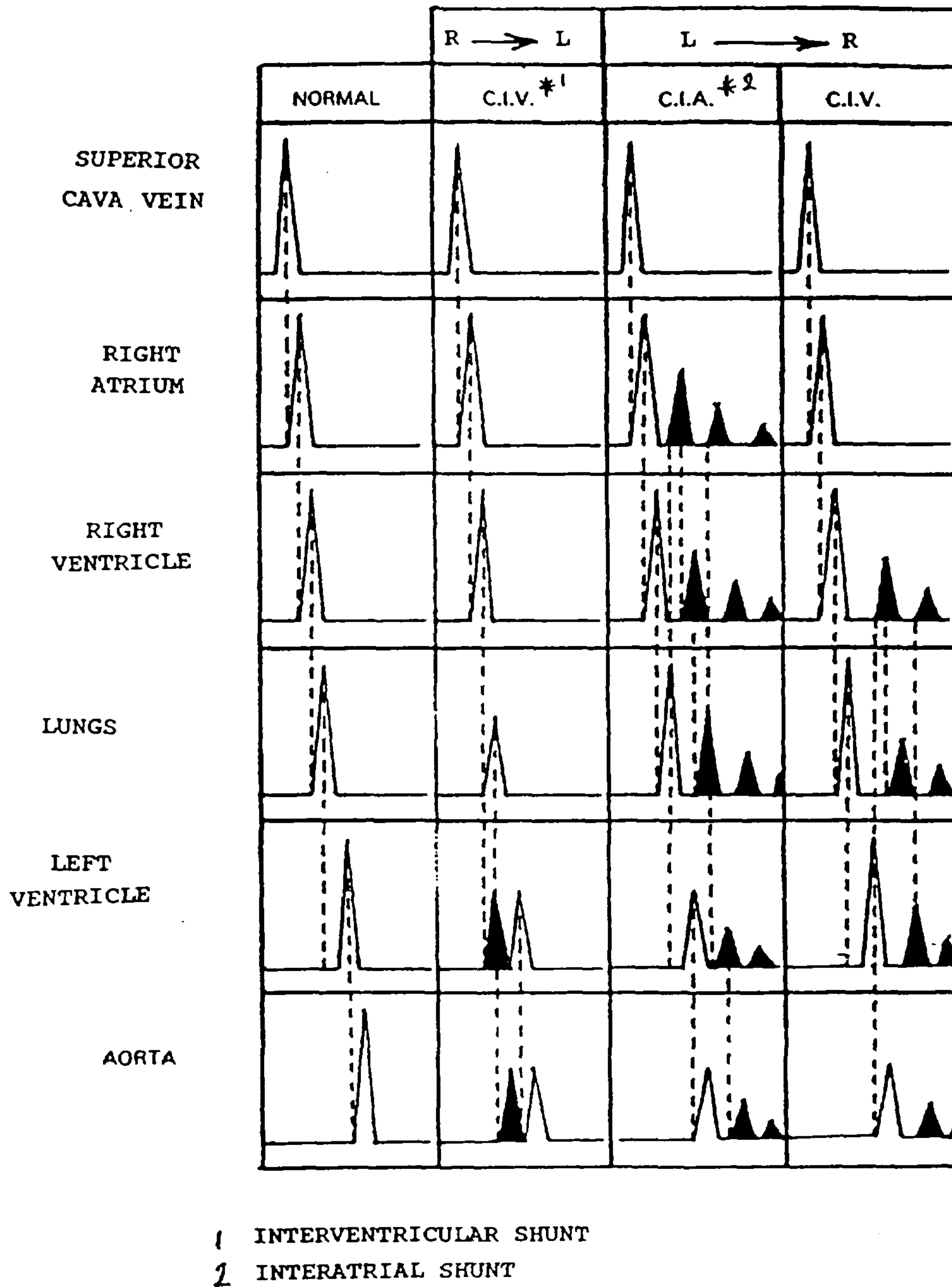


Fig. 24.5 Sequences for the appearance of radioactive peaks in superior cava vein, right atrium and ventricle, lungs, left ventricle and aorta. IVS = interventricular shunt; IAS = interatrial shunt. R - L = right-to-left shunt; L - R = left-to-right shunt.

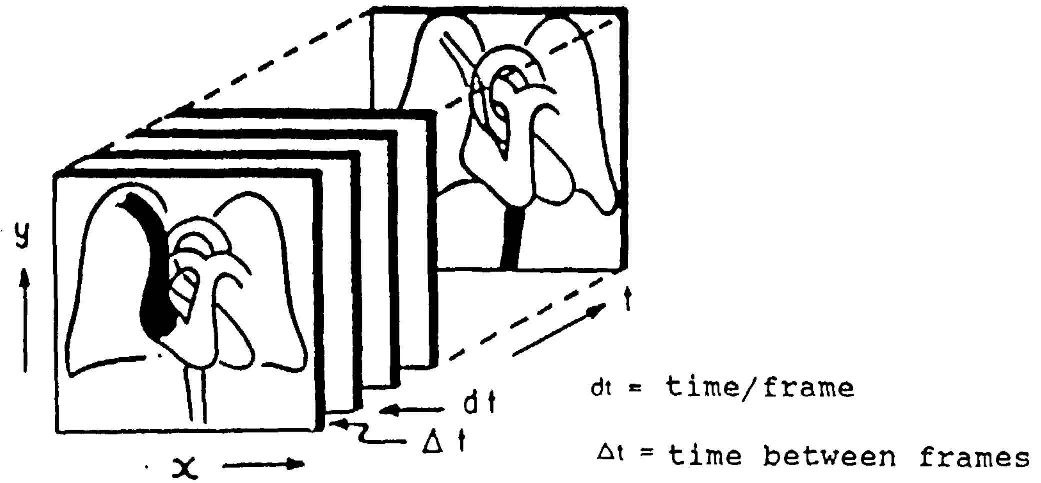


Fig. 24.6 Principle of first-pass quantitative radioangiocardiography. Each frame shows the image of the tracer during a particular period during its transit through the heart and great vessels.

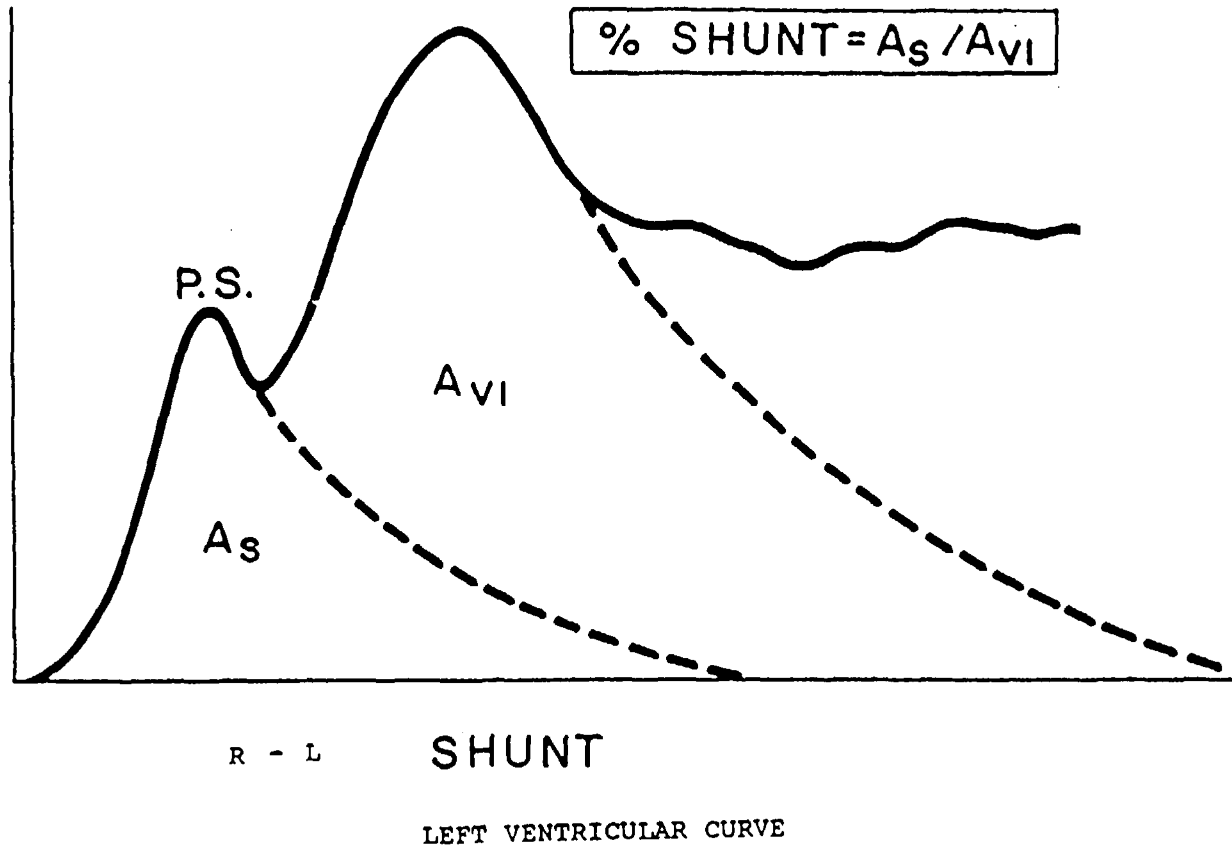


Fig. 24.7 First-pass radioangiographic curve in the left ventricle in a patient with a right-to-left shunt, evidenced by an early peak (PS) caused by the abnormal communication. The descending limbs of both peaks are extrapolated and the areas under each (A_s and A_{vi}) are measured. $Q_p / Q_s = A_s / (A_s + A_{vi}$.

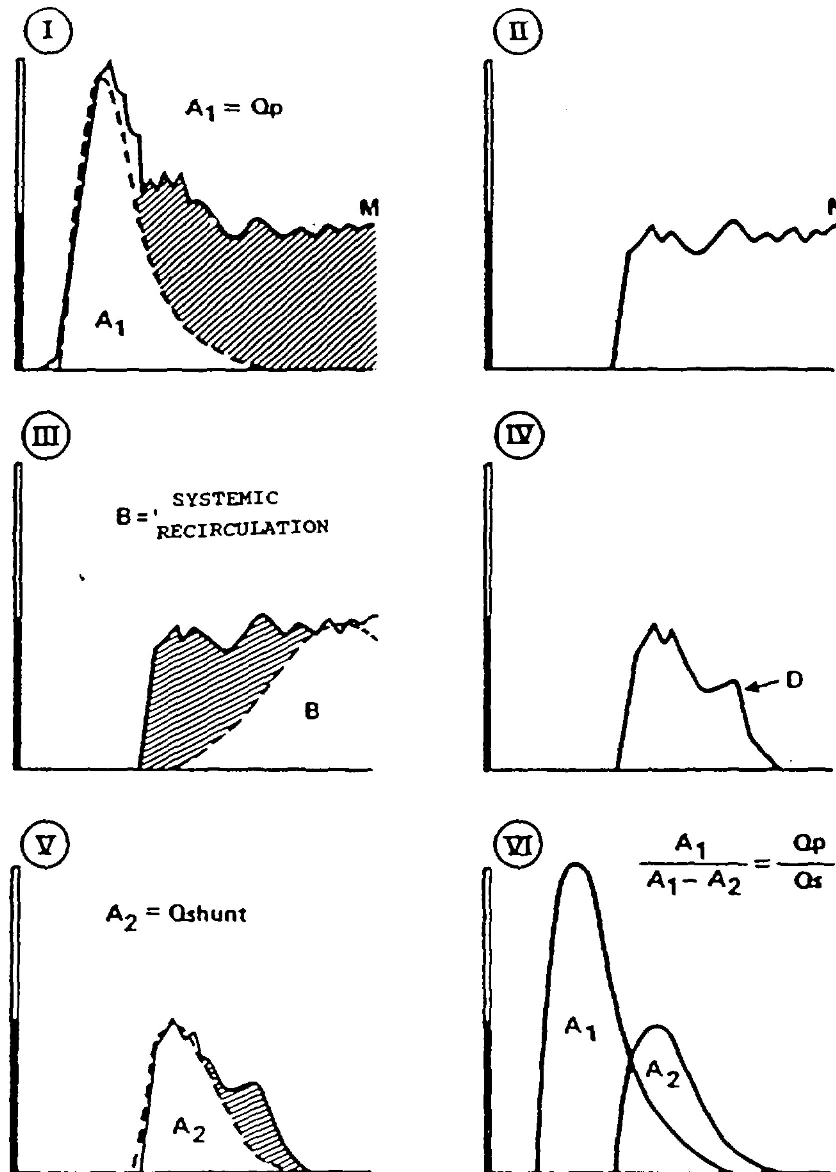


Fig. 24.8 Quantitation of a left-to-right cardiac shunt through the time / activity curve in the lungs during first-pass radioangiocardiography.

I. Gamma variate fit to isolate area $A_1 = Q_p$.

II. Differences between the original curve (M) and the gamma variate fit. Area under curve N includes bronchial circulation and shunted radioactivity (early recirculation).

III. Gamma variate fit corresponding to calculated total recirculation time. Area B = radioactivity from systemic recirculation in the lungs.

IV. Differences between curve N and curve B, isolating curve D.

V. Gamma variate fit to curve D to define area A_2, corresponding to the shunted tracer through the abnormal communication. $Q_p / Q_s = A_1 / A_1 - A_2$.

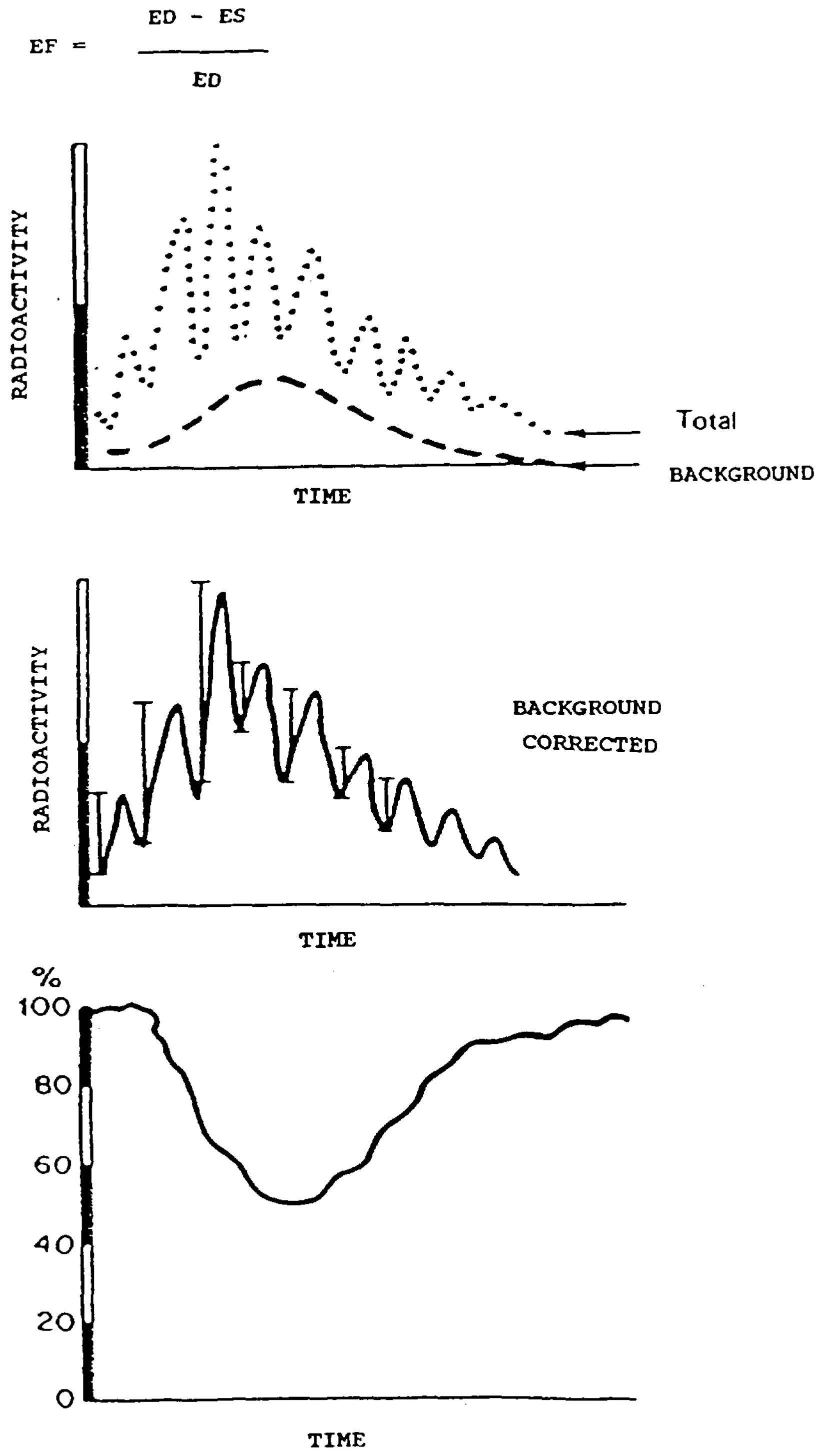


Fig. 24.9 Method for calculation ejection fraction from the first-pass radioangiographic curve at the left or right ventricle. (See text for explanation).

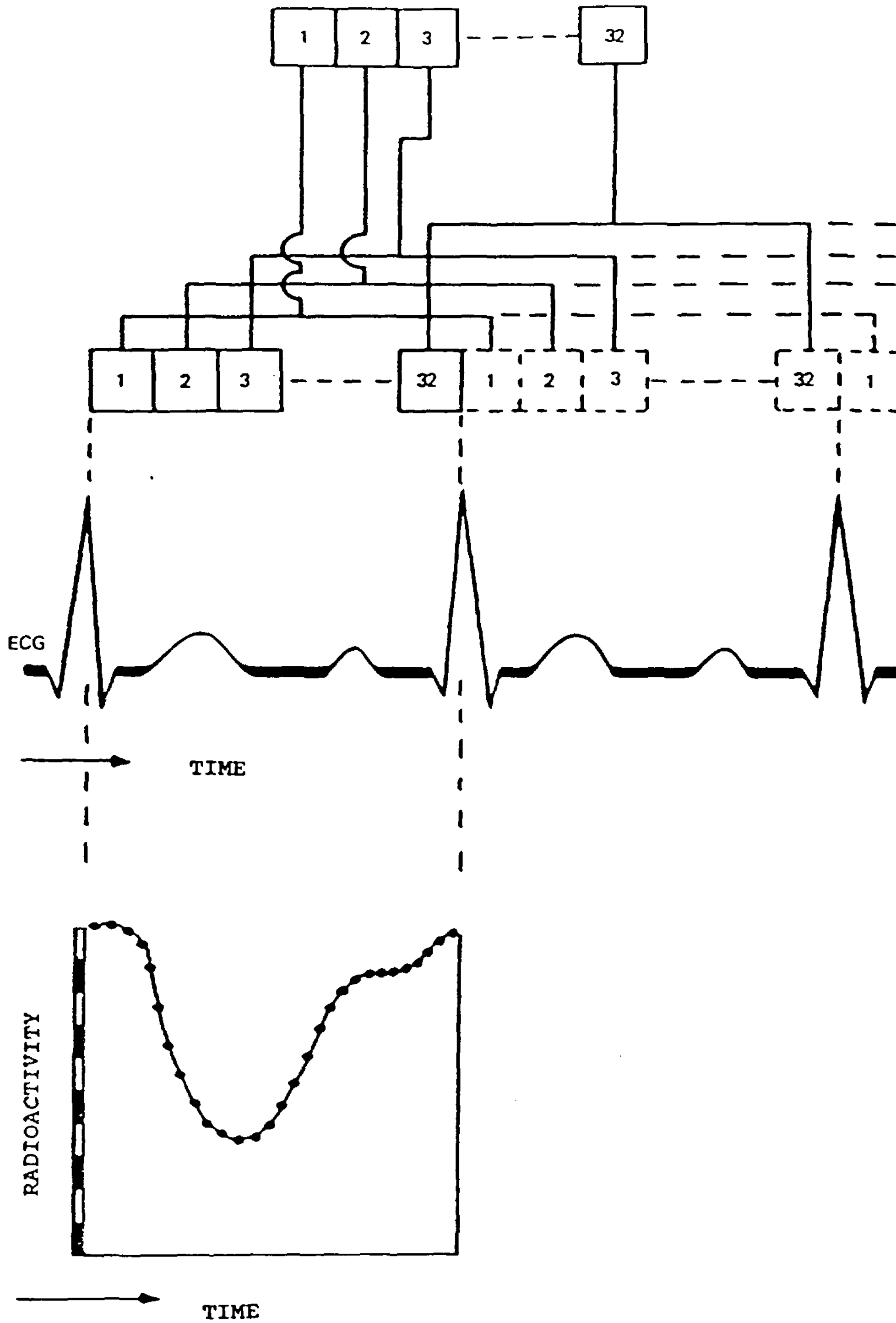


Fig. 24.10 ECG gated acquisition of the volumetric curve of the left ventricle during equilibrium radioangiocardiography. (See text for explanation).

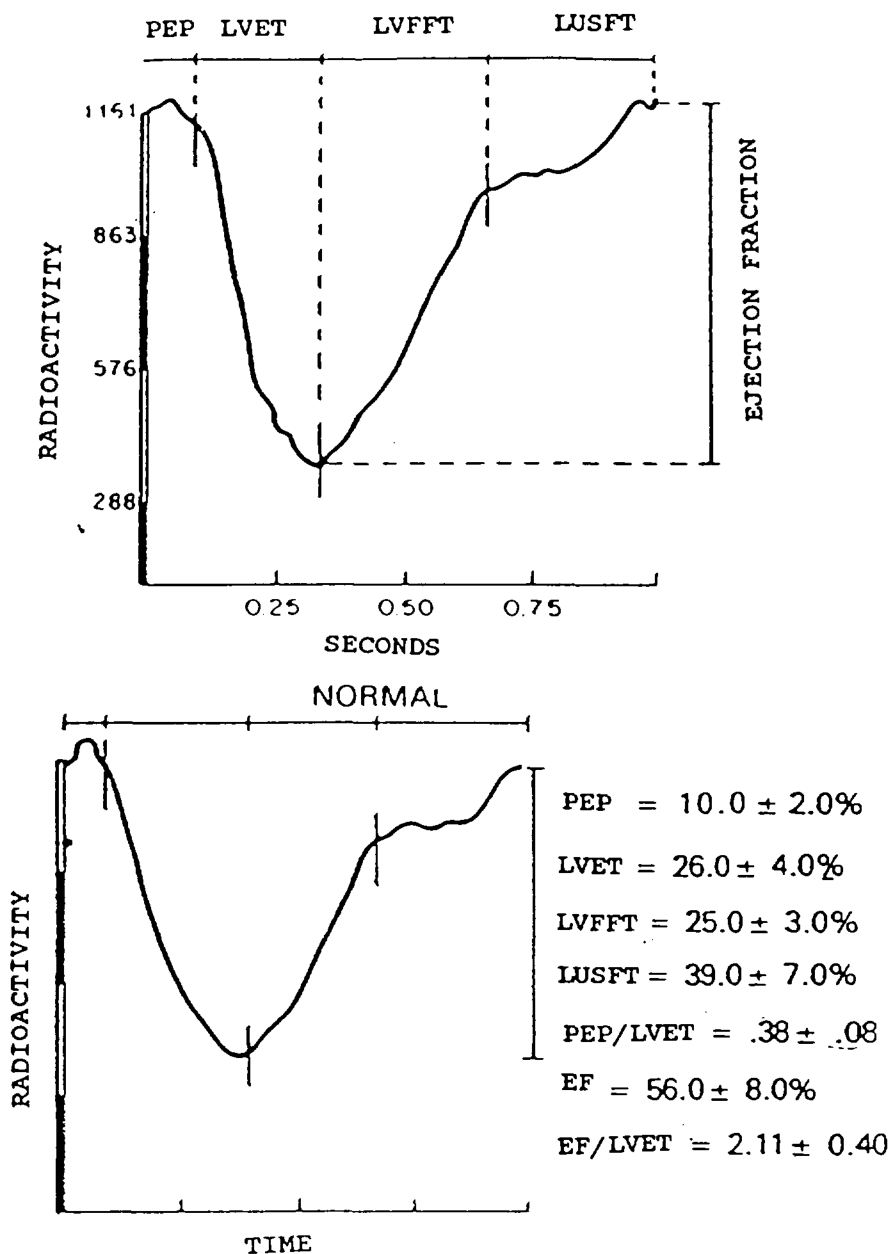


Fig. 24.11 Relative duration of the different segments of the ventricular volumetric curve during the cardiac cycle. PEP = Pre-expulsive period. LVET = LV ejection time. LVFFT = LV fast filling time. LVSFT = LV slow filling time. The point between LVFFT and LVSFT is called diatesis. EF = ejection fraction.

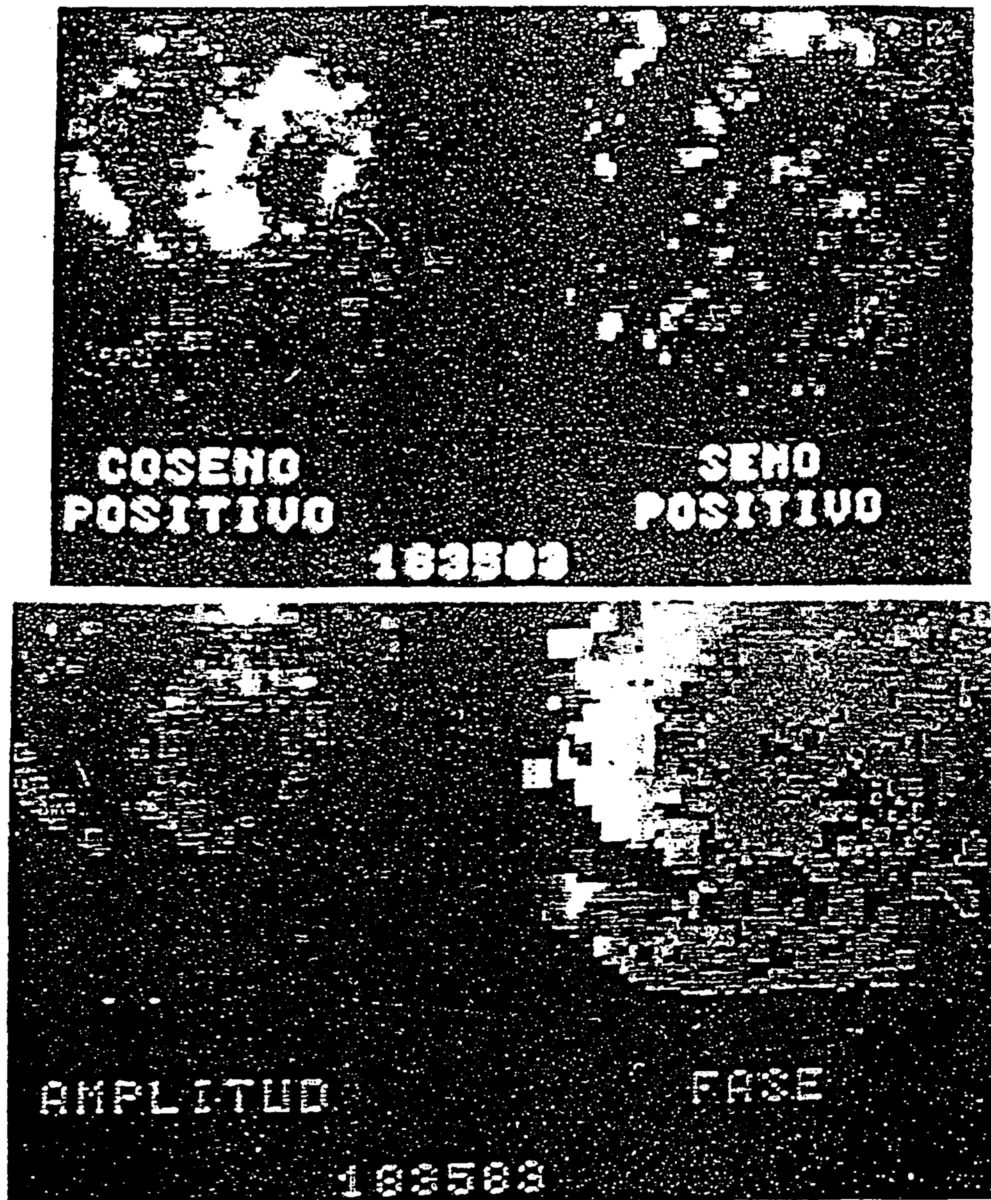


Fig. 24.12 Parametric images of the heart constructed by Fourier's analysis of the cardiac cycle in a pixel by pixel fashion. **Amplitude image** (upper left) is equivalent to stroke volume. It shows hypokinesis at the septum and upper region of the lateral wall, and akinesis at the lower region of the lateral wall. **Phase image** (upper right) showing dephasing of the ventricular wall motion at the lower region of the lateral wall, which is akinetic in the amplitude image. **Ventricular amplitude** (lower left) shows the amplitude of wall motion in both ventricles, evidencing the same hypokinesis and akinesis at the lateral wall (it is constructed by using the positive cosine). **Atrial amplitude** (lower right), shows amplitude of the atrial wall motion and paradoxical movement at the lower lateral wall as evidence of a ventricular dyskinesis (it is constructed by using the positive sine).

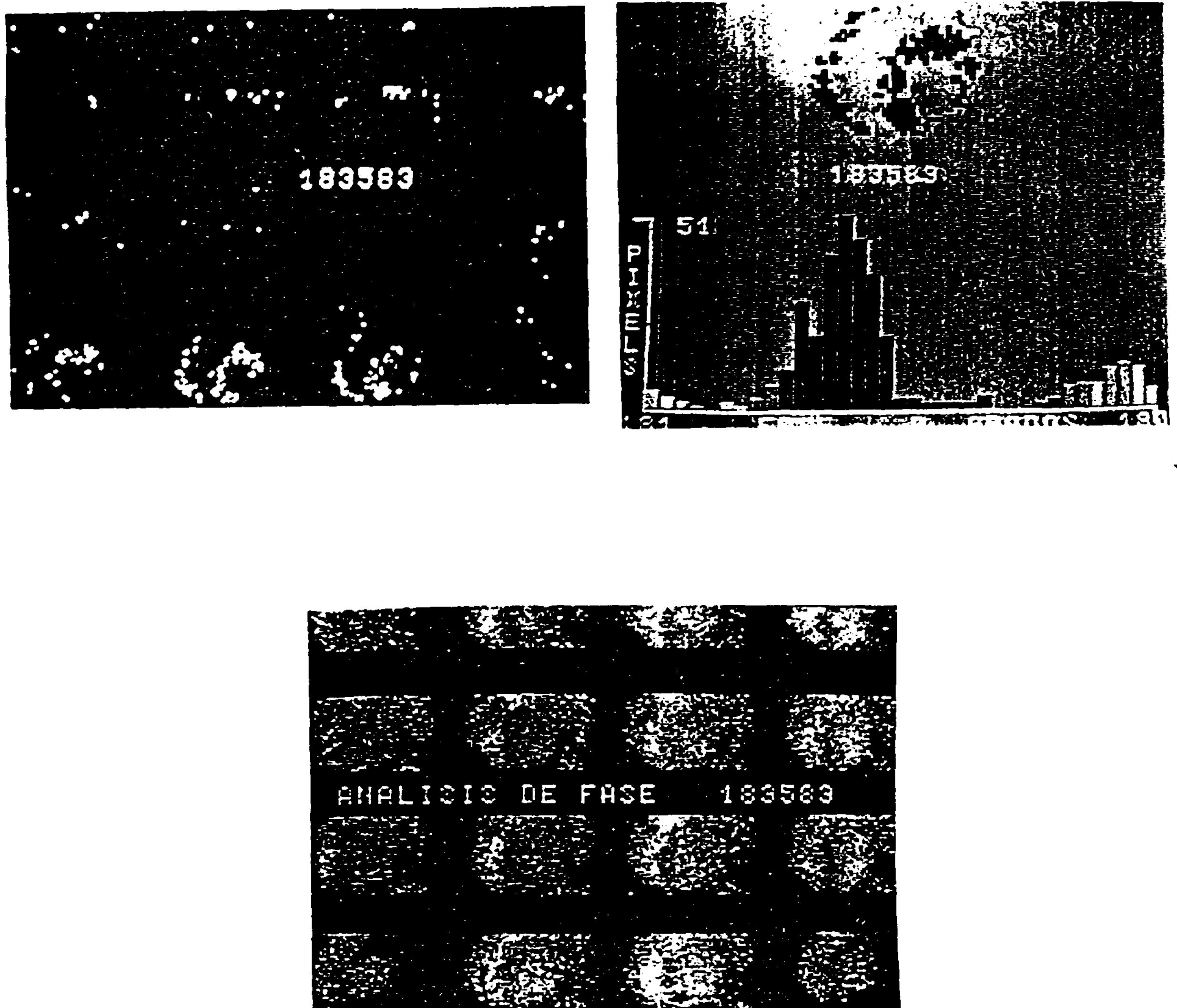
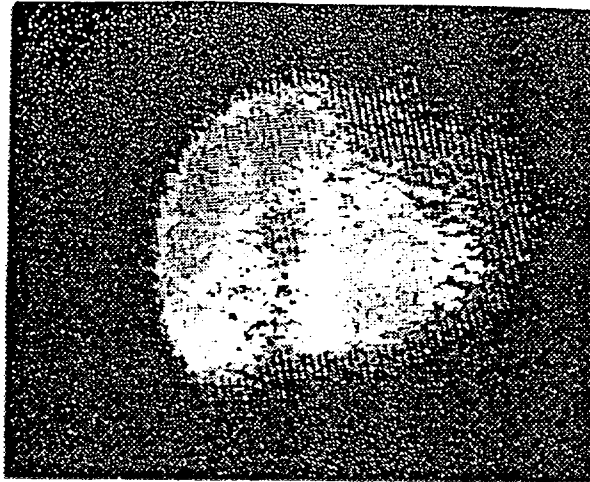
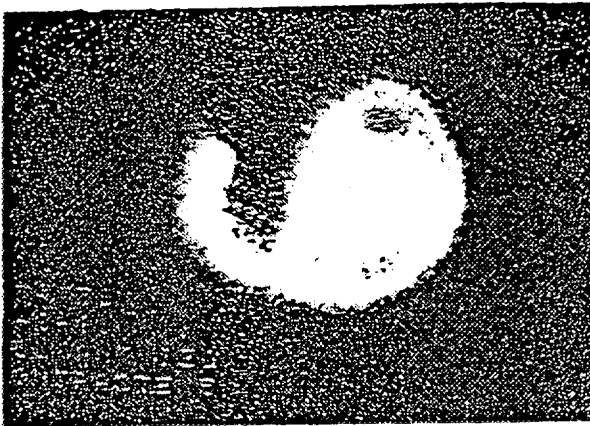


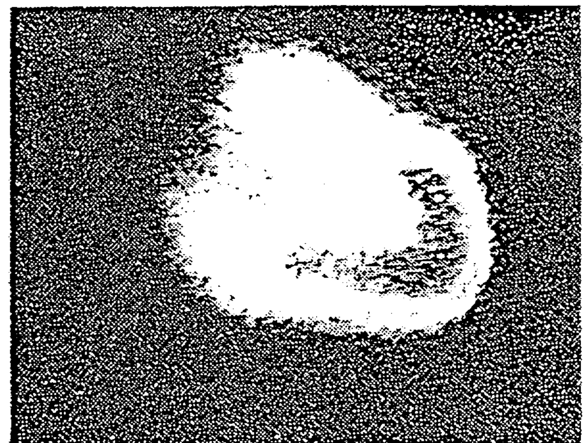
Fig. 24.13 Mechanism for the construction of the phase image. The pixels which are in phase (maximal amplitude) are marked with a different colour on each frame of the equilibrium gated radioangiocardiography corrected by background (Upper images). All pixels which are not in phase are deleted on each frame leaving alight only those pixels which are in phase. Each frame is given a particular colour following a predefined code (middle images). All frames of the e-rac are summed up in a single image (phase image) and the histogram showing the number of pixels in phase at each period of the cardiac cycle is displayed (lower image).



A



B



C

Fig. 24.14 Coronary capillary bed scintigraphy in three patients with diabetic coronary microangiopathy and normal coronary angiography.

A. Left lateral view showing a perfusion defect at the inferior wall near the left apex.

B. Left anterior oblique view, showing hypoperfusion at the septum and at the upper posterolateral wall.

C. Right lateral view, showing hypoperfusion at the anterior wall of left ventricle.

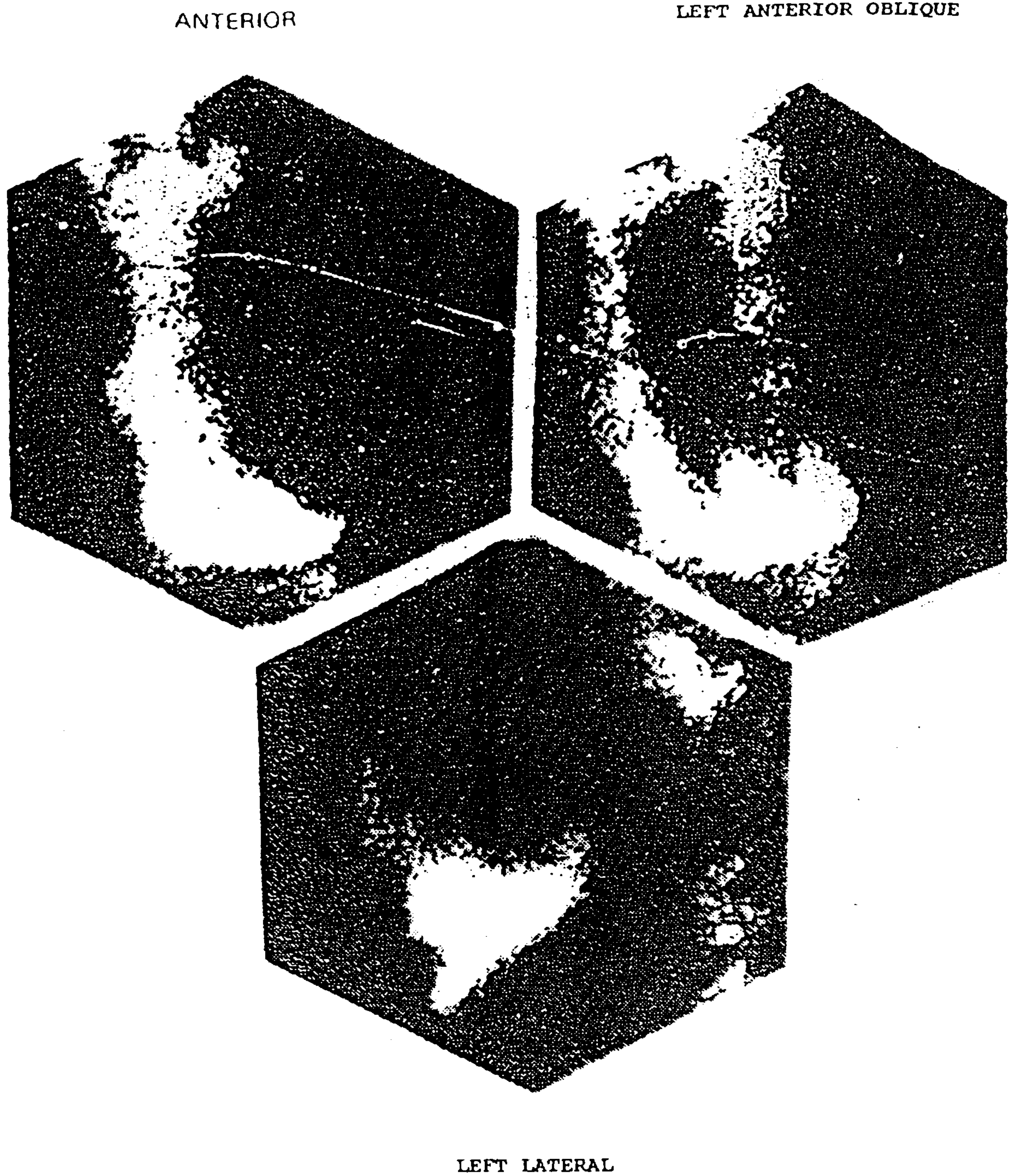


Fig. 24.15 **$^{99}Tc^{m}$-pyrophosphate myocardial necrosis scintigraphy** in a patient with an extensive acute myocardial infarction at the lateral, inferior and septal walls of left ventricle with an extension to the inferior and anterolateral wall of the right ventricle. This peculiar distribution of the tracer in both ventricles gives the left anterior oblique view the similarity to an inverted 3.

Chapter 25

NUCLEAR TECHNIQUES IN THE DIAGNOSIS OF LUNG DISEASES

Toyoharu Isawa

Introduction

In the late 50's, George V. Taplin was studying the function of the reticuloendothelial system (RES) by injecting aggregated particles of ^{131}I-human serum albumin. The RES is dispersed over many organs but is concentrated predominantly in liver, spleen and bone marrow. It has the important function of removing undesirable particulate substances from the circulation. Because particles of less than 0.5 to 1.0 μm in size are taken up by the cells of the RES he was measuring the disappearance rate of the injected particles from the general circulation and the gradual build-up of radioactivity in the RES cells (Kupffer cells) of the liver. For some reason or other, his laboratory on one occasion mistakenly made particles about 100 times larger than usual. Some impulse made him inject these mistakenly made larger particles into experimental animals to find out organs in the body in which they were trapped. Naturally the first capillary network, that the particles encounter after an intravenous injection is that in the lungs, and he found that most of the particles were, indeed, trapped there. The smaller particles were taken up by the RES cells of the liver. When a gamma ray scan was made, the mapping of the trapped radioactive particles showed up as images of the lungs and the liver. Working on this basis, Taplin developed a method of producing suitable sized albumin aggregates for lung imaging that would be safe for human use. He named them ^{131}I-MAA (macroaggregated human serum albumin). Because many fruitless endeavours had been made to find a lung imaging agent to facilitate the diagnosis of pulmonary embolism in early 60's, this serendipitous creation of ^{131}I-MAA was widely welcomed as the best and the only agent for perfusion lung imaging as soon as his first report was published in 1963.

Nobody would have thought, however, that his fortuitous production of ^{131}I-MAA in 1963 and his subsequent proposal of aerosol inhalation lung imaging in 1965 would revolutionize and widen the scope of pulmonary medicine to the extent that we enjoy today. Regarding aerosol inhalation lung imaging Pircher's contribution should not be forgotten, because he and his co-workers also proposed aerosol inhalation lung imaging almost simultaneously and independently.

Perfusion and inhalation lung imaging have not only facilitated the diagnosis of pulmonary embolism but also made it possible to see respiratory and non-respiratory lung function. The contribution of nuclear techniques to pulmonary medicine is immense and has been briefly outlined in the following pages.

CHAPTER 25

I. RESPIRATORY LUNG FUNCTION

1. Perfusion lung imaging

Lung function consists of pulmonary arterial perfusion, ventilation and diffusion of oxygen and carbon dioxide between perfusion and ventilation. Perfusion is equivalent to the distribution of the pulmonary arterial blood flow in the lungs that has returned from the entire body carrying the venous blood. The venous blood is to be replenished with oxygen in the lungs before being sent to the systemic circulation. Ventilation is the distribution of inhaled gas in the lungs to supply oxygen to the venous blood and remove carbon dioxide from it through diffusion. This process of gas exchange is called "respiration" **(Fig. 25.1)**.

1. How to study perfusion distribution in the lungs?

Currently $^{99}Tc^{m}$-MAA is used as a tracer for perfusion lung imaging instead of ^{131}I-MAA. The size of $^{99}Tc^{m}$-MAA ranges from 15 to 50 μm and is slightly larger than the red blood cells. Once slowly injected intravenously they are mixed in the right heart chambers and carried to the lung precapillary and capillary networks where they are trapped **(Fig. 25.2)**. It shows perfusion and inhalation aerosol deposition in the lungs in a normal subject. The latter, in normal subjects, is equivalent to ventilation distribution in the lungs.

(a) Body position

Because the lung is like a manometer, if a tracer is injected with the subject in the sitting position, the distribution of radioactivity represents the perfusion distribution in the lungs in the sitting position. If the tracer is injected in the supine position, the distribution of radioactivity represents the perfusion distribution in the lungs in the same supine position. In the sitting position, the lower lung regions show more perfusion per unit lung volume than the upper lung, but in the supine position, little difference exists in the distribution of perfusion between the upper and the lower lung fields. This is the "gravity effect" on perfusion in the lungs. If a tracer is injected with the subject in the right or left lateral position, the lower-positioned lung shows more perfusion distribution than that lung in either the supine or the sitting position due to the gravity effect. This is an important physiological fact that should be borne in mind in interpreting perfusion lung images.

(b) Lung volume

Perfusion distribution at different lung volumes can be studied if a tracer is injected at the lung volume determined by respective respiratory positions such as the residual volume (RV) or the total lung capacity (TLC) level. The perfusion distribution seen routinely in lung imaging is usually obtained at the lung volumes of resting tidal ventilation.

(c) Radioactive gases

Similar studies can be done using radioactive gases such as ^{133}Xe (xenon) and ^{81m}Kr (krypton) dissolved in saline and 5 % glucose solution, respectively. Breath-holding is required not only when the radioactive solution is injected but also when the lungs are being imaged. Otherwise radioactive gases would be exhaled from the lungs. Only one projection of the lungs is imaged at one time. In other words when the lungs are imaged from anterior, posterior and both right and left lateral projections, four injections are necessary and breath-holding is required each time. This is not practical. Usually better lung perfusion images with better statistics are obtained with ^{99}Tc^{m}-MAA than with radioactive gases.

(d) Multiple projections

Usually four view perfusion lung images are taken; anterior, posterior, right and left lateral views. Additional views like right and left anterior oblique or respective posterior oblique projections are taken if required.

2. Pathological factors influencing perfusion distribution in the lungs.

(a) Vascular obstruction

In pulmonary embolism blood clots (thrombi) obstruct the vascular lumen and the blood flow cannot pass through it. Lung regions distal to the obstruction has diminished or absent perfusion. **(Fig. 25.3)**.

(b) Parenchymal lung disease

In pneumonia, lung abscesses, tuberculosis or any parenchymal lung disease, vascular beds are involved in the disease processes to the extent that pulmonary arterial blood flow cannot pass through the vascular channels in the pathological region and, as a result, the regional perfusion is disturbed. In addition to structural changes in the vascular beds in parenchymal lung diseases, once the alveoli are filled with liquid or cellular material such as purulent discharge or cell debris, regional hypoxia ensues and the perfusion becomes diminished or absent there due to hypoxic vasoconstriction as described below.

(c) Vascular compression and/or invasion

In bronchogenic carcinoma, the pulmonary arterial beds are extrinsically compressed or invaded so that the perfusion is diminished or absent distal to the compression or invasion. In sarcoidosis pulmonary perfusion is not usually affected.

(d) Vascular stenosis or agenesis

In congenital pulmonary vascular anomaly like pulmonary artery stenosis, the perfusion distal to the stenosis can be diminished or absent. In pulmonary artery agenesis, there is no vascular channel for the blood to flow.

(e) Alveolar hypoxia (low oxygen tension).

In primarily obstructive airway diseases like pulmonary emphysema **(Fig. 25.4)**, bronchitis, bronchial asthma, panbronchiolitis, bronchiectasis, the perfusion is also decreased. Distal to bronchial obstruction due to intraluminal tumours or foreign substances such as a swallowed foreign body, perfusion is diminished or absent. In pulmonary emphysema, vascular destruction is associated with alveolar wall destruction and stretching of the vascular beds may contribute to the decrease in perfusion but alveolar hypoxia is the commonest cause for changes in perfusion in all these diseases.

Experimentally when hypoxic gas is inhaled, perfusion in the lung region is diminished due to hypoxic vasoconstriction or vascular narrowing. The same phenomenon is observed when ventilation is interrupted. In other words, the oxygen tension in the alveoli has been found to be the most important factor in regulating regional perfusion distribution. This physiological reaction can take place promptly in any of the regions in the lungs. Regional hypercapnia (high carbon dioxide tension) does not seem to contribute to such a hypoperfusion (decreased perfusion).

3. Pulmonary Embolism

In pulmonary embolism, parts of the pulmonary arterial system are blocked by thrombi so that pulmonary perfusion is absent in the regions distal to the thrombi **(Fig. 25.3)** and the gas exchange is impaired. Patients suffer from shortness of breath and chest pain. Unless the diagnosis is made promptly and the treatment started, it is usually a fatal condition.

Shortness of breath, chest pain, and bloody sputum are cardinal symptoms, but they are common to many other lung diseases that the clinical symptoms and signs are not in themselves helpful in diagnosing pulmonary embolism. Unfortunately chest x-rays cannot reveal the presence of emboli or underlying subtle vascular changes. Only pulmonary angiography using contrast media can show filling defects or narrowing of the vascular lumen, but angiography itself is not without risks. Perfusion images when combined with ventilation images (either with radioactive gases or radioaerosols) are indispensable to the diagnosis of pulmonary embolism, because in pulmonary embolism ventilation is not disturbed in embolic regions where perfusion is absent.

By taking multiple view lung perfusion images, naturally in areas distal to a blockade caused by emboli little perfusion is present and little radioactivity is seen as shown in Fig. 25.3. It is because of the simplicity of the technique that lung perfusion imaging with ^{131}I-MAA and now ^{99}Tc^{m}-MAA has enjoyed a wide acceptance. Diagnosis of pulmonary

embolism, even in emergency situations, is greatly facilitated by perfusion lung imaging. When done in tandem with radioaerosol inhalation lung imaging the sensitivity and the specificity for the diagnosis of pulmonary embolism is close to 100 %. Physiologically speaking, bronchoconstriction and hypoventilation can occur immediately after blockage of pulmonary arterial blood flow, but this phenomenon is transient and is known to disappear in dog experiments within six hours. Under clinical situations, six hours would have already passed before a doctor is ready to do the lung imaging.

Preoperative perfusion lung imaging is very helpful when a patient postoperatively develops symptoms and signs suspicious of pulmonary embolism. By comparing the preoperative and postoperative perfusion images in patients in whom pulmonary embolism is suspected, diagnosis of pulmonary embolism is greatly facilitated without doing inhalation lung images.

4. Perfusion Abnormalities in Other Lung Diseases

As mentioned earlier, regional perfusion can be reduced or absent, not only in pulmonary embolism, but also in parenchymal lung diseases and in airways diseases.

Besides the anatomical changes in the pulmonary vasculature, the lung perfusion is regulated by the alveolar oxygen tension. Ventilatory disturbances result in regional alveolar hypoxia which, in turn, constricts the regional pulmonary arterial beds to reduce the blood flow there. A regional decrease in perfusion ensues as soon as regional hypoxia takes place.

Parenchymal lung diseases can be recognized by chest X-rays as an area of increased density, but differentiation of pulmonary embolism or vascular diseases in general from airways disease by chest X-rays is usually extremely difficult. Here arises the importance of studying lung perfusion in conjunction with ventilatory function.

5. Assessment of Regional Lung function

It has been observed that the distribution ratio of the injected MAA in the right and left lungs corresponds closely with the oxygen consumption ratio. Perfusion lung imaging is, therefore, not limited to the diagnosis of pulmonary embolism as originally envisaged, but also offers a new means of studying regional pulmonary function. Obviously lung function or gas exchange cannot exist without perfusion. Where in the lungs the function is preserved? An answer to this question is vital when considering various alternatives for the treatment of the patient. The actual sites of poor or absent perfusion can be shown and quantitated by this simple, safe and non-invasive perfusion lung imaging procedure.

In countries like Japan where the incidence of pulmonary embolism is extremely low, perfusion lung imaging is widely used to study regional lung function. Before a lung operation, for example, perfusion lung imaging and the quantitative determination of the

perfusion ratios in the right and left lungs are essential for the evaluation of regional pulmonary function. Perfusion lung imaging by nuclear techniques has completely replaced complicated invasive procedures for preoperative lung function evaluation.

6. Safety of perfusion lung imaging

Because MAA particles are intravenously injected and trapped in the lung precapillaries and the capillary beds, the fear that MAA injection itself might be hazardous, causing microemboli in the pulmonary vascular beds is reasonable and understandable. Actually in the '60s when the MAA particles were not commercially available and made by the user in his own laboratory, there were indeed several accidents because the injected particles often contained particles larger than 150 μm in diameter. There were other reported incidents of adverse reactions in patients with severe pulmonary hypertension.

Human lungs have about 300 million arterioles and 380 billion capillaries. If we assume that 5 mg of MAA, containing 0.8 million particles whose sizes are smaller than 20 μm in diameter and 0.2 million particles whose sizes are over 20 μm, is injected, the possibility of blockade is in 1 in 35 000 capillaries and one in 1500 arterioles or precapillaries. Usually less than 1 mg of MAA is injected and the safety margin is much greater than this assumption. Taplin reported that if the particles are less than 35 μm in diameter, there is absolutely no haemodynamic effect either on the systemic or the pulmonary circulation even after injecting 40 mg of MAA per kg body weight in the dogs. When the particle size is larger than 80 μm, injection of 25 mg of MAA per kg of body weight can cause depression of the systemic blood pressure and elevation of the pulmonary artery pressure. In this sense, MAA is one of the safest radiopharmaceuticals and the fear about its safety is unjustified. MAA can be practically used in any patient without any kind of apprehension. Making MAA preparation in one's hospital radiopharmacy should be undertaken only if strictest quality control is possible.

2. VENTILATION LUNG STUDIES

Both radioaerosols and radioactive gases such as ^{133}Xe and ^{81}Kr^{m} can be used for studying ventilation distribution in the lungs (**Fig. 25.2**). Aerosols are appropriate for taking multiple views following the inhalation, whereas gases are more suitable for studying the dynamic aspect of ventilation.

1. How to study ventilation in the lungs?

(a) Radioactive gases

(i) Single breath method

In practice the single breath method is most frequently used; radioactive gases are inhaled from the residual volume (RV) to the total lung capacity (TLC) levels by a single breath and

then the breath is held for 10 sec or so followed by breathing air. Then the inhaled radioactive gases are washed out.

(ii) Tidal breathing method

Instead of inhaling radioactive gases in a single breath, tidal breathing is done from a container of radioactive gases. At a certain point the circuit is switched to breathing air following a breath-holding period and radioactive gases are washed out.

(iii) Equilibration method

Radioactive gases are inhaled from a container in a closed circuit from a given respiratory level such as the RV or the functional residual capacity (FRC) to the total lung capacity (TLC) level and the breath is held to determine the distribution of ventilation in the lungs. Then the subject keeps breathing the radioactive gases until an equilibrium state of radioactivity is reached between the whole circuit and the subject's lungs and then holds his or her breath at the TLC level to determine the lung volume. Then the circuit is switched open to permit breathing air and the washout phase is recorded. $^{81}Kr^{m}$ gas is not suitable for this kind of study because of its short physical half life. In all the above methods the exhaled gases need to be collected.

(b) Radioaerosols

Radioaerosols are inhaled in resting tidal breathing until radioactivity, enough for a good image, deposits in the lungs. For this purpose, deposition of, at least, one to two mCi in the lungs is necessary. The radioaerosol inhalation imaging shows the distribution of the deposition of the radioaerosols in the lungs. The main purpose of the aerosol studies is to know whether or not the regions of interest in the lungs are aerated and how aerosols deposit in these regions as well as in the entire lungs. If inhaled aerosols deposit in the lung regions, that means that the ventilation is present there.

Radioaerosols are generated either with jet nebulisers or with ultrasonic nebulisers. They are commercially available and those that consistently produce particle size less than 3 μm in mass median diameter, are acceptable for clinical use. **Table I** shows the size of aerosol particles produced by various aerosol generators. As described later, BARC(Bhabha Atomic Research Centre) nebuliser, a jet nebuliser made by assembling needles, test tube and an air compressor, originally developed in India is quite good for routine use and can be even fabricated in one's own laboratory. The Appendix I shows a construction diagram of the BARC nebuliser.

TABLE I. MASS MEDIAN DIAMETER (Dm) AND GEOMETRIC STANDARD DEVIATION (σg) UNDER STANDARD CONDITIONS (TEMPERATURE 37°C, RELATIVE HUMIDITY: 100%, AND TEST AGENT: $^{99}Tc^{m}$-ALBUMIN) MEASURED BY CASCADE IMPACTOR (ANDERSEN SAMPLER)

NEBULIZER	Dm (μm)	σg
Jet Nubulizers		
OEM-1 (USA)	1.96	1.65
OEM-2 (USA)	1.19	1.86
Ultravent (USA)	1.04	1.71
Penicillin nebulizer glass (Japan)	1.76	1.70
BARC with reservoir (India)	0.84	1.73
BARC without reservoir	1.57	1.80
Ultrasonic nebulizers		
Mistogen EN-142 (USA)	1.93	1.52
Omuron-NE-U11 (Japan)	1.62	1.50
Devilbiss (USA)	1.78	1.60

2. Significance of ventilation studies

Ventilation is usually disturbed in various parenchymal and chronic obstructive lung diseases but not affected in vascular diseases such as pulmonary embolism.

In a region of the lung where the ventilation is primarily disturbed, washout (disappearance rate) of inhaled radioactive gases is prolonged, and radioactivity remains longer in the affected region than in the healthy regions of the lungs.

An image of the distribution of inhaled aerosols is not, strictly speaking, an image of the ventilation in the lungs especially in the presence of complicated obstructive airways disease. The word "inhalation imaging" is more appropriate than "ventilation imaging". With radioaerosol imaging, only the deposition of the inhaled particles in the lungs is seen, as there is no washout as in the case of gases. Despite this limitation, aerosol deposition patterns are helpful in distinguishing the lung regions with good ventilation from the regions which are poorly ventilated. They are also useful in diagnosing the nature of the underlying disease. Whereas a central deposition pattern indicates an emphysematous type of obstructive airways

disease, non-homogeneous or peripheral patchy distribution suggests a bronchitic type of disease. Most patients with obstructive lung diseases, however, show both kinds of patterns combined in different proportions (Fig. 25.5).

3. Agents used for ventilation studies

Except for ultra-short half-life radioisotopes produced by the cyclotron, ^{133}Xe and $^{81}Kr^{m}$ are exclusively used as radioactive gases for ventilation studies. Radioactive gases for inhalation are mixed with either air or oxygen.

Agents used for radioaerosol production are $^{99}Tc^{m}$-albumin, $^{99}Tc^{m}$-DTPA, or $^{99}Tc^{m}$-phytic acid. The aerosols are produced with either an ultrasonic nebulizer or a jet nebulizer. In addition to the airflow rate and the shape of the airways, the size of inhaled aerosols is an important factor in determining the aerosol distribution in the lungs. The size of aerosols is different with different instruments as shown in **Table I.**

4. Combined Perfusion and Inhalation Lung Imaging

Images of the distribution of inhaled radioaerosols or radioactive gases give a good idea of the ventilatory status and the degree of functional disturbance in the lungs. A comparison of inhalation lung studies with perfusion images can show how well regional perfusion matches with regional ventilation. If ventilation is present in non-perfused regions in patients with normal chest X-rays, those lung regions can be diagnosed as having some kind of vascular diseases including pulmonary embolism (Fig. 25.3). If perfusion is absent or abnormal in lung regions where inhalation studies also show evidence of abnormal ventilation, those lung regions are diagnosed as having some kind of airways disease where alveolar hypoxia has induced regional perfusion abnormalities by hypoxic vasoconstriction (**Figs. 25.6 and 25.7**).

It is clear from this that lung imaging is not complete without images of both the perfusion and the ventilation systems. Obtaining the latter is difficult in many developing countries because of the unavailability of the labelled gases. The use of radioaerosols now makes it possible to do total lung imaging in these countries and diagnose many acute and chronic disorders of the lungs.

In a co-ordinated research programme (CRP) sponsored by the IAEA over the period of 1987-1990 to promote lung imaging in developing countries, a jet nebulizer originally developed at Bhabha Atomic Research Centre (BARC) in India has been used in ten countries in the Asia and Pacific region. The nebulizer contains a series of hollow needles and a compressed air pump. As shown in **Table I**, the mass median diameter of aerosol produced by the BARC nebulizer was 0.84 um with geometric standard deviation of 1.73, if the nebulizer is used with a reservoir placed between the aerosol generating part and the mouth piece. The size of aerosol produced by BARC nebulizer is appropriate for studying the distribution of inhaled aerosols in the lungs as a simulation of ventilation distribution. Both $^{99}Tc^{m}$-DTPA and $^{99}Tc^{m}$-albumin can be used to prepare radioaerosols in this kind of nebulizer.

Once the aerosols are inhaled and imaged, injection of three to five times as much $^{99}Tc^{m}$-MAA can follow to give simultaneous perfusion images. Normally, except in emergency situations, perfusion imaging is done initially and is followed 24 hours later by aerosol inhalation lung studies.

Recently an instrument called "Technegas" has been developed in Australia. This produces an aerosol less than 200 nm in size. The principle of the aerosol generation is as follows; a carbon crucible that has adsorbed $^{99}Tc^{m}O_4^-$ is instantly heated to 2500 degrees in the atmosphere of argon (Ar) gas and $^{99}Tc^{m}$ tagged carbon particles are generated. Inhaled aerosol penetrates better to the lung periphery than conventional aerosols generated ultrasonically or by jet nebulizers and the alveolar deposition ratio is about 85 %, while it is about 40-50 % with the inhaled aerosol generated by ultrasonic and jet nebulizers.

III. NON-RESPIRATORY LUNG FUNCTION

Apart from ventilation and perfusion, a lung function that has nothing to do with gas exchange or respiration can also be studied by using aerosols (**Fig. 25.8**). They are mucociliary clearance function, pulmonary epithelial permeability and few other non-respiratory lung functions.

1. Mucociliary Clearance Function.

Mucociliary clearance is the first line of defense of the lungs against undesirable material inhaled inadvertently. How to measure and evaluate mucociliary transport and clearance mechanism has been a long-standing problem. A nuclear technique has solved this problem to some extent and mucociliary clearance function can now be studied by inhalation of radiolabelled aerosols. Nuclear methodology has revealed what the mucociliary clearance mechanism is like in vivo. What it involves is a sequential measurement of the distribution of inhaled aerosols in the lungs over a period of time and analysis and comparison of the changes in radioactivity in the sequential images.

Bronchial mucus is transported upwards toward the larynx by the interaction of the mucous layer and the ciliary beating motion in order to get rid of:

(a) mucus itself
(b) intrinsically produced cell debris,
(c) extrinsically inhaled particulates and
(d) chemical substances dissolved in the mucus so that the lungs and the airways are kept clean.

In pathological conditions, this upward transport has been found to be disturbed in various ways.

Mucociliary clearance takes place only on the ciliated airways but inhaled aerosols deposit not only on the ciliated airways but also on the non-ciliated airways and the alveolar space. Thus radioactivity deposited in the non-ciliated airways should be excluded from the evaluation of mucociliary clearance function. The radioactivity remaining in the lungs at 24 hrs after the inhalation is defined as the radioactivity deposited in the non-ciliated airways including the alveoli. Aerosols deposited in the non-ciliated airways including the alveoli, are cleared by other mechanisms than mucociliary clearance. Macrophages play an important role in clearing aerosols that deposit in the non-ciliated space of the lungs. In order to separate the non-ciliated portion of the lungs including the alveolar space from the ciliated airways, the percentage of the net radioactivity in the lungs at 24 hours after inhalation of aerosols (minus the body background) versus the total initial radioactivity is defined as alveolar deposition ratio (ALDR). The following parameters are also defined and calculated as shown in **Fig. 25.9**: lung retention ratio (LRR), airway deposition ratio (ADR), airway retention ratio (ARR), airway clearance efficiency (ACE) and ALDR. Using radioaerosol of mass median diameter of 1.93 um with geometric standard deviation of 1.52, the ALDR can be calculated by the following formula:

$$\mathbf{ALDR(\%) = -48.08 + 0.47 \times FEV_{1.0}\% + 0.59 \times LRR60.}$$

Here $FEV_{1.0}\%$ is forced expiratory volume in one second divided by forced vital capacity in % and LRR 60 is lung retention ratio LRR) at 60 min in %. In addition to the ALDR and the LRR, airway deposition ratio (ADR), airway retention ratio (ARR) and airway clearance efficiency (ACE) are calculated to quantitate the mucociliary clearance function in the lungs. These ratios would differ according to the size of aerosols inhaled.

The mucociliary clearance mechanisms can be visualized by a cinematographic display of sequential images of the lungs after aerosol inhalation. This procedure is called " radioaerosol inhalation lung cine-scintigraphy ". In normal subjects, the mucus flow is steady and cephalad in direction, but in pathological states four main abnormal transport patterns are seen;

(i) stasis or a transient stopping and starting of mucus globs in the airway,

(ii) regurgitation or a retrograde transport of mucous globs in a reverse direction to the original site,

(iii) straying of mucus globs to either the ipsilateral or the contralateral lungs and

(iv) zigzag or spiral path of mucus transport (**Fig. 25.10**).

In lung diseases like obstructive airways disease, bronchogenic carcinoma, or bronchiectasis, all the four above abnormal transport patterns are seen to a varying degree. In pulmonary vascular diseases, mucus clearance function is well preserved without abnormal transport patterns, unless obstructive lung disease is also present concurrently.

Studies using this technique have shown, for example, that beta 2-stimulators do not facilitate mucus transport as widely suggested by commercial advertisements. However, their pharmacologic action as bronchodilators can be definitely confirmed by radioaerosol inhalation lung imaging.

2. Pulmonary epithelial permeability

The aerosol ^{99}Tc^{m}-DTPA, a small molecular chelating substance, disappears from the lungs rapidly after inhalation. It is not yet well understood how it is cleared from the lungs, but it is now accepted that measurement of its clearance rate from the lungs can serve as a test of pulmonary epithelial permeability. Smoking and interstitial lung diseases make it easier for the inhaled aerosols to pass through the pulmonary epithelial membrane and the half time of clearance (T½) becomes shorter. **(Fig. 25.11)**. The technique provides dramatic evidence of damage caused by smoking for periods as short as few weeks, even before the subject has any symptoms. The T½ becomes shorter when patients develop interstitial pneumonitis induced by radiation therapy or drugs and lengthens when this condition improves.

^{99}Tc^{m}-HMPAO (hexamethylpropylene amine oxime) which is a lipophilic ^{99}Tc^{m}-complex (MW 380), normally used for measuring cerebral blood flow, can also be used as aerosols and its disappearance seems to be related to the degree of pulmonary epithelial damage.

Pulmonary vascular permeability instead of pulmonary epithelial permeability can be studied by measuring radioactivity in the lungs following injection of radioactive albumin solution.

3. Other Radiopharmaceuticals for lung studies

(a) ^{123}I-IMP (^{123}I-Iodoamphetamine)

The agent ^{123}I-IMP is used to study cerebral blood circulation. When it passes through the lungs following intravenous injection, a significant portion of it is retained in the lung. Whether it reacts with "amine receptors" in the lungs is not yet established but it seems to have some affinity for the endothelial cells especially when they are damaged. Clearance of radioactivity is delayed from the lung areas where pulmonary arterial perfusion is disturbed. The true significance of its lung retention is not clear but suggests that some kind of non-respiratory lung function, probably affecting the pulmonary vascular beds, is the cause. This problem needs further study, but unfortunately this radiopharmaceutical is not likely to be easily available in the developing countries.

(a) ^{67}Ga-citrate (^{67}Gallium-citrate)

This agent was first developed as a tumour imaging agent. Indications for the gallium scan for some types of tumour are clear, but for other types its use is debatable. A positive uptake is seen in a wide range of benign lung conditions such as infections, interstitial lung

diseases, sarcoidosis, pneumoconiosis, and interstitial fibrosis. In interstitial lung diseases, radioactive uptake in the lung increases initially when it is active and decreases when the disease process is slowed down or stabilized. Again, it is not clear where in the lungs this agent is picked up, and most of the diagnostic information that it provides is non-specific in nature. It is also relatively expensive and not easy to obtain in the developing countries.

FUTURE PROSPECTS

With the help of nuclear techniques not only respiratory but also certain non-respiratory functions can be investigated and functional images of the lung can be obtained from which lung function can be analyzed in quantitative and dynamic terms both globally and regionally in the lungs. Further exploration is needed to extend the coverage to the metabolic or biochemical aspects of non-respiratory functions of the lungs.

Lung studies by nuclear techniques have been mostly neglected so far in the developing countries because " total lung imaging " was not possible. The availability of radioaerosols had now provided means to do complete lung studies in these countries. IAEA's effort to make radioaerosol techniques more widely available in the Asian countries has been most noteworthy.

Pulmonary tuberculosis is still prevalent in the developing countries, scourge of smoking is becoming increasingly wide spread and atmospheric pollution is on the rise as these countries race towards industrialisation with insufficient technical and financial resources. These conditions would provide a fascinating backdrop of infective, cancerous and pollution-induced conditions of lungs where lung imaging techniques would have a large scope of providing useful service.

Present day lung imaging can show the ravages of a disease process by visualizing the state of perfusion, ventilation and certain aspects of non-respiratory functions but does not always indicate a specific disease. For example, a "coin lesion" due to either tuberculosis or lung cancer produces very similar findings. Even if a small abnormality is found on chest X-ray pictures, and even if malignancy is highly suspected on the basis of all other investigations, the definitive diagnosis is often elusive, because no pathologic material is accessible by any of the non-invasive methods. However, there is now hope for a specific diagnosis of lung cancer or malignancy in general by nuclear medicine techniques. It has become possible to produce, at least in vitro, "cancer specific" monoclonal antibodies. If a lesion could be diagnosed as malignant by nuclear imaging using radiolabelled monoclonal antibodies in vivo, the benefits would be invaluable.

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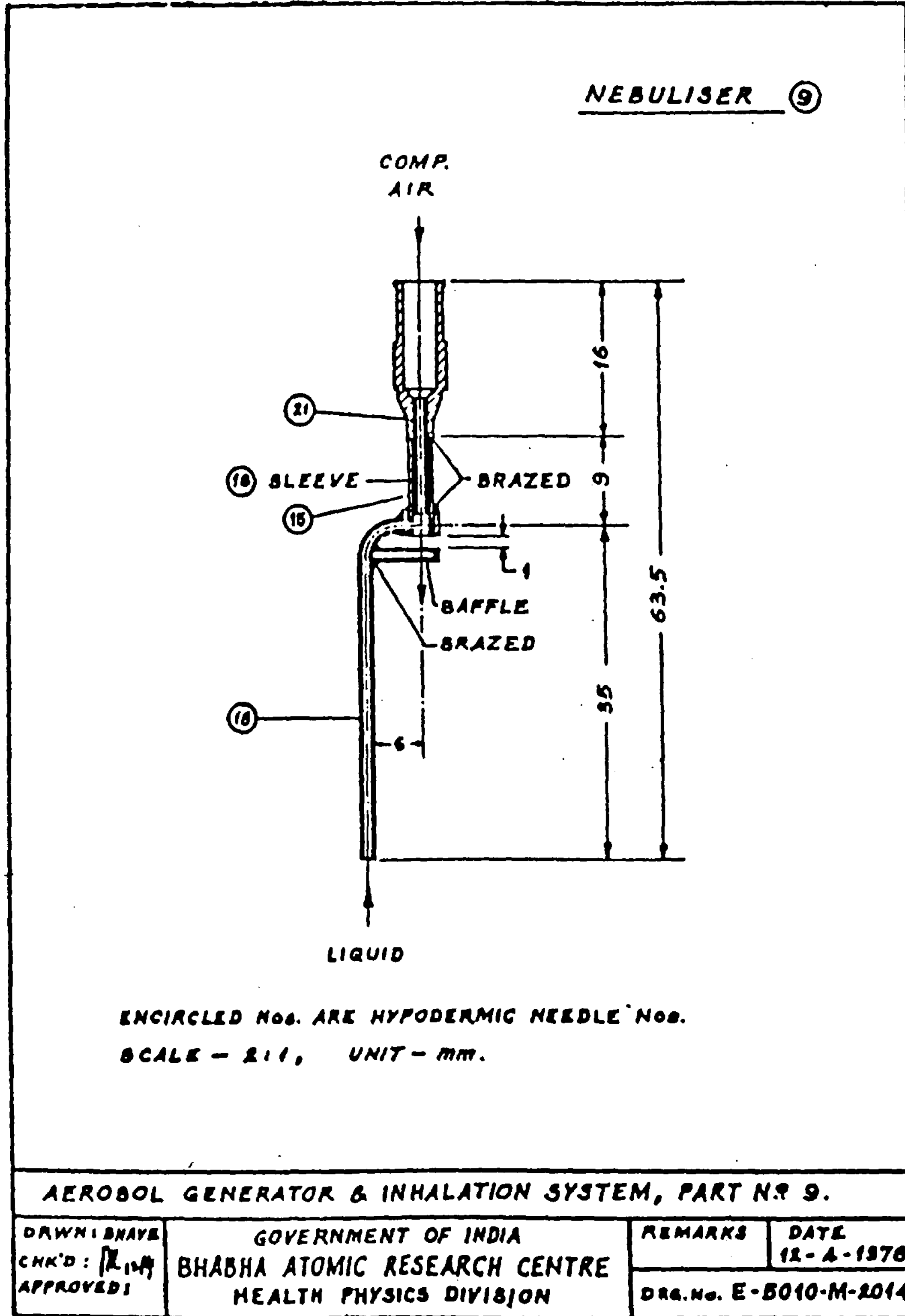
Appendix I



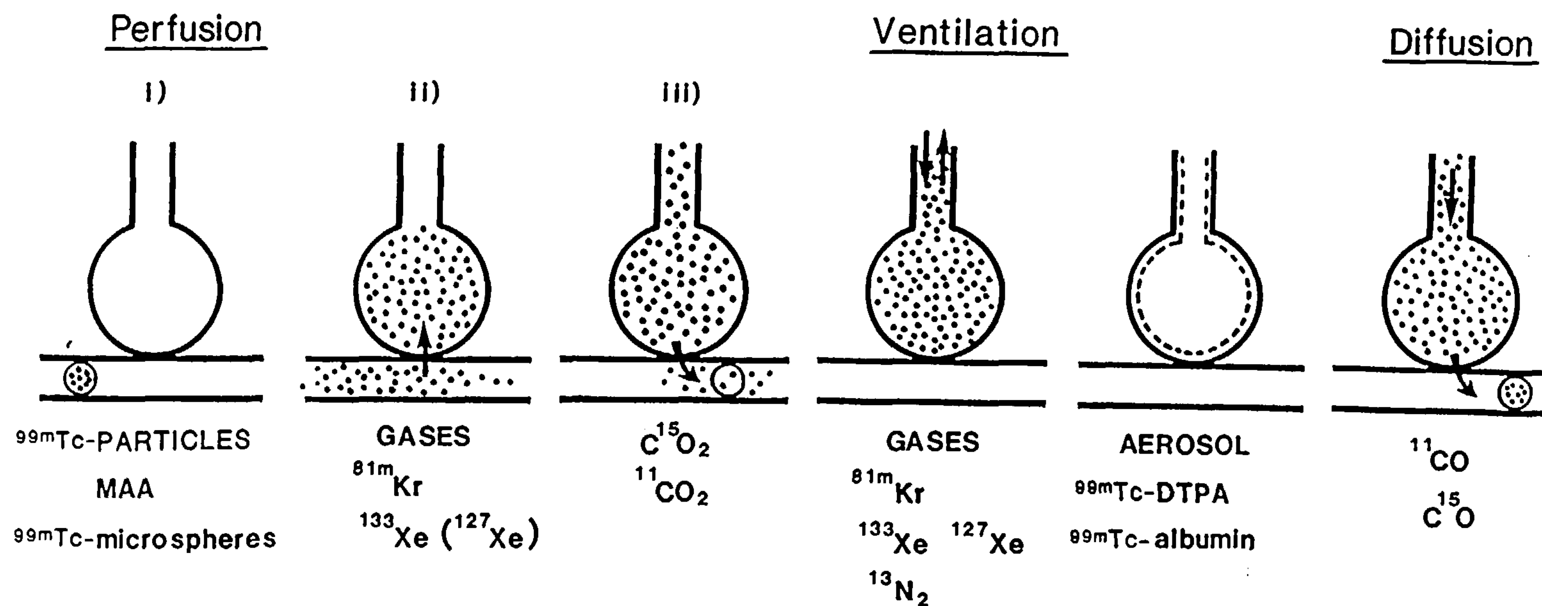


Fig. 25.1 Schematic diagrams of various respiratory functions and radionuclides used for studying them.

NORMAL SUBJECT

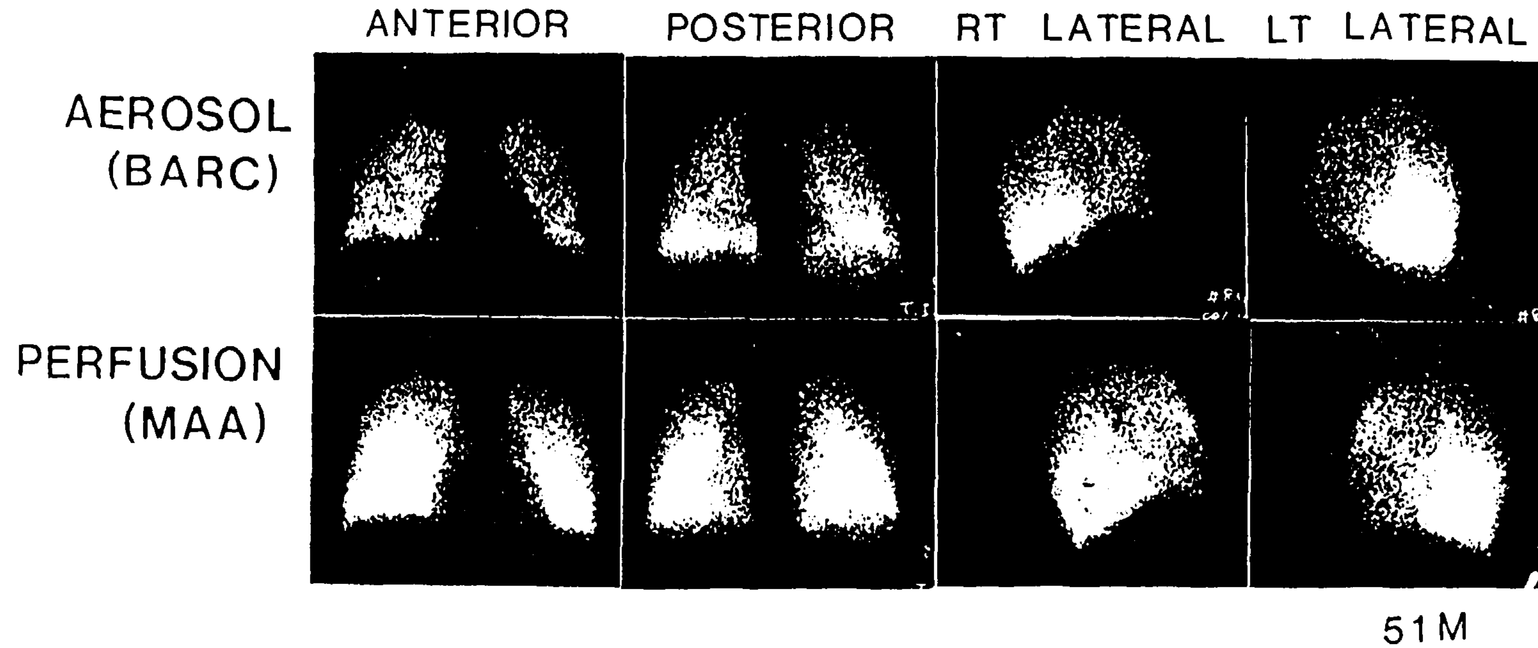


Fig. 25.2 A 51 year old normal subject. Radioaerosol inhalation (upper row) and perfusion (lower row) lung studies in anterior, posterior, and right and left lateral views. Practically no distinction can be made between aerosol inhalation and perfusion lung images in any of the four projections.

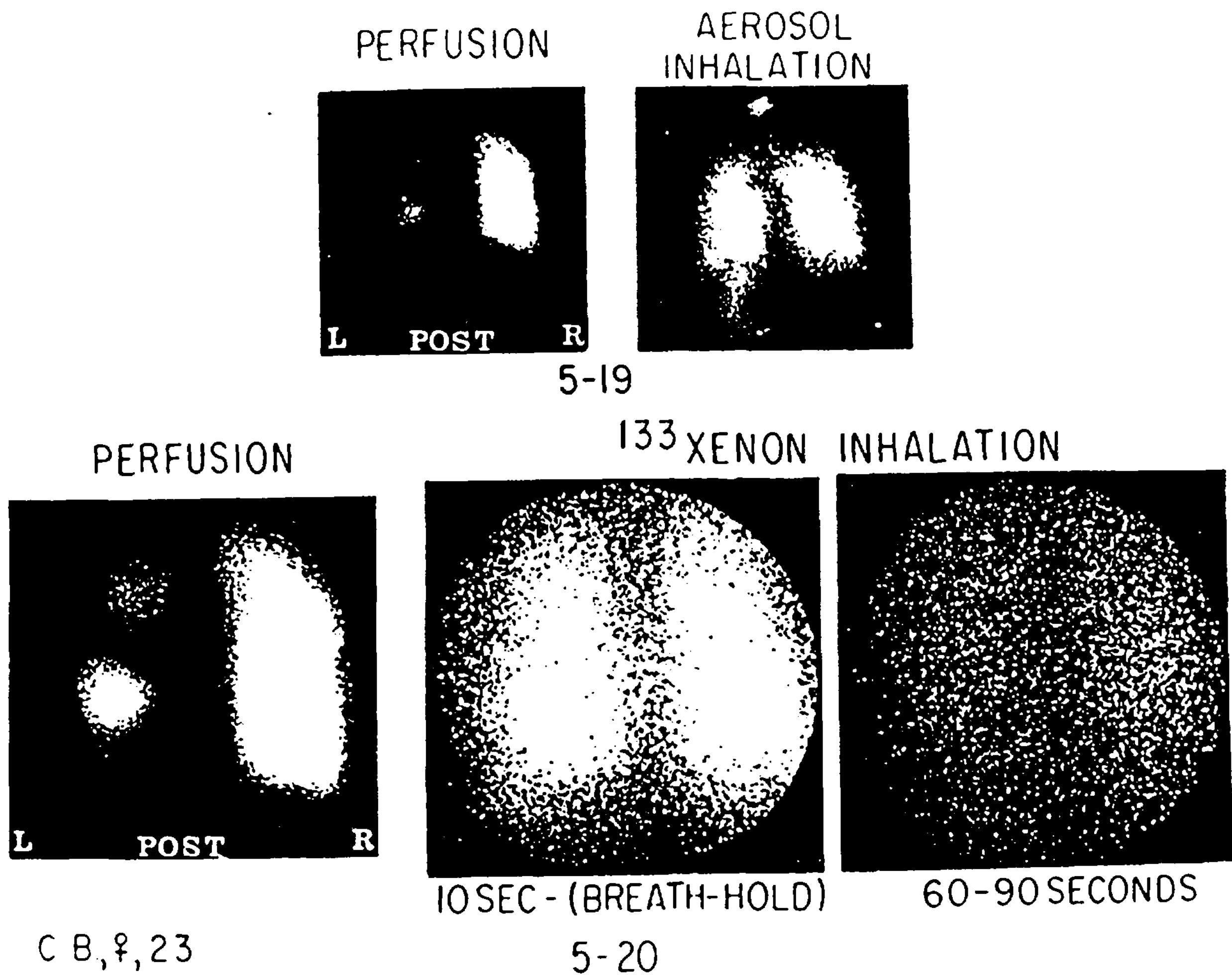


Fig. 25.3 A 23 year old woman with massive pulmonary embolism in the left lung. Posterior perfusion, and radioaerosol inhalation lung images (upper row) studied on May 19, and posterior perfusion and xenon inhalation (breath-bold) and washout images (lower row) studied on the following day (May 20). In the left lung where perfusion is greatly diminished, inhaled aerosol deposits normally. Inhaled xenon gas distributes homogeneously by single breath method. Furthermore no evidence of xenon gas retention can be detected in either lung, especially in the left lung during the washout phase, indicating the presence of normal ventilation without bronchoconstriction in the embolic left lung.

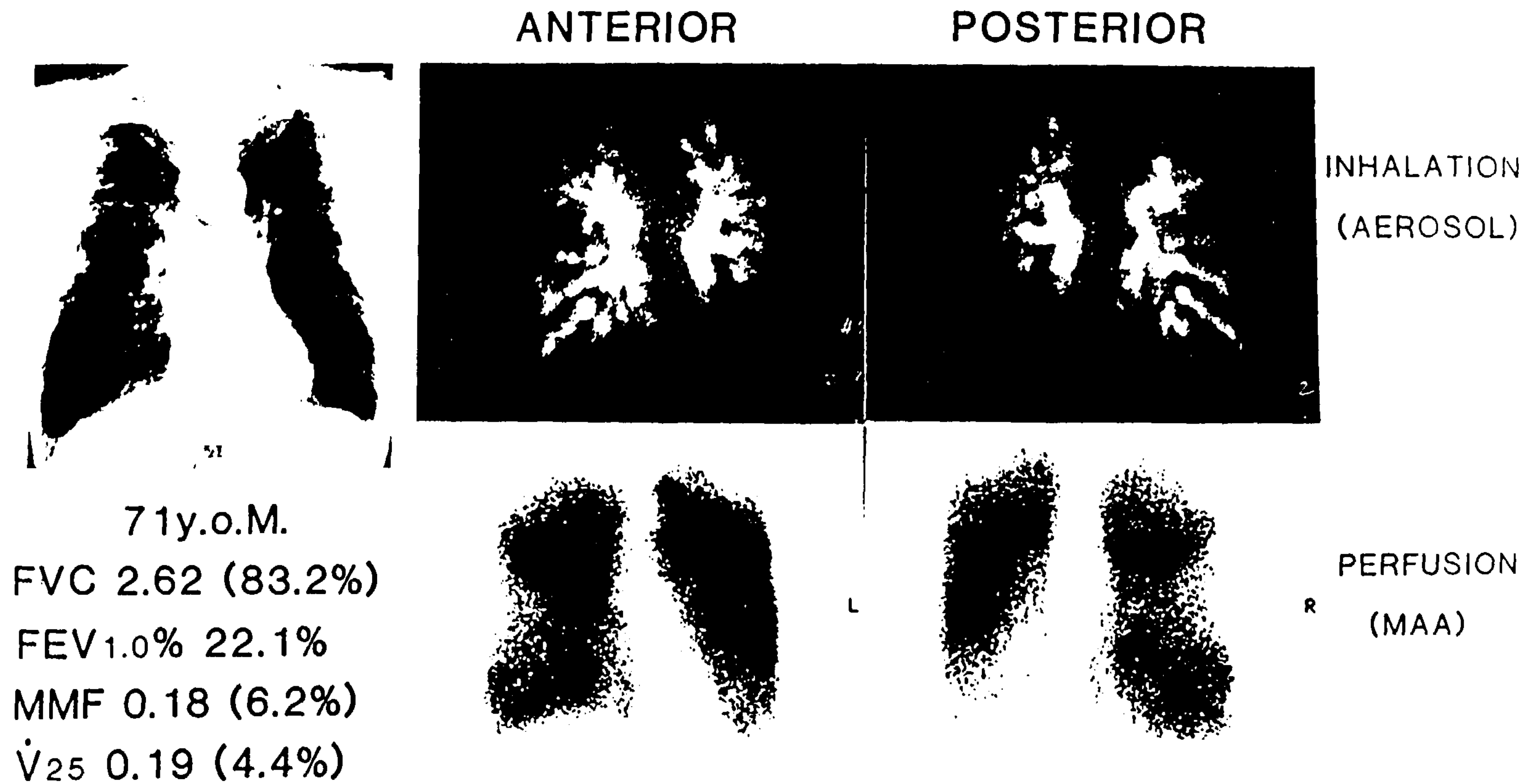


Fig. 25.4 Perfusion and radioaerosol inhalation lung images in a 71 year old patient with chronic obstructive pulmonary disease. Note inhomogeneously decreased perfusion in the right and left lungs and excessive deposition of inhaled aerosol in the large airways (central pattern) and patchy inhomogeneous deposition in the peripheral lungs, making mixed central and peripheral pattern.

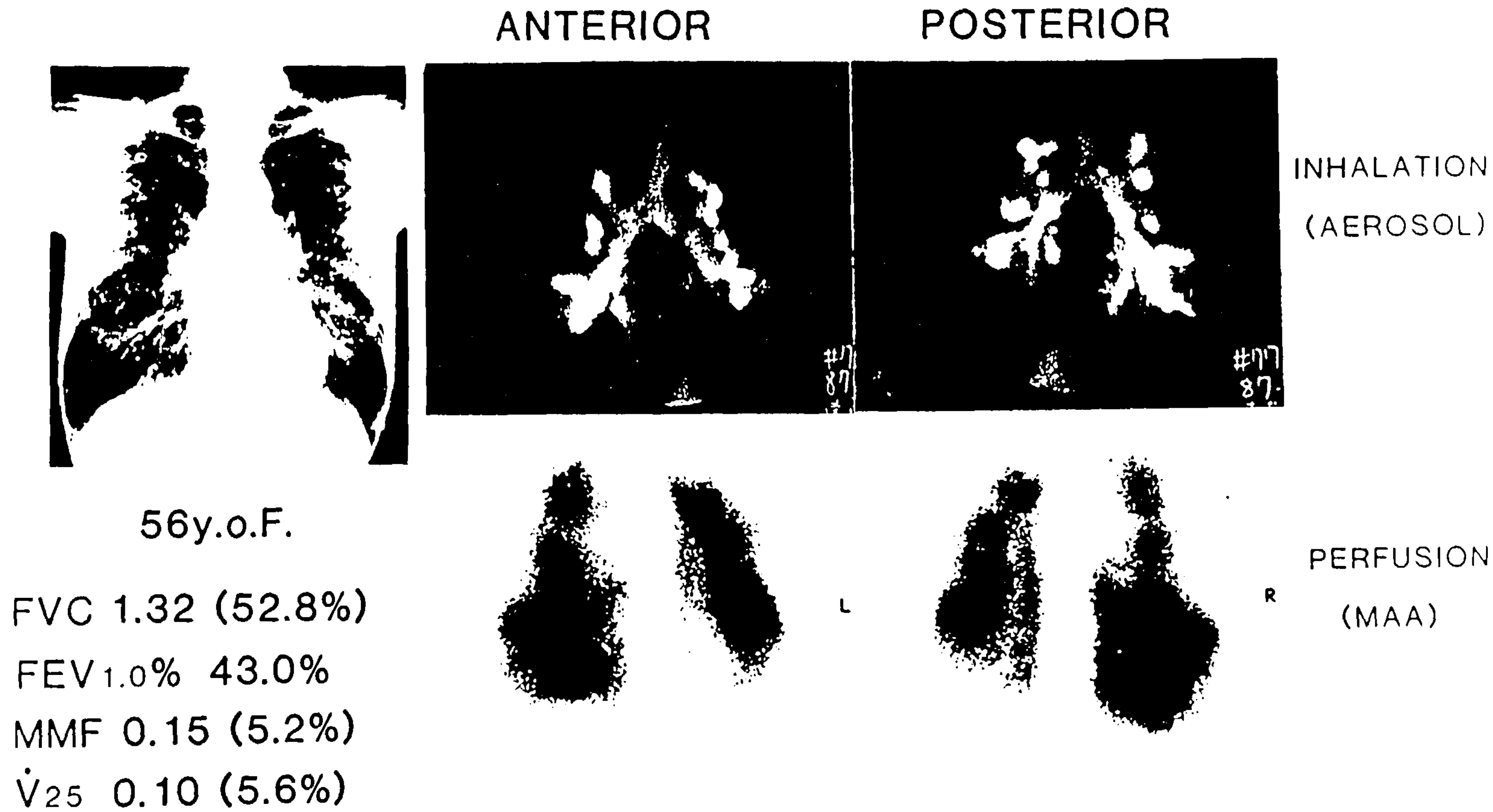


Fig. 25.5 A 56 year old female with diffuse panbronchiolitis complaining of dyspnea for a long time. Diffuse panbronchiolitis is a diffuse lung disease of the transitional zone characterized by diffuse nodular densities, hyperinflation and hyperlucency on chest X-ray film and decreased vital capacity, forced expiratory volume and hypoxemia. Aerosol inhalation lung images indicate central deposition pattern as could be seen in emphysematous patients and perfusion is greatly diminished in lung regions where aerosol deposits less.

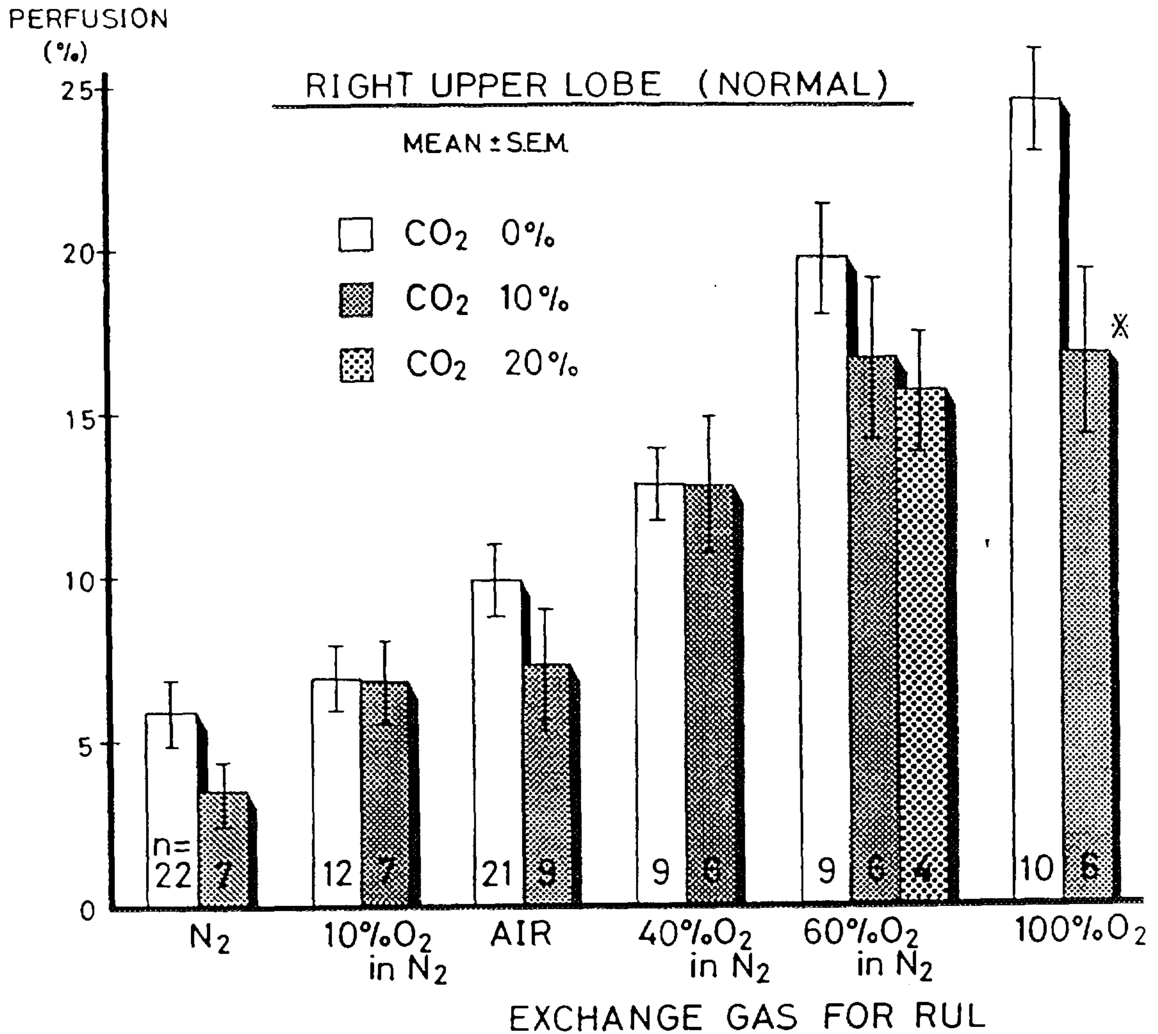


Fig. 25.6 The right upper lobe of normal dog was artificially ventilated with gases of various oxygen concentrations. The regional perfusion distribution was diminished due to regional hypoxic vasoconstriction when the lobe was ventilated with gases of less oxygen tension than that of air. The regional perfusion distribution was increased because of vascular recruitment when it was ventilated with gases of higher oxygen tension than that of air.

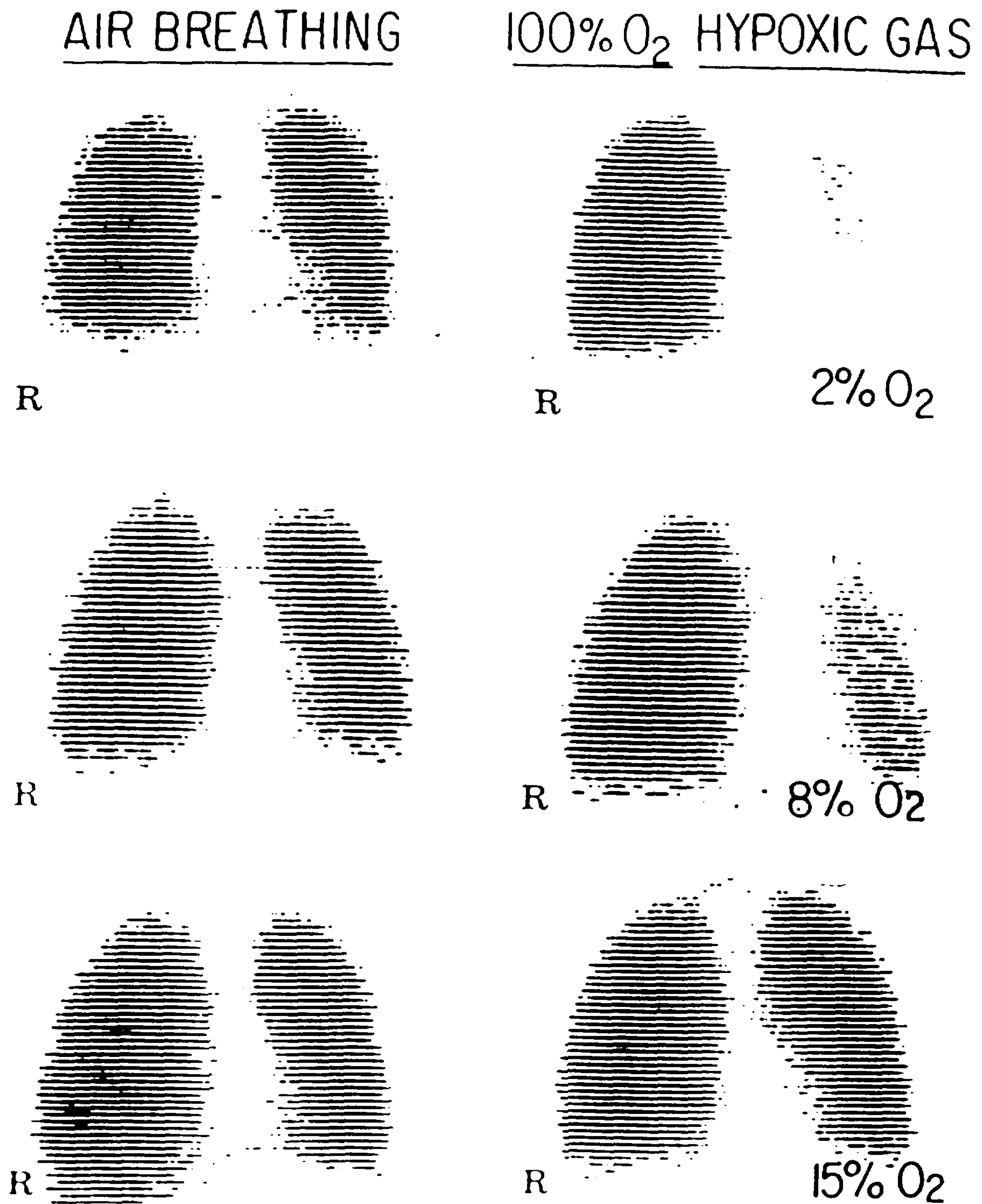


Fig. 25.7 As compared with controls on the left hand side, perfusion in the left lung became diminished when the left lung was ventilated with hypoxic gases like 2%, 8% and 15% oxygen, while the right lung was ventilated with 100% oxygen.

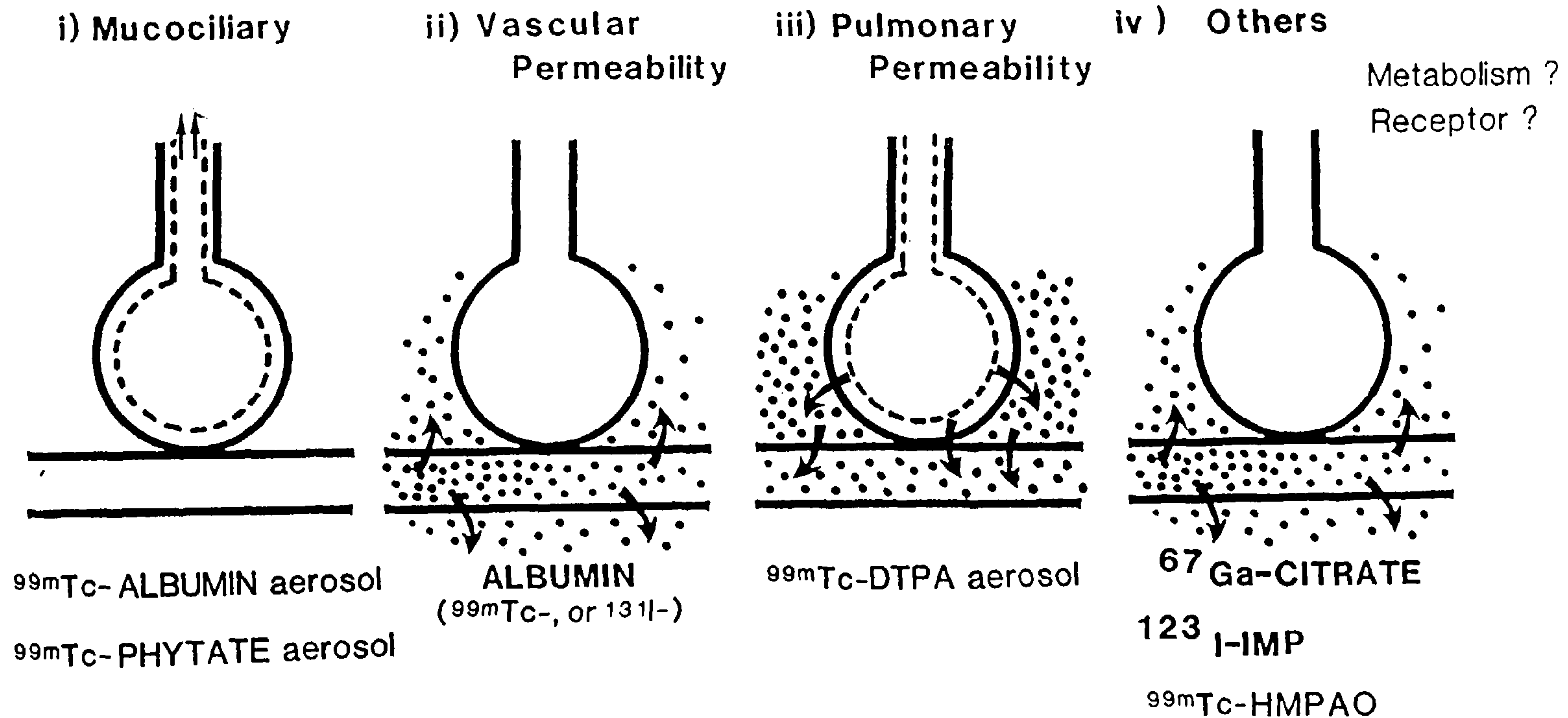


Fig. 25.8 Schematic diagrams of various kinds of nonrespiratory lung function and radionuclides to study with.

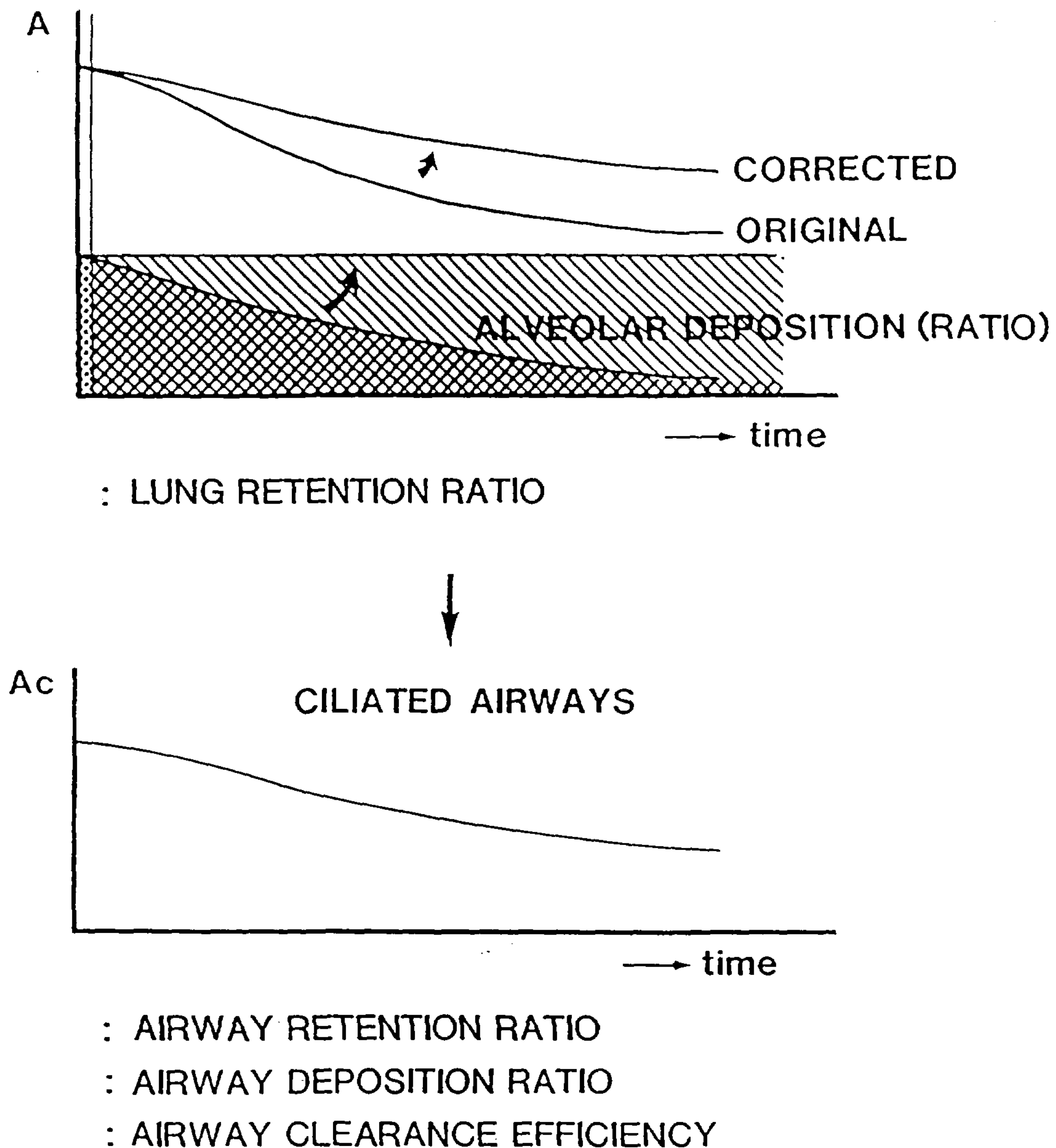


Fig. 25.9 Time activity curves constructed from sequential measurement of radioactivity. Alveolar deposition is the amount of radioactivity remaining in the lungs at 24 hours after body-background is subtracted. Lung retention ratio (LRR) is calculated from the corrected time activity curve. After the amount of alveolar deposition is subtracted, time activity curve only from the ciliated airways is constructed. Airway retention ratio (ARH), airway deposition ratio (ADR), airway clearance efficiency (ACR) are calculated.

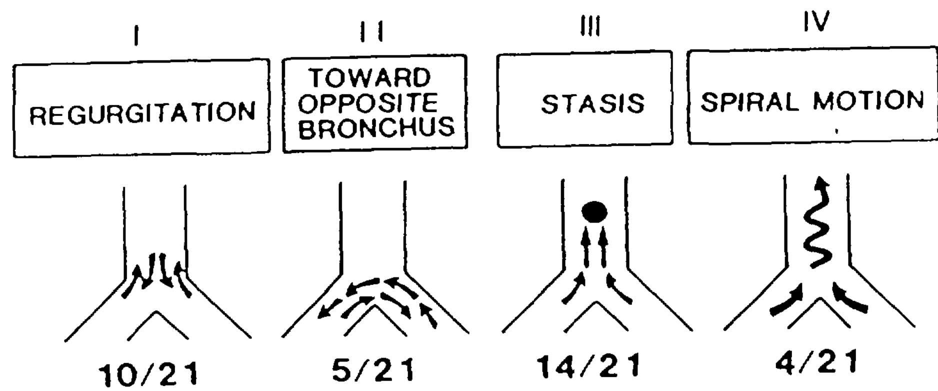


Fig. 25.10 Four abnormal mucous transport patterns on the trachea. More or less similar patterns are seen on the ciliated airways. Numbers below the diagrams indicate approximate frequency in patients with COPD (18).

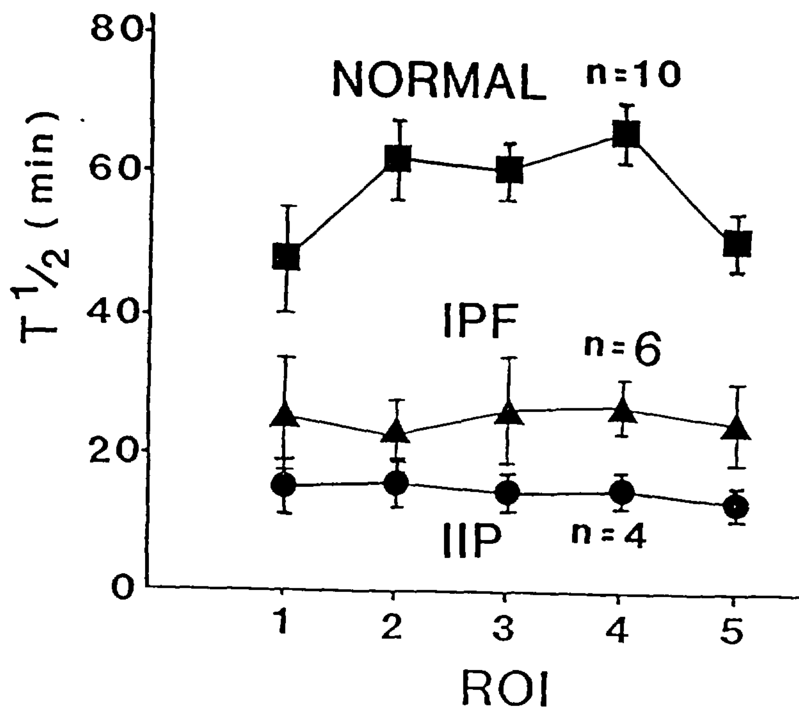


Fig. 25.11 Half clearance time (T½) (mean ± S.E.) of ten normal subjects, six patients with idiopathic pulmonary fibrosis and four patients with idiopathic interstitial pneumonitis. T½ was measured at five different regions of interest (RO1). There was a statistically significant difference between them, indicating more permeable pulmonary epithelium in interstitial lung diseases. RO1 1: the left whole lung, RO1 2: the right whole lung, RO1 3: the upper third of the right lung, RO1 4: the middle third of the right lung and ROI 5: the lower third of the right lung parts of the lungs. In any lung regions (from one to six) T½ is smaller in the latter than the former.

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Chapter 26

NUCLEAR HAEMATOLOGY

Johan S. Masjhur

Introduction

Nuclear techniques have been applied to study, diagnose and treat various haematological disorders for more than five decades. Two scientists are regarded as pioneers in this field, i.e. John Lawrence who in 1938 used ^{32}P to treat chronic myeloid leukaemia and George Hevessy who used ^{32}P labelled erythrocytes to measure blood volume in 1939. At present, many nuclear medicine procedures are available for diagnosis and therapy of a variety of haematological disorders.

Although nuclear techniques are somewhat complex, they give direct and quantitative assessment of the kinetics of blood elements as compared to other non-isotopic haematological tests.

Basically, equipment required for nuclear haematology is very simple such as well scintillation counters to measure radioactivity in blood samples. More sophisticated equipment like rectilinear scanner or gamma camera is required when imaging is necessary.

An overview of the basic principles and clinical applications of nuclear haematology is given in this chapter.

Radionuclides and blood elements labelling

Labelling of various blood elements is crucial in many of the haematological applications of nuclear techniques. Commonly used radionuclides for this purpose are ^{51}Cr, $^{99}Tc^{m}$ and ^{111}In.

Ideally, an isotopic label for use in haematology should have the following characteristics:

(a) The label should neither alter the function nor the life span of the cell.

(b) The label is not eluted or reutilized after (patho)physiological destruction of the cell.

(c) The label should preferably be a gamma emitter with appropriate energy for optimum counting and/or imaging and a half life appropriate to the parameter being studied.

There are two types of cell labelling, i.e. **random** and **cohort** labelling. Cohort labelling, sometimes known as **pulse labelling**, is performed on cell precursors. The labelled precursors will appear in the circulation as labelled young cells and will remain in circulation

throughout the life-span of the cell. This technique enables us to study the rate of cell production, its kinetics, its absolute life span, the mechanism for its destruction and its final fate in the human body. Unfortunately there is no ideal radionuclide for cohort labelling. Radioactive iron, perhaps, is the only radionuclide which has most of the above qualities, but it is currently not routinely available in the developing countries.

In random labelling, the radionuclide labels all cells of different degrees of maturity in a blood sample taken from peripheral circulation and treated with anticoagulant prior to labelling. This method is useful for mean cell survival determination and requires separation of unbound label from the labelled fraction (usually carried out by centrifugation).

Erythrocyte labelling

Erythrocyte labelling with ^{51}Cr. ^{51}Cr in the form of sodium chromate is the radionuclide most commonly used for erythrocyte labelling. Chromate ion will be transported across the erythrocyte membrane when incubated with blood previously treated with anticoagulant. Inside the erythrocyte, chromate ion (Cr +6) will be reduced into chromic ion (Cr +3) which is readily bound to the beta chain of the haemoglobin. Prior to (re)injection, the unbound chromium should either be washed or reduced into Cr +3 by the addition of ascorbic acid.

Labelling efficiency of ^{51}Cr can be as high as 90% and is not affected by washing and dialysis. Several other factors can reduce labelling efficiency i.e:

(1) prolonged incubation of ^{51}Cr with blood,

(2) presence of stannous ion (or other reducing agents),

(3) excessive calcium ion concentration,

(4) increased ACD pH (if ACD is used as an anticoagulant) and

(5) low specific activity of ^{51}Cr.

Normally, the whole blood is used for erythrocyte labelling. However, if the number of leucocytes exceeds 25 000/m^3 or the number of thrombocytes exceeds 500 000/m^3, the buffy coat/layer formed upon centrifugation must be removed prior to labelling. Otherwise leucocytes and thrombocytes will also be labelled, making it difficult to study the fate of erythrocytes alone.

^{51}Cr labelled erythrocytes can be used for measurement of erythrocyte and total blood volume, assessment of red cell survival and spleen sequestration, measurement of occult blood loss and in-vivo cross matching.

There are several constraints associated with the use of ^{51}Cr i.e:

(a) Only 10% of ^{51}Cr disintegrations are emitted as gamma ray of 320 keV. This low photon yield with high energy gamma ray is not satisfactory for scintigraphy at an acceptable radiation dose.

(b) ^{51}Cr is eluted from labelled normal erythrocytes at a daily rate of 1%.

(c) half life of 28 days is too long for short procedures such as blood volume determination.

Erythrocyte labelling with $^{99}Tc^m$. $^{99}Tc^m$ labelled erythrocytes are used for cardiovascular imaging especially the gated study, detection and localization of gastrointestinal bleeding, measurement of erythrocyte volume and spleen imaging.

Erythrocyte labelling with $^{99}Tc^m$ which is normally supplied as sodium pertechnetate (Tc 7+) can be carried out in vivo, in vitro or a combination of both. All methods of labelling require the presence of stannous (tin 2+) ion. Both ions can freely diffuse through the erythrocyte membrane, and inside the erythrocyte pertechnetate ion is reduced by the stannous ion which is readily bound to haemoglobin, possibly to the beta chain. Reduced $^{99}Tc^m$ and oxidized tin cannot pass through the membrane. To avoid binding of stannous ion with erythrocyte cells, EDTA is sometimes added for in-vitro labelling method. In the in vivo method, both stannous ion and $^{99}Tc^m$ are injected into the body and labelling takes place inside the body. In the in vitro method, venous blood is taken and labelled aseptically outside the body. The labelled erythrocyte preparation is then reinjected to the patient. In the in vitro method, the patient is first injected with stannous ion (pretinning) and afterwards a small amount of venous blood is sampled for labelling outside the body. The labelled erythrocyte will then be reinjected.

Labelling efficiency can decrease in the presence of certain drugs e.g. antimicrobial agents, anticonvulsants, antihypertensives, cardiac glycosides, tranquillizers, heparin and anti-inflammatory drugs. Diseases and treatments which cause formation of anti-erythrocyte antibodies also reduces labelling efficiency.

Erythrocyte labelling with labelled DFP. Diisopropyl fluorophosphate (DFP) can be used for erythrocyte labelling using the random or cohort technique. Radionuclides that are commonly used as label are ^{32}P, ^{3}H or ^{14}C. In the random technique, labelled DFP binds to the cell membrane. Neither elution is observed during the first 24 hours nor there is any significant damage to the cells. However, as this label is a pure beta emitter, it is impossible to carry out external detection or in vivo counting.

When used as a cohort label, labelled DFP is taken up by cells in the bone marrow. To eliminate uptake of labelled DFP by circulating cells, the binding sites in the latter should be saturated by prior injection of cold DFP.

Leucocyte labelling

Random labelling of leucocytes with ^{3}H thymidine. ^{3}H thymidine is a cohort label for leucocytes, it is taken up by marrow myeloid precursors and will appear as circulating young granulocytes two to three days afterwards. This method is useful for obtaining data on the kinetics of (patho)physiological characteristics of human granulocytes. However, as thymidine is bound to DNA, it can potentially produce genetic damage. Also, the absence of gamma emission has made localization study impossible.

Random labelling of leucocytes with $^{99}Tc^{m}$. The leucocytes can be labelled with $^{99}Tc^{m}$ sulphur colloid; around 30 to 40% of $^{99}Tc^{m}$ sulphur colloid is phagocytosed by granulocytes in vitro. The use of this method has been hampered by indications that the labelling process damages the granulocytes, and by the technical difficulties in separating the $^{99}Tc^{m}$ labelled granulocytes from unbound radiocolloid and from radiocolloid bound loosely to cell membrane (Price and McIntyre, 1984). However, some workers have claimed that they succeeded in efficiently labelling leucocytes with $^{99}Tc^{m}$ and used it for detection of inflammatory processes such as pelvic inflammatory disease, inflammatory bowel disease, abdominal abscess, wound abscess, etc. (Labelling of leucocytes with $^{99}Tc^{m}$-HMPAO has been described in Chapter 27.)

Random and cohort labelling of leucocytes with other labels. The method has been experimentally carried out to study the kinetics of granulocytes. ^{32}P DFP acts as a random label when incubated with purified granulocyte preparation in vitro. ^{51}Cr sodium chromate is also an effective label of purified granulocytes in vitro. Leucocytes labelled with ^{111}In oxine is useful for imaging of abscess and local inflammatory process.

Platelet labelling

Platelet can be labelled with ^{35}S sodium sulphate, ^{32}P DFP or ^{51}Cr sodium chromate. More recent workers have also reported the use of ^{111}In oxine (8-hydroxyquinoline).

Platelet labelling using ^{35}S sodium sulphate. When injected intravenously, a part ^{35}S sodium sulphate will enter megakaryocyte and act as a partial cohort label of platelets. Because of its beta emission and long half-life ^{35}S, gives quite high absorbed radiation dose to the patient.

Platelet labelling using ^{32}P DFP. ^{32}P DFP can also label platelets through binding with cell membrane enzymes, however its use is limited for survival study only.

Platelet labelling using ^{51}Cr sodium chromate. ^{51}Cr is widely used as a random label for platelets to assess platelet survival and platelet localization through external counting. The difficult part of this in vitro technique is that it requires isolation, labelling and reinjection of platelets without significant damage to the platelets.

Platelet labelling with ^{111}In oxine. This has been used to study platelet kinetics and organ localization in vivo.

Albumin or plasma labelling

Among the long lived radionuclides, ^{131}I and ^{125}I are the most common radioisotopes used for plasma labelling. ^{125}I labelled human serum albumin (^{125}I HSA) is the radiopharmaceutical of choice for determining plasma volume. ^{125}I HSA can be obtained as a commercial kit. ^{131}I-HSA can also be used for this purpose.

Short lived radionuclides that can be used for plasma labelling include ^{99}Tc^{m} which binds albumin. ^{113}In^{m} in the form of indium chloride in gelatin solution at acid pH binds plasma transferrin in vivo. The complex formed is very stable and used for blood pool imaging. Both radionuclides are also used for determination of plasma volume.

CLINICAL APPLICATIONS

Diagnostic studies

In vitro and non imaging in vivo techniques

Erythrokinetics

Erythrokinetic studies using radionuclides are more complex than their indirect simple counterparts, such as haematocrit determinations. However, the former has the advantage of being direct and quantitative, which is essential in certain cases such as differentiation between relative and absolute polycythemia when haematocrit is only moderately elevated.

Erythrokinetics is maintained and regulated, among other things, by bone marrow, which at steady state conditions replaces aging cells, cells lost due to bleeding or pathophysiological destruction. There are three components of erythrokinetic studies i.e. measurement of total blood volume (TBV)/ red cell volume (RCV) / plasma volume (PV), red cell production and their destruction.

Measurement of total blood volume (TBV) / red cell volume (RCV) / plasma volume (PV). Total blood volume is a heterogenous compartment comprising plasma and cellular fraction. Therefore, blood volume can be determined indirectly from plasma or erythrocyte volume provided the haematocrit value is available. However, more accurate result is obtained if total blood volume is calculated through separate measurements of plasma and erythrocyte volumes since this technique eliminates error due to possible difference in total body haematocrit compared to peripheral haematocrit normally used which can vary widely.

Plasma or erythrocyte volume or any other compartment in the human body can be measured based on dilution principle, where the volume in question is calculated from the concentration of an inert labelled tracer added in an accurately measured amount, mixed homogeneously within the compartment being measured according to the following formula:

$$V = Q/C$$

V = Volume of the compartment being measured
Q = Quantity of the tracer injected into the compartment
C = Concentration of diluted tracer after equilibrium

This formula is valid only if the following conditions are fulfilled:

(a) Tracer should be distributed homogeneously within the compartment.

(b) There should not be any loss of tracer during the process unless the loss can be measured accurately.

(c) At equilibrium, tracer distribution should not change.

(d) Tracer should not affect the compartment in any way.

(e) Tracer can be easily and accurately measured.

Measurement of plasma volume. Plasma volume (PV) is measured by injecting labelled human albumin. If the label is ^{125}I, free iodine content should be less than 2%, while the protein concentration should be around 2 gram/dl. Slow extravascular diffusion of albumin may introduce up to a 3% error if the measurement is carried out ten minutes after the injection. In certain diseases, where the rate of protein loss is great or there is increased vascular permeability, there may be rapid loss of tracer. In this case, serial measurements up to 60 minutes post-injection should be carried out, an initial concentration of diluted tracer should be found out by extrapolation of the tracer disappearance curve to zero time. Another problem that merits attention is the continuous exchange of intravascular albumin with its extravascular counterpart. Therefore, plasma volume measurement using albumin is always slightly higher compared to plasma volume measured by exclusively intravascular protein such as fibrinogen.

$^{99}Tc^{m}$ can also be used as a label, however, care should be taken to avoid formation of $^{99}Tc^{m}$ colloid or free pertechnetate. Rapid clearance of these compounds cause falsely elevated plasma, and hence, blood volume.

For red cell volume (RCV) calculation is based on the following formula:

$$RCV = TBV \times Hct \times 0.91 \times 0.98$$

Total blood volume (TBV) can be estimated from plasma volume using the following formula:

$$TBV = PV / [1 - (Hct \times 0.91 \times 0.98)]$$

Plasma volume can be calculated based on the following formula:

$$PV = \frac{\text{Total activity of injected labelled albumin}}{\text{Activity/ml of plasma sample at equilibrium}}$$

0.91 is a correction factor to take into account the difference between peripheral haematocrit(Hct) and total body haematocrit in normal cases where the number of platelets and leucocytes are also normal. This factor can vary between 0.82 to 1.0 or more, especially in splenomegaly.

0.98 is another factor to correct for plasma trapped within the erythrocyte fraction if haematocrit is determined by centrifugation. This of course does not apply if haematocrit determination is done by counting or sizing.

Determination of red cell (erythrocyte) volume. Red cell volume is best determined using autologous ^{51}Cr labelled erythrocytes, and is calculated by the following formula :

$$RCV = \frac{\text{Total activity of injected labelled erythrocytes}}{\text{Activity/ml of red blood sample at equilibrium}}$$

Total blood volume can be estimated from the following formula:

$$TBV\ (ml) = RCV/Hct \times 0.91 \times 0.98$$

while plasma volume is:

$$PV = TBV - RCV$$

Erythrocyte volume can be expressed in several ways i.e.:

(a) in terms of the total volume (ml) in comparison with the volume expected from a normal subject with the same body weight and height;

(b) in terms of volume per body surface area (ml/m2);

(c) in terms of volume per kg of body weight (ml/kg). The last expression is used most widely, however it has a disadvantage of negative bias in obese persons due to the hypovascular nature of fat tissue.

Normal Blood Volume Values (ml/kg)		
	Males	Females
TBV	70 (55-80)	65 (50-75)
RCV	30 (25-35)	25 (20-30)
PV	40 (30-45)	40 (30-45)

Determination of erythrocyte destruction

Assessment of erythrocyte destruction using isotopic methods can be carried out **directly** by random or cohort labelling, or **indirectly** by measuring the rate of erythrocyte production or haem destruction. Direct methods enable us to obtain information on the nature of shortening of red cell life span, independent of red cell age, while indirect methods give information on mean life span.

Cohort method is based on biosynthetic incorporation of label into maturing erythrocytes. Therefore, the labelled cells are approximately of the same age, comprising about 5% of total circulating erythrocytes. Labels used for this techniques include ^{14}N or ^{15}N labelled glycine, ^{55}Fe or ^{59}Fe. Disadvantages of this method are lengthy sampling period and reutilization of label causing difficulties in interpretation.

Random method. The most widely used label is ^{51}Cr. Other radionuclides which can be used are ^{32}P, ^{3}H or ^{14}C labelled DFP or ^{14}C-cyanate.

Determination of erythrocyte life span

Erythrocyte life span is measured by reinjection of ^{51}Cr labelled autologous erythrocytes. Twenty four hours post-injection and every alternate days for three weeks afterwards, about 5 ml of blood is taken from the patient. Accurate aliquots of each sample are counted at the end of the sampling period and the disappearance curve is plotted, either using a semilogarithmic paper or a computer program. From the disappearance curve, the half life of red cell survival is extrapolated. During this period of the survival study, haematocrit should not fluctuate grossly.

The blood sample to be referred as zero time (100% survival) should not be taken earlier than 24 hours post injection as within this first 24 hours, there may be up to 9% of ^{51}Cr label loss. The cause of this, whether due to early elution or red cell destruction due to labelling process, is not known.

Normal erythrocyte life span is about 120 days. This means that on an average, daily loss of cells is 0.8%. Therefore the expected half life of erythrocyte survival is about 55 to 65 days. However, the actual values observed in normal persons are shorter, ranging from 25 to 35 days. This is due to the fact that disappearance of blood radioactivity is not only due to cell death but also due to elution of ^{51}Cr from labelled cells at an average rate of 1% per day. Although this apparent shortening of the life span does not seem to reflect the true pathophysiological conditions of erythrocytes, this study still has many useful clinical applications.

Red cell survival study is meaningful only under steady state conditions, i.e. there is no loss or addition of erythrocytes due to factors other than pathophysiological death or production. Constancy of haematocrit is an indicator of steady state. Inaccuracy, for instance, will be observed, if during the study, the patient received blood transfusion or experienced bleeding. If steady state condition is difficult to attain, erythrocyte survival data can be estimated from a plot of whole blood counts without haematocrit correction.

Correction factors for ^{51}Cr elution are supplied in the publication of International Council for standardization in haematology (ICHS) to calculate the mean red cell life span, which should be 45 to 60 days. Without elution correction, one obtains a ^{51}Cr red cell half life, which is normally more than 23-24 days. Counts per minute per millilitre of red cells are plotted against time. It is determined from the curve whether the disappearance of erythrocytes is linear or exponential (use both semilogarithmic and linear graph paper). If it fits in linear graph, half life can be extrapolated by the line to zero activity, while if exponential (straight line on semilog paper), mean survival can be calculated by multiplying the half time with 1.44. If the line is not linear or exponential, use weighted mean least square computer program.

Spleen sequestration studies

This study, which is normally carried out in conjunction with erythrocyte survival study, is a surface body counting method using external probe. The probe is positioned on the precordium, liver and posterior or lateral spleen. Each position is counted every alternate days for about two weeks for two to five minutes to reach a 3% counting statistical precision.

To count the precordium, the probe is placed on the third left intercostal area at the border of the sternum with the patient in the supine position. For the liver, the probe position should be on the right ninth and tenth ribs along the midclavicular line. As for the spleen, the patient should lie prone with the detector between the left ninth and tenth rib, two thirds of the distance between the midline and the left edge of the body. In an elderly patient or patients with chronic pulmonary diseases, the lower position of diaphragm requires some adjustment of the probe position. As the positioning of the detector should be precise, the exact detector location should be accurately marked on the patient and protected with a transparent tape.

In normal subjects, the ratio of spleen to liver radioactivity is approximately 1 : 1. In patients with splenomegaly, the ratio increases up to 2 : 1 and even 4 : 1. Instead of this ratio, the spleen to liver ratio, when the blood radioactivity has reached half of its original level, should be taken into account when assessing spleen sequestration. Although spleen to precordium ratio can also be used to assess spleen sequestration function (in sequestration, the ratios are initially elevated but don't rise further with time), however, when contemplating splenectomy, spleen to liver ratio is a more accurate reflection of spleen sequestration.

Progressive accumulation of ^{51}Cr labelled red blood cells is a good guide for a decision about splenectomy. However, splenectomy may be useful despite the absence of sequestration, when there is synthesis of anti-erythrocyte antibodies by the spleen, which is not possible to demonstrate by ^{51}Cr studies.

Determination of erythrocyte production

Iron metabolism studies

Ferrokinetic studies are useful for evaluation of iron metabolism, because the technique enables us to find out about erythrocyte production as well as the relative roles of bone marrow and other extramedullary centres in total erythropoiesis.

Complete ferrokinetic study is only possible if ^{59}Fe is used. For a satisfactory evaluation of iron metabolic pathways, radioactive iron should be completely bound to circulating transferrin by prior incubation with donor plasma especially in patients with reduced iron binding capacity such as those with haemochromatosis, haemosiderosis, haemolytic anaemia, aplastic anaemia and patients with recent blood transfusions. If unbound iron is administered to these patients, the iron will not be held in the circulation. Therefore, determination of serum iron level and iron binding capacity should be determined first in any ferrokinetic study.

Ferrokinetic studies normally consist of plasma radio-iron disappearance curve (PID), red cell radio-iron incorporation and external in vivo surface counting.

Clinical applications of erythrokinetic studies

Pre-operative, intra-operative or post-operative total blood volume determination is widely applied for effective management of patients likely to have massive bleeding during surgery as in open heart surgery or major orthopaedic surgery. Total blood volume determination is also useful for management of patients after major trauma, severe congestive heart disease and patients with chronic renal disease.

Studies on erythrokinetics are useful for evaluation and management of polycythemia and anaemia. Studies on red cell production and destruction can reveal the pathogenesis of these diseases.

Thrombocyte studies

In vivo platelet kinetic study is useful in evaluating primary platelet disorders (thrombocytopenia, thrombocytosis), hypercoagulable states (TTP), and vascular disorders such as atherosclerosis in which the extent of disease may correlate with shortened platelet survival. With ^{111}In oxine labelled platelets, sites of active thrombus formation can be localized in vivo with good sensitivity.

The use of ^{51}Cr labelled erythrocytes for compatibility testing

Labelled erythrocyte can be used for compatibility testing in blood transfusion if serological evaluation shows incompatibility between donor and recipient. Such a problem arises due to the presence of cold antibody which shows in vitro activity at 30 to 37 centigrade. In addition, labelled erythrocytes can also be used to find out the presence of unexplained haemolysis after transfusion.

The procedure recommended by ICSH involves injection of 0.5 ml of donor erythrocytes labelled with 20μCi of ^{51}Cr. Prior to injection, the labelled erythrocytes should first be washed and resuspended in 10 ml of saline. Blood samples are taken 3, 10 and 60 minutes after injection from the antecubital vein opposite to the injection site of labelled erythrocytes.

If donor erythrocyte is compatible with the recipient, counting rate of blood sample 60 minutes post-injection should be around 99.5% with coefficient of variation between replicates not more than 1.1% and between duplicates not more than 0.8%. The lowest normal value of the 60 minute sample is 94.5%. According to ICSH the normal range is between 94 to 104%.

In case of emergency when a compatible donor is not available, transfusion with minimal risk can still be considered provided survival at 60 minutes is not less than 70% and plasma radioactivity at 10 and 60 minutes is not more than 3% of the total radioactivity injected. In this case, the antibody concentration is probably very low that destruction of incompatible erythrocytes is negligible. Although the 24 hour survival is high, the occurrence of haemolytic reaction is still likely.

Vitamin B_{12} absorption (Schilling test)

This is the most commonly used test to diagnose vitamin B_{12} malabsorption. The test is based on the principle that in cases of vitamin B_{12} malabsorption, little radioactivity is found in the urine after loading of the body with parenteral vitamin B_{12} (1 mg) following oral administration of 1μc radioactive vitamin B_{12} (^{57}Co); because in that condition vitamin B_{12} binding proteins are still unsaturated due to the absence of vitamin B_{12} in the serum.

Normally 24 hour urine will contain more than 10% of given radioactivity. When there is deficiency of intrinsic factor, less than 6% of radioactivity is excreted within 24 hours. In repeat study normally carried out two to five days later with the intrinsic factor given

together with the tracer, radioactive excretion will be normal. Albeit not diagnostic, a value less than 6 - 10% is suggestive of pernicious anaemia.

Since the test depends on the glomerular filtration of vitamin B_{12}, false positive result may occur if renal function is impaired. The dose of stable vitamin B_{12} (associated with the radioactive B_{12} as a carrier) should not exceed 1.0μg since at higher dose low fractional absorption may occur bypassing the intrinsic factor specific binding mechanism.

In clinical practice, Schilling test is normally carried out in three stages i.e.:

(a) In the first stage the patient is given radioactive vitamin B_{12} to determine whether absorption is normal.

(b) The second stage comprises giving radioactive vitamin B_{12} together with intrinsic factor to assess the influence of this factor on vitamin B_{12} absorption.

(c) After two months of vitamin B_{12} therapy, the third stage is carried out in the same manner as the second stage. The intestinal mucosa damaged by previous lack of vitamin B_{12} should have returned to normal by this time. If this test shows normal absorption but in vitro assay shows lack of intrinsic factor, diagnosis of pernicious anaemia can be confirmed with dysfunction of ileum secondary to lack of vitamin B_{12}.

Radioassay in haematology

Radioassay of vitamin B_{12}

Diagnosis of vitamin B_{12} deficiency can be confirmed if the serum level of vitamin B_{12} is lower than 100 pg/ml. Determination of serum vitamin B_{12} can be carried out using radioassay technique based on the competitive inhibition of labelled vitamin B_{12} binding to intrinsic factor which is added as the binder.

Reliable commercial kits for vitamin B_{12} assay are widely available. However, care should taken in choosing a kit. While some kits do measure serum cyanocobalamine (vitamin B_{12}), other kits are inappropriately labelled for vitamin B_{12} assay as they, in fact, measure total corrinoids (vitamin B_{12} plus its analogues in serum).

It should be noted that in patients with liver or myeloproliferative disorder, vitamin B_{12} binding proteins in serum are elevated. When serum vitamin B_{12} is assayed, the result is, therefore, high. However, since the binding of vitamin B_{12} is irreversible, the tissue concentration of vitamin B_{12} is actually low and there is an overall biochemical vitamin B_{12} deficiency. In this case, as well as in conditions where serum vitamin B_{12} is artificially

elevated e.g. recent injection of vitamin B_{12}, assay of red cell vitamin B_{12} is required. The technique is similar to that for the serum.

During pregnancy, serum vitamin B_{12} tends to fall while the binding capacity of its transport protein tends to rise. This phenomenon seems to be similar to that shown by serum iron.

Radioassay of intrinsic factor

In pernicious anaemia, inadequacy or absence of intrinsic factor secretion can be demonstrated by measuring the capacity of intrinsic factor in gastric juice to bind added radioactive vitamin B_{12}. Separation of the tracer bound by intrinsic factor can be carried out using charcoal adsorption technique or guinea pig intestinal mucosa homogenate. The latter, although less popular, has the advantage of also measuring the transfer of vitamin B_{12} to its specific receptor in the intestines.

Diagnosis of folate deficiency

Folate deficiency can be diagnosed through measurement of folate level in serum and red cells by radioassay techniques and performing radiofolate absorption test. For an accurate diagnosis, interpretation should be carried out after evaluating vitamin B_{12} levels.

USE OF LABELLED BLOOD ELEMENTS FOR IMAGING STUDIES

Spleen imaging

The spleen is a reticuloendothelial organ, which together with the liver, is responsible for filtration of foreign particles from the circulation. The spleen also has an immunological function. During the third until the sixth month of foetal life, spleen also serves haemopoietic function. This function reappears during severe pathological conditions such as advanced myelofibrosis or severe haemolytic anaemia.

Spleen scintigraphy is performed 15-20 minutes after intravenous injection of 3-5 mCi of $^{99}Tc^{m}$-labelled sulphur microcolloid. Spleen scintigram can also be obtained using damaged red cells, either by heat or excessive tinning, labelled with $^{99}Tc^{m}$ or ^{51}Cr.

Indications for spleen scintigraphy include assessment of spleen size, evaluation of abdominal mass in the upper left quadrant, assessment of focal defect, accessory spleen and functional asplenism.

Bone marrow scintigraphy

Locations of erythropoiesis and iron storage can be depicted by imaging with ^{52}Fe and ^{59}Fe. However, constraints associated with the use of both radioactive irons have made $^{99}Tc^{m}$

sulphur colloid more popular. $^{99}Tc^{m}$ antimony sulphide colloid, with a particle diameter of 1 to 13 nm or 5 to 12 nm has been shown to have higher marrow uptake than $^{99}Tc^{m}$ sulphur colloid by a factor of two to three within one hour post-injection, with correspondingly less hepatic uptake.

The variables which may influence the degree of colloid uptake by marrow include type of the colloid, function of the rest of RES (largely spleen and liver), colloid charge, particle number, the presence of stabilizers, surface-acting agents, plasma opsonin, the chemical nature of the colloid surface, concomitant liver and/or spleen disease (especially as the disorder relates to impaired blood flow), altered marrow distribution and blood flow, and increased phagocytic efficiency by marrow.

At birth, bone marrow is present within all bones, and replacement with fat tissue starts at the age of seven to eight years at the distal extremities. After this age it is abnormal to find bone marrow beyond the lower two thirds of the diaphysis of femur.

In normal adults, erythropoiesis occurs in the whole middle part of the skeleton (ribs, vertebra, sternum, pelvis, scapula and clavicula) and the caudal part of the sacrum. The presence of radioactive iron within the spleen during the initial spleen imaging shows extramedullary erythropoiesis in the organ. Serial imaging can demonstrate whether the erythropoiesis is productive or not, as well as spleen erythrocyte sequestration. Abnormal pattern of bone marrow due to myelofibrosis, radiotherapy or aplastic anaemia can also be seen from the spleen image.

Absence of colloid uptake indicates defective marrow stroma or microenvironment. An appropriate response to erythroid aplasia can be seen by expansion of RE marrow into the distal extremities. Fibrosis of axial marrow and extramedullary haematopoiesis especially in the spleen and liver, can be due to "spent" polycythemia vera and agnogenic myeloid metaplasia. Bone marrow scan may be used for localization of active marrow for aspiration and biopsy, and to determine how much marrow remains in the body if splenectomy is being considered. Other clinical application of marrow scan is in differential diagnosis of pancytopenia; damage from radiation and/or chemotherapy can also be differentiated from hypersplenism with active marrow compensation.

In aplastic anaemia, no colloid uptake by bone marrow is observed. Expansion of reticuloendothelial cells to the distal extremities indicates adequate response to erythroid aplasia.

In "spent" polycythemia vera and agnogenic myeloid metaplasia, axial marrow fibrosis and extramedullary haematopoiesis may occur especially in the liver and spleen. Splenomegaly can cause leucocyte, LYe and erythrocyte sequestration, or pain due to infarct or of its large size. To estimate the amount of marrow remaining in the body after splenectomy, clinicians can find out locations of active bone marrow for biopsy and aspiration from marrow imaging.

Thrombus localization using ^{111}In-oxine labelled thrombocytes

Scintigraphy of thrombi deposits with ^{111}In-oxine labelled thrombocytes is useful for localization of active thrombi which is clinically unidentified.

Scintigraphy is performed 24, 48 and up to 96 hours after an intravenous injection of 300μCi of labelled thrombocytes. Most active thrombi can be localized within 24 hours post-injection, however more sensitive results are obtained after 24 hours, especially in areas where blood pool activity decreases and target to background ratio improves such as within the heart. The most optimal result is obtained by combination of 173 and 247 keV peaks using medium energy collimator for five to ten minutes.

Positive results have been found for thrombi localized in left ventricle, coronary arteries, abdominal aortic aneurysm, prosthetic arterial grafts and rejection of renal transplants.

Labelled leucocytes for localization of infection

Studies using labelled granulocytes can be helpful in determining the localization of infection. Labelling can be carried out using ^{32}P-DFP, ^{51}Cr, ^{99}Tc^{m}, ^{67}Ga or ^{111}In-oxine. Labelled leukocyte scans assist in proving the diagnosis or excluding a diagnosis of abscess or inflammatory condition infiltrated with polymorphonuclear leucocytes. This subject is dealt with in another chapter at great length.

THE USE OF RADIONUCLIDES FOR TREATMENT OF HAEMATOLOGICAL DISORDERS

Treatment of polycythemia vera

Polycythaemia vera is a chronic proliferative disease of erythroid precursors in bone marrow which persistently causes increasing red cell numbers, haemoglobin and haematocrit. Erythrocytosis is commonly accompanied by leucocytosis, thrombocytosis and splenomegaly. Without adequate treatment, haematocrit can increase up to 70% or more. Blood viscosity becomes very high causing vascular catastrophes such as myocardial infarction, stroke, arterial and venous thrombosis or life threatening bleeding. This disease can develop into myelofibrosis or acute leukaemia, however both conditions can also appear as a result of treatment. Treatments include phlebotomy, ^{32}P administration or chemotherapy. ^{32}P or chemotherapy can increase the median life span by 11 to 15 years, while phlebotomy by only 7 to 9 years. The incidence of leukaemia after phlebotomy is only around 1%, while after ^{32}P or chemotherapy it increases up to 10 to 20%.

^{32}P in the form of sodium phosphate is usually administered intravenously with an initial dose of 2.3 mCi (85 MBq) per square meter of body surface. The upper limit of the starting dose is 5 mCi (185 MBq). Otherwise, a standard dose of 3 mCi is also acceptable. Blood

counts are performed every three to four weeks, and if after 12 weeks there is no definite response, more ^{32}P is given with a dose increase of 25% to 30%. This formula is repeated every 12 weeks with a maximum dose of 7 mCi (260 MBq) until an adequate response is obtained. If necessary, phlebotomy can be performed to control haematocrit. A haematologic response is inadequate if haematocrit increase reaches above 47% during a 12 week follow-up, or if leucocyte or platelet count decrease is less than 25%.

If remission occurs, blood counts are performed every eight weeks to detect relapse. Remission can last from several months up to several years. If no response is obtained after 9-12 months of ^{32}P therapy, treatment strategy should be changed to phlebotomy or phlebotomy and chemotherapy (using busulfan or hydroxyurea).

Treatment of essential thrombocythaemia (idiopathic or primary thrombocytopenia, haemorrhagic thrombocythaemia, megakaryotic leukaemia)

Essential thrombocythaemia is an idiopathic myeloproliferative disorder characterized by increase in thrombocytes in the circulation. In addition to chemotherapy (with busulfan or melphalan), this disease can also be treated with ^{32}P.

An intravenous injection of ^{32}P at a dose of 2.9 mCi (110 MBq) per square meter of body surface is given. The initial dose should not exceed 5 mCi (185 MBq). LYe count is done every four to six weeks, and repeat doses are given with a three month interval until an adequate response is achieved, i.e. thrombocytes less than 450 000/mm3. On repeat administration, the ^{32}P dose should be increased by 25% up to a maximum dose of 7 mCi. If after six months or the third dose of ^{32}P no response is obtained, treatment should be changed to chemotherapy.

With the above protocol, complete remission is achieved in 63% of patients and partial remission in 37% at the end of the first treatment year.

Appendices I to VII from Price and McIntyre, 1984
Appendix VIII from Van Nostrand and Silberstein, 1985

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CHAPTER 26

Appendix I

Red cell labelling procedure with ^{51}Cr (ICSH, 1980)

1. Ten ml patient's blood is collected in a sterile tube or plastic labelling bag that contains 1.5 ml of ACD (NIH A) solution. The blood is centrifuged, the plasma is withdrawn, and 0.5μCi/kg (--20 kBq/kg) of high specific activity ^{51}Cr sodium chromate is added.

2. Incubate the blood at room temperature for 15 minutes with occasional gentle mixing.

3. Wash the cells twice with isotonic saline to remove unbound ^{51}Cr.

4. After the second centrifugation and decantation, the original volume is restored with isotonic saline.

5. A measured aliquot of the labelled RBCs can be used for red cell survival and sequestration studies.

Appendix II

In vitro $^{99}Tc^m$ red blood cell labelling

(Brookhaven National Laboratories method)

1. Draw 4 ml of the patient's whole blood into a heparinized syringe, and transfer to the kit Vacutainer with a lyophilized stannous citrate mixture containing 2 μg of tin. The blood is incubated with these reagents for five minutes.

2. Add 1 ml of 4.4% EDTA (disodium or calcium disodium salt), mix, and centrifuge the tube upside down at 1300 g for five minutes.

3. Withdraw 1.25 ml of the packed RBC's, transfer to a vial containing 1 - 3 ml $^{99}Tc^m$ pertechnetate, and incubate with gentle mixing for ten minutes.

The red cells are now ready for injection. Alternatively, they can be heated at 49°C for 15 minutes and used to scan spleen.

Appendix III

In vivo $^{99}Tc^{m}$ red blood cell labelling

1. Reconstitute commercial stannous pyrophosphate containing 2 to 4 mg of stannous ion with normal saline, and inject into the patient an aliquot containing 10 to 20μg of tin per kg body weight.

2. After 30 min, inject the required quantity of $^{99}Tc^{m}$ pertechnetate (usually 10 to 25 mCi or 370 to 925 MBq). Red blood cell labelling occurs almost immediately.

Note: It is important to inject the stannous pyrophosphate shortly after reconstitution to avoid oxidation of the tin.

Appendix IV

In vitro $^{99}Tc^m$ red blood cell labelling

1. Inject 0.5 mg stannous ion intravenously from a reconstituted commercial stannous pyrophosphate kit.

2. Insert a 19-gauge butterfly infusion set into an appropriate vein. Attach a four-way stopcock, and flush from a syringe containing ACD.

3. Approximately 20 min following injection of the tin, withdraw 3 ml of blood into a 5 ml shielded syringe containing 10 to 25 mCi (370-925 MBq) $^{99}Tc^m$ pertechnetate.

4. Flush the tubing with ACD solution. After ten minutes of incubation with gentle agitation at room temperature, the labelled red blood cells are injected through the infusion set.

Appendix V

The procedure for measuring red cell volume

1. Draw 10 ml blood under sterile conditions. Add 6 ml blood to 2 ml ACD in a sterile tube, and mix well. Anticoagulate the remaining 4 ml with EDTA, and pipette a 2 ml aliquot for background count.

2. Inject 35μCi (--1 MBq) ^{51}Cr sodium chromate into the ACD-blood tube, and incubate 45 min at room temperature with continuous gentle rotation or agitation.

3. After incubation, centrifuge at 1000 g for 10 min. Remove the supernatant with a spinal needle, and discard as radioactive waste. Usually, 85 to 90% of the tracer remains labelled to the red cells.

4. Resuspend red cells gently in sterile normal saline, almost filling the incubation tube.

5. Recentrifuge and wash one more time with normal saline, then resuspend in normal saline to approximately the original volume.

6. Measure exactly 1.0 ml of the labelled cells, dilute to 1000 ml with distilled water, then pipette two 2.0 ml aliquots for gamma counting.

7. With the patient in a basal resting state for 30 to 60 min, inject exactly 5.0 ml of labelled cells intravenously. With the patient still supine, draw 5-ml EDTA blood samples at 10 and 40 min from opposite arm. Obtain the patient's height and weight on the same day.

8. Check the injection site with a hand radiation monitor to be sure that there is no extravasated radioactivity compared with the opposite arm.

9. From each blood sample, pipette 2.0 ml for gamma counting and perform a microhaematocrit measurement on the remainder.

10. Calculate red cell volume (RCV) (ml) for each blood sample as follows:

RCV = (net cpm/ml std) x (Hctvx0.98)x1000x5/(net cpm/ml) blood sample

Then, as indicated above total blood volume (TBV) is:

TBV (ml) = RCV/Hctv x 0.91 x 0.98

And, plasma volume (PV) is:

PV = TBV - RCV

11. If the 10- and 40- min calculations are similar, utilize the 10-min RCV value and compare with standard tables for accuracy. If the 40-min RCV value is significantly larger, and if the patient does not have and aggressive haemolytic process or bleeding, which would result in significant loss of red blood cells over a 30-min period, the 40-min value is considered to be more accurate. This decision is particularly appropriate if the patient has marked splenomegaly which causes delayed equilibration.

12. When autologous red blood cells are difficult to obtain (e.g. with severe cold haemagglutinin or haemolysin), type-O Rh negative donor cells can be labelled for the study.

13. If time or facilities do not permit washing the red cells to remove the unbound ^{51}Cr add 50 mg of ascorbic acid to the labelling vial at the end of the incubation period. This procedure reduces the unbound chromate to the chromic ion and thereby prevents further labelling of erythrocytes. The whole blood is then injected. Also, a standard of the plasma from the labelled blood to be injected must be prepared, and a plasma sample must be counted from each of the patient's blood samples to correct for free circulating radioactivity. Washing the labelled cells prior to injection is the preferred method, however, and it eliminates the need for ascorbic acid.

Appendix VI

The procedure of measuring plasma volume

1. The patient must be in a basal metabolism state (recumbent) for 30 to 60 min prior to the study. Determine height and weight that day, and draw 5 ml of blood (EDTA) prior to the study for a 2.0 ml plasma background counting sample.

2. Inject intravenously 5μCi (185 kBq) ^{125}I HSA that has been carefully measured from a commercial supplier's vial.

3. Monitor the injection site with a hand radiation monitor and compare with the opposite arm for any evidence of isotope extravasation.

4. At exactly 10 min, draw 5 ml blood (EDTA) from a vein other than that injected. Centrifuge, and pipette 2.0 ml plasma for gamma counting.

5. If there is reason to suspect accelerated plasma albumin clearance, draw additional blood samples at 20, 30, and if appropriate, 60 min. In such a case, the zero-time plasma ^{125}I count is determined by extrapolation from the several timed samples plotted on semilogarithmic graph paper.

6. Pipette 5μCi ^{125}I HSA into a 1000-ml volumetric flask almost filled with normal saline. Fill to exactly 1000 ml, mix well, and immediately pipette two 2.0-ml counting standards. Since albumin can adhere to glass, siliconized glass ware is recommended, or carrier albumin or a detergent can be added to the dilution.

7. Plasma volume (PV) is calculated from the 10-min blood sample count as follows :

PV (ml) = net cpm x 1000 std / net cpm plasma sample

Appendix VII

Schilling test

1. After an overnight fast, the patient is given 0.5μCi (18.5 kBq) ^{57}Co labelled vitamin B_{12} orally in 0.5μg total vitamin B_{12}. A diluted standard is made, and two 2.0-ml samples are pipetted into counting tubes for dose standards. The patient begins two consecutive 24 hour urine collections after the oral dose.

2. One to two hours later, 1.0 mg of nonradioactive vitamin B_{12} is injected intramuscularly or subcutaneously.

3. At the end of the 48 hour urine collection, each 24 hour collection is mixed well, its total volume is measured, and two 2.0-ml aliquots are counted in a properly calibrated well counter with the two standard tubes.

4. The percentage of injected dose excreted over each 24 hour period is calculated. Normal patients excrete greater than 10%, and patients with malabsorption excrete less than 6%. Excretion of 6 to 10% is considered suspicious, but not diagnostic.

5. If the result is low or suspicious, the test is repeated a few days later, using 0.5μCi ^{57}Co labelled B_{12} precomplexed to human IF. Both oral preparations are available in standard commercial kits.

CHAPTER 26

Appendix VIII

The ^{32}P regimen for treatment of Polycythemia Vera

(Polycythemia Vera Study Group)

1. Induction is performed with 2.3 mCi/m^2 intravenously, with the initial dose not exceeding 5 mCi.

2. Twelve weeks later, if phlebotomy is again required to bring the haematocrit down to 45% and less than a 25% decrease in platelet or leukocyte count has occurred, or if the platelet count still exceeds 600,000/ul, a second ^{32}P dose is given. This dose may be increased at 12-week intervals by 25% of the initial dose, to a final limit of 7 mCi per dose. ^{32}P is no longer given when the haematocrit has stabilized without phlebotomy. Unmaintained remission may last up to 9 years, but the modal duration is about a year.

3. For relapse, i.e. with haematocrit in excess of 45%, phlebotomy is employed and ^{32}P is given at the previously effective dose.

4. Administration of ^{32}P is contraindicated if the platelet count falls below 100,000/μl or the leukocyte count is less than 3,000/μl. Phlebotomy alone is employed to treat an elevated packed cell volume under these circumstances, until these counts normalize.

5. If haematologic control has not been achieved after one year of ^{32}P treatment, another form of therapy is indicated. A review of the total dose of ^{32}P required with a variety of treatment regimens during the first 5 years of therapy found an average total of 2 mCi/m^2/year.

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Chapter 27

RADIOLABELLED BLOOD ELEMENTS TECHNIQUES AND CLINICAL APPLICATIONS

Matthew L. Thakur

" Everything looks possible for those people
Who are willing to try it, "
-Jean-Louis Etienne

Introduction

Over the past few years, in nuclear medicine, the diagnostic applications of radiolabelled blood elements in general, and of radiolabelled white blood cells in particular, have become increasingly popular. This is primarily due to the introduction of lipid soluble ^{111}In-oxine as an agent, which not only is an excellent and a reliable tracer for blood cells but also enables the investigators to study the in vivo cell kinetics and map the localization of labelled cells by external gamma scintigraphy. The tracer has the modest half life of 67 hours and decays with the emission of two gamma photons (173 and 247 kev) in high abundance. This technique has provided a powerful tool to study the in vivo cell kinetics in health and localize abnormal lesions in diseases which invoke intense focal cellular concentration.

Although ^{111}In-oxine remains the most commonly used agent for labelling blood cells, it's use in the developing countries is likely to be limited because it is produced in a medium to high energy accelerators, which are not readily available in these countries. The applications of radiolabelled blood cells are so numerous and so useful in patient care, that intense research effort is in progress to develop ^{99}Tc^{m} labelled agents. Preliminary evaluation of some of these agents, has shown some limitations in comparison to ^{111}In-oxine but this approach has provided a host of new agents for labelling blood cells.

Another limitation of all these agents, is that they require separation of blood cells in vitro following the collection of patient's blood. This inconvenience has prompted investigators to produce monoclonal antibodies specific for surface glycoproteins of blood cells which can be radiolabelled and administered intravenously (i.v.) to the patient directly. The labelled antibodies would interact with a specific cell type in vivo, thereby obviating the need for blood withdrawal and in vitro cell labelling. Many such antibodies have been already evaluated, both in animals and patients.

This field, thus far, has been the subject of hundreds of publications. It is difficult to encompass all facets of its enormous growth and discuss every aspect of the subject, within the confines of this Chapter. I have made an attempt to limit myself to the high-lights of the techniques, a brief discussion of some of the new agents, routine and novel applications of white blood cells, platelets and lymphocytes, and an outline of possible future directions. At the end of this chapter, a list of selected reading material is given which, hopefully, will provide answers to any specific queries that a reader might have.

Cell labelling techniques

The techniques can be divided into two groups; in vivo labelling and in vitro labelling.

In vitro labelling

In vitro labelling is the most commonly used approach in cell labelling. In this technique, blood is drawn from a patient in a suitable anticoagulant, cells are separated by selective centrifugation, washed free of plasma and then incubated with a radioactive agent. The general format of this procedure is illustrated in **Fig. 27.1.**

The need for labelling the blood cells in the absence of plasma, stems from the fact that most lipophilic chelates prepared for this purpose are indiscriminative in that they label all cell types rather than just the specific ones. The agents are also weak chelates and in the presence of plasma bind to transferrin or other lipoproteins. Transferrin bound radioactivity will not diffuse through the cell membrane and can not label blood cells. Washing platelets free of plasma also diminishes their aggregatability.

The early search in the development of agents better than ^{111}In - oxine was aimed at the preparation of agents that will label cells in the presence of plasma and in replacing ^{111}In with ^{99}Tc^{m}. Several such agents have been prepared. Some of them are listed in **Table 1.**

The most promising compounds from this long list are ^{111}In-oxine and ^{99}Tc^{m} HMPAO (Hexamethyl propylene amine oxime). Both are lipid soluble agents. Using ^{111}In-oxine and another lipid soluble agent ^{111}In-Mercaptopyridine-N-oxide, it has been shown that the lipid soluble complex diffuses passively across the cell membrane and ^{111}In binds to cytoplasmic components. This provides a firm intracellular label. ^{99}Tc^{m}-HMPAO, also a lipid soluble agent, is expected to behave similarly. However, presumably, ^{99}Tc^{m} gets reoxidized intracellularly and is eluted from the labelled cells. This radioactivity then distributes in the patient, in the intestinal tract, thyroid, kidneys and bladder. The intestinal activity has been observed in 27% of the patients imaged four hours after the administration of labelled cells and in 100% of those imaged at 24 hrs. One way to alleviate this problem is to image the patients earlier than four hours post-injection. This however results in high false negative images. Both early and late imaging are therefore done and diagnosis is made after comparing the images obtained at both time periods.

Labelling Procedure

Basically there are four separate procedures to label blood cells; two for labelling leucocytes (one for labelling with lipid soluble agents and the second one for labelling with phagocytic agents), one for labelling platelets and the other for labelling lymphocytes. The latter two use only lipid soluble agents. All procedures that use lipid soluble ^{111}In-oxine or ^{111}In-merc were initially studied in my laboratory. A brief outline of the basic labelling procedures followed in our laboratory is described below.

Labelling Granulocytes

a) With lipophilic agents

The term granulocytes refers to a mixed population of all white blood cells (excluding platelets) which are referred to in the literature as white blood cells (WBC) or leucocytes. For separating leucocytes from the whole blood, many variations of the basic technique are reported from laboratory to laboratory. The most common method **(Fig. 27.2)** consists of drawing with 19 G needle, 30 ml venous blood in a sterile disposable syringe containing 200 units of heparin. The syringe is then hung upright in a sterile area, usually a laminar flow hood, and red blood cells are allowed to sediment spontaneously. Sedimenting agents such as hydroxyethyl starch or methyl cellulose have been used but only rarely do we find the use of such agents necessary. Leucocyte rich plasma is then carefully expelled in two round bottom sterile test tubes and centrifuged at 450 g x 5 minutes, using a horizontal swing rotor. The leucocyte poor plasma is separated and stored. The cell button which usually appears red due to contaminating red cells is suspended in 5 ml 0.9% NaCl or sterile isotonic phosphate buffer pH 7.0. The radioactivity is then added to it drop by drop and the mixture incubated for 30 minutes at room temperature. The cells are then centrifuged again, washed once with plasma, and resuspended in plasma for reinjection. The yield with ^{111}In-oxine is generally greater than 80% but that with ^{99}Tc^{m} HMPAO is variable. Most investigators use 400-500 uCi ^{111}In but 5-15 mCi ^{99}Tc^{m}. In order to obtain high labelling efficiency with ^{99}Tc^{m} HMPAO some investigators use 80 ml blood. After administration of the labelled cells, the liver and spleen receive the highest radiation dose because up to 60 % of the administered radioactivity is promptly deposited in these two organs.

b) Labelling by phagocytosis

In this type of labelling, polymorphonuclear leucocytes are allowed to engulf radioactive particles, 1.5 - 2.0 u in diameter. Two agents, ^{99}Tc^{m} albumin colloid and ^{99}Tc^{m} stannous fluoride colloid have been explored most. White cells are separated as described previously and are incubated in plasma with 10-15 mCi of ^{99}Tc^{m} colloid at 37°C for 15 min. Monocytes will also get labelled. Some particles will adhere to the cell surface and will come down with the cells during centrifugation carried out to eliminate non-bound radioactivity. As much as 40% of the activity can be surface bound, which in vivo, might separate and subsequently accumulate in the liver and spleen. Up to 70% of the injected activity has been found in the liver soon after injection. Following phagocytosis, the cells are carrying radioactive particles but may have diminished functional ability.

This labelling technique uses less expensive and commonly available ^{99}Tc^{m} colloid. It can be easily set up in a developing country. It has been tried clinically in many patients, with acute appendicitis. The reported results have been remarkably acceptable with 89 % sensitivity, 92 % specificity and 92% accuracy. Experience with this agent is limited in patients with osteomyelitis and other soft tissue abscesses.

Labelling Platelets

Subtle differences also exist in various techniques for platelet separation and labelling procedures. Most commonly however, (Fig. 27.3) 34 ml venous blood is drawn in a sterile syringe containing 6 ml anticoagulant, (Travenols or equivalent). Usually the blood to anticoagulant ratio of 6:1 is maintained and no smaller than a 19 G needle is used. Smaller needle may damage platelets and induce haemolysis. In both events ADP is released which promotes platelet aggregation.

The blood is then divided in two equal volumes in two sterile conical test tubes and centrifuged at 180 g x 15 min., again in a horizontal swing rotor. Platelet-rich plasma is separated and platelets are harvested by further centrifugation at 2000 g x 10 min. Platelet poor plasma is then separated without disturbing the button. Platelets are then suspended in a salt balance media. Several media such as Tyrodes solution, modified Tyrodes solution, and perhaps more easily available I:I::ACD(A): plasma (10%) have been employed. Two important points must be kept in mind. One is to avoid plain isotonic or phosphate buffered saline as a medium because platelets suspended in these solutions have reduced aggregability (Fig. 27.4). Secondly one must make sure that the pH of the medium is between 6.2 to 6.6, preferably 6.4. pH below that range will alter platelet viability and above that will tend to clump the platelets making it difficult to suspend them uniformly. As in the leucocyte labelling procedure, platelets are washed once, free of plasma, resuspended and labelled using a ^{111}In or ^{99}Tc^{m} lipophilic agent. Most investigators resuspend the labelled platelets in ACD: plasma for injection. Because of a non-physiologic pH and the presence of ACD, platelet aggregation studies can not be carried out on platelets suspended in this media. Post-labelling aggregation studies are somewhat complicated for routine use and most of the investigators prefer to rely on careful adherence to the preparation procedure.

Although platelets labelled with ^{99}Tc^{m} have been used for imaging studies, their use for kinetic studies suffers from the short half-life of ^{99}Tc^{m} as well as its spontaneous elution from the labelled platelets.

Labelling Lymphocytes

The procedure for labelling lymphocytes differs from that of labelling the mixed population of white blood cells in two ways. First, in the cell separation and second, in the radioactivity concentration. Because of the susceptibility of these mononucleated cells to the radiation toxicity induced by the Auger electrons from the decay of ^{111}In, it is recommended that 10^8 lymphocytes be labelled with no more than 20 μCi ^{111}In. If a whole body scan of the patient is required, a minimum of 150 μCi ^{111}In is needed. This would require close to 10^9 isolated lymphocytes. These have been obtained by using a cell separator connected to the patient's arm. The contaminating platelets are then eliminated by passing the collected buffy coat through a sterile glass bead column and the lymphocytes are then purified over the Ficoll Hypaque gradient. Lymphocytes are then harvested, washed with PBS, suspended in PBS and labelled with ^{111}In-oxine to yield approximately 20 μCi/10^8 cells.

This complexity has limited to some extent the clinical applications of labelled lymphocytes. In a routine leucocyte preparation, the contaminating lymphocytes also get labelled with much higher quantity of radioactivity than 20 μCi/10^8 cells. It is estimated that each cell receives more than 17 000 rads. With such a high radiation dose, lymphocytes do not survive but the neutrophil function is not affected.

Due to the spontaneous elution of Tc, studies with Tc labelled lymphocytes are not common.

Quality Control

The quality control tests for labelled cells should consist of a sterility test and cell function tests. The sterility tests on blood samples obtained from patients with bacterial infection may not be meaningful. However, all patients; e.g. patients with vascular thrombosis may not fall into this category. One must make sure that the labelling procedure is carried out in a sterile area using sterile containers. A certified laminar flow hood is the equipment of choice. However, a home-made plexiglass box equipped with a U.V. lamp for sterilization has been successfully used. Alternatively working carefully around a Bunsen burner may be acceptable. The rapid Limulus sterility test can not be easily carried out on blood samples and one must rely upon the bacteriological tests. However, such tests do not provide results until long after the preparations have been injected and serve only as a guideline, warning caution against any reagents or procedural failures.

Chemotaxis is one of the most acceptable tests for the assessment of PMN function; however this too is not a quick test that can be done before the preparation is injected. The aggregability test for platelets is rapid, reliable and quantitative. Most investigators believe that it is a worthwhile platelet function test. Many researchers, however, argue that the results of this test do not necessarily correlate with in vivo platelet survival or with imaging results achieved.

In vitro labelling: potential risks

The blood cell labelling techniques are increasingly used clinically in the developed countries because of the diagnostically helpful information that they provide. There is an inherent danger of handling blood from patients with positive HIV, hepatitis or bacterial infection. This poses a constant risk to individuals handling the blood. The possibility of such a danger is much more in a developing country where adequate testing facilities are unavailable and where the incidence of all these infections in the population is expected to be quite high. The risk could be eliminated only if agents given to the patients would label the desired type of blood cells selectively in vivo.

A number of such agents have been recently developed, many with a commercial potential. Such agents may or may not be available at present in the developing countries. However, this chapter would not be complete without their brief description, a partial list, their potential virtues and possible limitations in routine clinical practice.

In vivo cell labelling

These agents can be broadly classified in two groups; specific and non-specific. They are administered directly to a patient or imaging abnormal lesions and thereby eliminate the need for drawing patient's blood and labelling the blood components in vitro. The most common specific agents, in that they are specific in their interaction with only a certain type of blood component, are monoclonal antibodies tailored to interact with certain antigenic sites available on blood cells or other blood components. In the use of such specific agents one relies upon the high affinity constant between the antibody and the antigen. It is assumed that the blood components associated with the radioactive antibody molecules then carry the radioactivity to the lesion under examination.

Non-specific agents

The non-specific agents, in principle, do not rely upon blood component carrier mechanism. Perhaps one of the most important non-specific agent given to patients directly is ^{111}In or ^{99}Tc^{m} labelled human polyclonal immunoglobulin (HIgG) to image inflammatory foci. Excellent results with >90% sensitivity and specificity have been reported. Among the currently explored directly administered agents for imaging abscesses or inflammation, ^{99}Tc^{m} HIgG has drawn the most attention. ^{99}Tc^{m} is not only less expensive and easier to obtain than ^{111}In but it also delivers less radiation dose to the subject. With ^{99}Tc^{m} HIgG, the imaging can be done six to eight hourss post-administration, compared to that of 24 hr or longer with In-111 HIgG, The recently reported results from a Singapore group, for example, are highly encouraging and set an example for its use in other developing countries.

The HIgG is labelled with ^{99}Tc^{m} by the cyclic anhydride DTPA bifunctional chelating agent technique. Serum albumin as a stabilizing carrier and stannous chloride as a reducing agent for ^{99}Tc^{m} are added to the HIgG. The recent techniques of labelling antibodies with ^{99}Tc^{m} by the direct antibody sulphhydryl group methods are perhaps simpler, and easier to use. We, in our laboratory, routinely label HIgG with ^{99}Tc^{m} by reducing a small number of disulphide groups to sulphhydryls using ascorbic acid as a reducing agent. We believe that it has several advantages over the other methods. Reference to this could be found in the suggested list of reading at the end of this chapter.

In addition to the nonspecificity of this agent, there are several other disadvantages that should be noted. Perhaps one of the primary mechanism by which this agent accumulates in the inflammatory lesions is the infiltration of radioactivity into the interstitial space due to increased capillary permeability. If this is the mechanism then this agent may also be taken up in certain tumours, thereby, like ^{67}Ga citrate, raising diagnostic ambiguities about the lesions in which they localize.

HIgG also has a long half clearance time giving very high blood activity, as evident in the images given in the Singapore group publication. This blood pool activity contributes to the delayed imaging times as late as 24 hr post-injection. Using $^{99}Tc^{m}$ labelled human neutrophil specific antibody, we have obtained positive abscess images within three hours of administration in all five patients studied (**Fig. 27.5**). In all $^{99}Tc^{m}$ labelled proteins, some spontaneous elution of the tracer is inevitable. The radioactivity then goes to the kidneys, bladder, thyroid and the intestines. This distribution of radioactivity makes the interpretation of scans difficult. The ability of an agent to image lesions successfully at an early time after injection is important as well as convenient. Modern preparations of HIgG are very safe and have a proven track-record of being used in massive doses as an anti-infective agent in patients with burns and immunodeficiency.

Specific agents

During the past four years or so, several agents specific for a variety of blood components have been developed. Monoclonal antibodies specific for neutrophils, platelets, lymphocytes, or fibrin have been radiolabelled and infused directly into the blood stream. These antibodies, or their fragments, have been labelled with 111-In, 123-I or $^{99}Tc^{m}$. The antibodies are not only designed to interact with specific cellular blood elements but also with other blood components such as fibrin and fibrin fragment B. The antibody molecules in the blood stream are then expected to interact with the cell surface glycoprotein without altering the cell's physiologic properties. The scintigraphic imaging is done later at a suitable time. Many of them have been evaluated in patients for imaging infection, inflammatory process, vascular thrombi and for the determination of the rejection episode of transplanted organs. This approach and its early success has added new momentum to the research in this field and it is expected that the need for labelling blood cells in vitro may soon be declining.

Despite the success and the excitement, some investigators have expressed concern that the use of murine protein in humans may lead to the formation of HAMA (human antibodies against mouse antibody). HAMA production may be particularly dangerous in those patients who may otherwise be expected to have normal life span. The repeated use of antibodies in these patients could therefore be inadvisable and perhaps unsuccessful. However, studies have thus far shown that the HAMA response in patients receiving less than a mg of MAb is infrequent than in those patients receiving multi- mg quantities. Most studies for imaging haematological disorders of the kind pertaining to this chapter, have used less than a mg MAb which hopefully will eliminate this problem. It is expected that the choices of the agents will not be based upon the price or availability of the agent but instead may be made on the basis of the sensitivity and the specificity of these agents, which must be evaluated thoroughly.

Several antiplatelet antibodies have been evaluated in patients with vascular thrombi and excellent results have been obtained. It has been reported that $^{99}Tc^{m}$ labelled antibody fragment clears rapidly from circulation and helps to enhance the lesion to background ratio. Although this phenomenon is clearly favourable to imaging, it makes one wonder as to why

the platelets labelled with such antibodies (assuming a major proportion of the antibody molecules bind to circulating platelets) clear from circulation so rapidly.

Antibodies specific for CD - 2 lymphocytes antigens detect rheumatoid arthritis effectively and those against CD - 4 antigens diagnose rejection episode in transplanted kidneys by their extensive accumulation in the organ even before the clinical symptoms become apparent.

Clinical applications

Leucocytes

It is reasonable to argue that in the history of nuclear medicine, no single radiopharmaceutical has found as many applications as ^{111}In- oxine, which till today, remains the most suitable agent for labelling cellular blood elements. The pathophysiological responses against disease invoke intense cellular infiltration and often complicated biochemical reactions. Many such responses have been thoroughly examined. The studies have provided a strong basis for application of radiolabelled blood elements in scintigraphic localization of abnormal lesions. During the past 15 years or so, the feasibility studies of numerous such applications have been carried out using ^{111}In labelled leucocytes, platelets and lymphocytes. Some of these applications are listed in **Table II.**

Probably the most common among these applications are those which use radiolabelled autologous leucocytes. Leucocytes withstand in vitro labelling manipulation well and allow investigators to image lesions within 24 hrs after the injection. The labelling technique is efficient and presents little problem in studying paediatric population or neutropenic individuals. A general rule of thumb is that lesions actively harbouring blood cells will accumulate radioactivity. Walled off abscesses or the lesions of chronic osteomyelitis may be difficult to localize. Histologic studies have shown that the lesions of chronic osteomyelitis, for example, attract more mononuclear cells than polymorphonuclear leucocytes. These can be detected more readily by labelling a larger number of those cells. This is achieved by drawing 80 ml of blood for labelling rather than the usual 30 ml. With ^{111}In labelled cells, there is virtually no radioactivity found in the gastrointestinal tract. This makes it convenient to study the patients with inflammatory bowel disease. However, patients with intestinal bleeding may complicate interpretation and serial imaging may be necessary. The usual lack of uptake of labelled leucocytes in tumours normally allows one to distinguish malignant lesions from infection. However, the literature is full of examples in which intense radioactivity accumulation in infected tumours has been reported. Imaging infarcted myocardium is feasible yet uncommon since the kinetics of leucocyte infiltration requires imaging to be performed within 24 hrs of the onset of chest pain. In subsequent images fewer true positive scintigram have been obtained.

Although many such applications are routine, certain diseases tend to be more regional. Ascariasis and filariasis, for example, is more frequent in developing and tropical countries

than in the industrial, western part of the World. The occurrence of this infestation Is usually associated with elevation of eosinophils in circulation. The so called tropical eosinophilia supposed to be associated with filarial migration is associated with intense eosinophilia, sometimes as high as 70 to 80 %. Because of their usual small number and difficulty in their separation in pure form from blood, there are very few attempts to label eosinophils and study their kinetics in a patient and investigate their interaction with helminths. Many such regional diseases provide unique opportunity to study the cell pathophysiology non-invasively, for basic knowledge as well as for patient care.

It is our hope that the availability of monoclonal antibodies specific for blood cells, and the ability to label them with a variety of radionuclides without alteration of their biochemical properties, will simplify the technique for applications in routine diagnosis and add to the vital patient care. Needless to add that the simplification of cell labelling procedure will also promote cell kinetic studies previously complicated by the possibility of their modulated biological behaviour. Many such studies have already begun and encouraging results have been obtained.

An example is given (Fig. 27.5) from our preliminary studies in which 100 ug of IgM antibody, specific for lacto-N-fucopentoase antigenic determinant on human neutrophils, was labelled approximately with 5 mCi $^{99}Tc^{m}$ and given to patients suspected of ongoing inflammatory disease. The figure shows an intense uptake of radioactivity in the right lower abdomen within 30 min, after the administration of anti-body. Perforated appendicitis was evident at surgery. Approximately 40% of the circulating activity was associated with neutrophils. At three hrs post-injection the bone marrow activity was estimated to be approximately 7% and the bladder activity to be less than 1%.

Platelets

The number of experimental and clinical applications of radio- labelled platelets perhaps exceed the number of applications explored with any other blood cells. The literature indicates, however, that the labelled platelets are employed for fewer routine applications than for example radiolabelled leucocytes. One of the major difficulties in the routine scintigraphic applications of platelets may be traced to their long survival time which results in high blood background activity and compels investigators to perform imaging several days after the labelled platelets have been administered. For imaging intracardiac thrombosis, for example, patients have been imaged for up to 5 days in order to allow the blood pool background radioactivity to minimize. It is probably for the same reason that, the visualization of small lesions of bacterial endocarditis or coronary artery thrombosis has been less reliable and infrequent.

The clinical problems associated with hypercoagulation are treated with anticoagulating agents. This can reduce platelets aggregability and also their deposition on the lesions under examination. In one study on imaging pulmonary embolism (PE), for example, heparin prevented scintigraphic visualization of PE but its effect was reversible. Contrary to this,

lesions of atherosclerosis are imaged in several patients receiving dipyridamole known to reduce platelet aggregability.

Several successful studies have been carried out on the determination of platelet survival and on the site of platelet destruction in the management of patients with idiopathic thrombocytopenic purpura (ITP). A renal uptake index has been developed in order to be able to predict a rejection episode of transplanted kidneys before the evidence of clinical symptoms so that an aggressive regimen of therapeutic intervention could be warranted. The scintigraphic determination of platelet kinetics in patients with transient thrombocytopenia during surgery and its reversal by protamine sulphate illustrates the feasibility of such studies that previously were almost impossible to perform.

Several antiplatelet anti-bodies and antifibrin antibodies and some antibody fragments labelled with ^{123}I, ^{111}In as well as with $^{99}Tc^{m}$ have been prepared and evaluated in patients with deep venous thrombosis. Results have been encouraging not only because the agents were able to localize thrombi but reportedly there was a rapid clearance of radioactivity. Since the clearance time of platelet bound radioactivity should be determined by survival of platelets and not by the in vivo elimination of the radiolabelled antibody, this phenomenon is interesting. Such antibodies may be highly useful in imaging vascular thrombi and the lesions of atherosclerosis but may have limited applications in platelet kinetic studies.

Lymphocytes

In principle, with ^{111}In or $^{99}Tc^{m}$ labelled autologous lymphocytes, it is possible to image all kind of lymphoid malignancies. Enlarged lymph nodes in patients with Hodgkins Disease have been successfully imaged and tumours have been localized with high specificity and sensitivity. The lymphocyte is a ubiquitous cell with a heterogeneous population. In animals like rats and sheep lymphocytes are obtained from the thoracic duct, from the spleen in mice, and from the footpad lymph nodes in hamsters. The number of lymphocytes in circulating blood is relatively small but it is the most convenient place to obtain lymphocytes in humans. The presence of lymphocytes in the blood, spleen, bone marrow, lymph nodes, thoracic duct and other non-lymphoid tissue makes the cell migration pattern complex but interesting to examine. Not much has been studied of their migration pattern in a state of disease. Radiolabelled LAK cells appear to migrate in the malignant lesions within a few hours after administration. Their migration in transplanted hearts at the time of rejection episode has been studied in animals and more recently in transplanted kidneys in man. However, for the latter application; radiolabelled antibody specific for lymphocyte determinant CD - 2 appear to be a better way.

Labelling lymphocytes in vivo is not only convenient but the surface label of radiolabelled antibody is expected to exert less radiation risk to these mononuclear cells than the intracellular label like ^{111}In-oxine. Such a surface label is highly desirable particularly for these dividing cells. It is reasonable to anticipate that the availability of such an in vivo tracer for lymphocytes will promote new investigation not only in scintigraphic diagnostic

studies but also in the studies of the migratory properties of lymphocytes in health and disease.

Conclusion

In conclusion, I can say that this field is still in the infancy and the full scope of the applications of the labelled blood cells is not yet fully realized. Hardly any work in this area has come from the developing countries. The techniques are relatively simple and does not need any costly equipment which is normally not available in a nuclear medicine department of a hospital. The vast variety of infectious diseases that are prevalent in these countries would provide numerous opportunities to an inquiring mind to pursue research in understanding the interplay between the invader and the host's defence. In developed countries there has been an undue emphasis on devising scintigraphic applications. The basic kinetic studies can provide a lot of useful information even without scintigraphy. What better physiologically compatible tracer there can be than labelled blood cells? I hope that the international organizations will help in transferring this technology to the developing countries where it is most needed and where this kind of technology will find its greatest challenge and true fulfilment.

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TABLE I. RADIOACTIVE AGENTS FOR IN VITRO LABELLING	
In-111-oxine In-111-Tropolone In-111-Merc (2-Mercapto-Pyridine-N-Oxide)	Tc-99m MDP, PYP (Pre-tinning) Tc-99m Colloid (Albumin, Sulphur, SnF2) Tc-99m HMPAO Tc-99m Porphyrine

TABLE II. DIAGNOSTIC APPLICATIONS OF RADIOLABELLED BLOOD CELLS

Cell Type	Applications
Leucocytes	Soft tissues abscesses. Inflammatory foci. Inflammatory bowel disease. Infections of orthopaedic implants Osteomyelitis Intracranial abscesses Rheumatoid arthritis Myocardial infarction
Platelets	Venous Thrombosis Intracardiac thrombosis Renal transplant rejection Bacterial Endocarditis Pulmonary Embolism Coronary Artery Thrombosis Atherosclerotic lesions.
Lymphocytes	Lymphoid tissues Malignant tumours Transplanted organ rejection Rheumatoid Arthritis

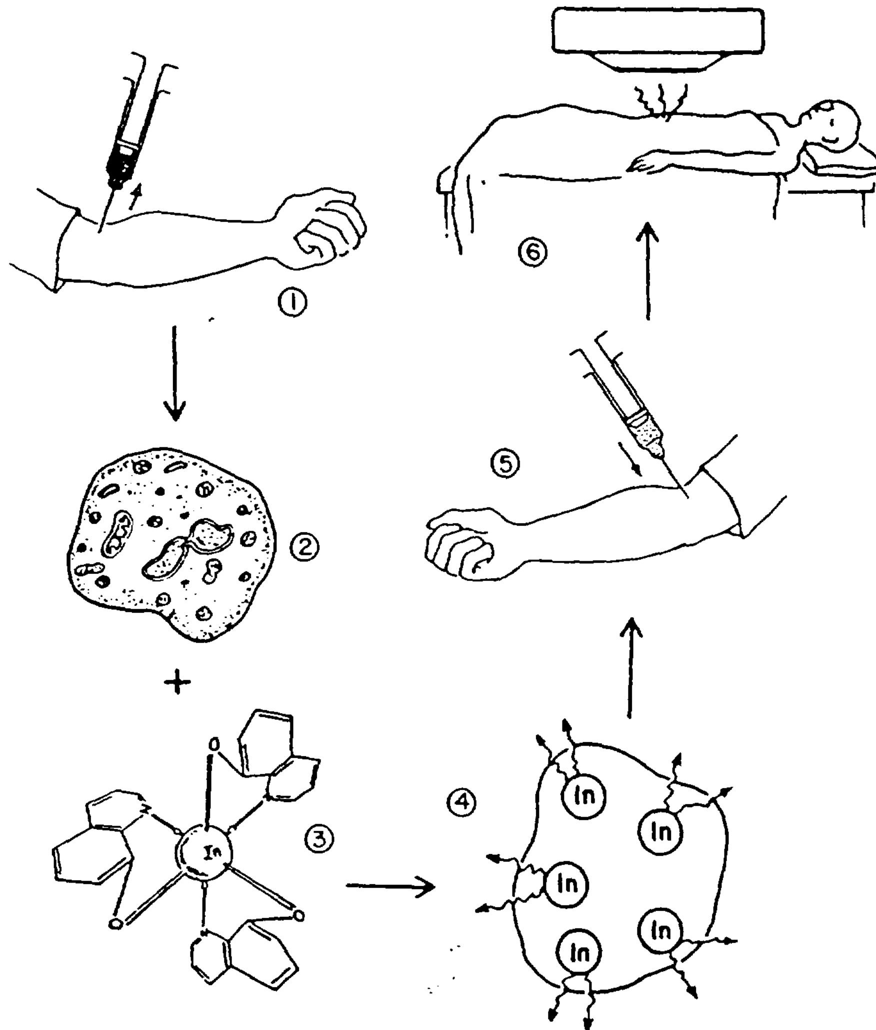

Fig. 27.1. A schematic presentation of a general procedure for imaging patients with radiolabelled autologous blood cells.

1.	venous blood is drawn,
2.	cells are separated,
3.& 4.	cells are labelled with In-111-oxine,
5.	injected back to the patient and
6.	patient imaged later.

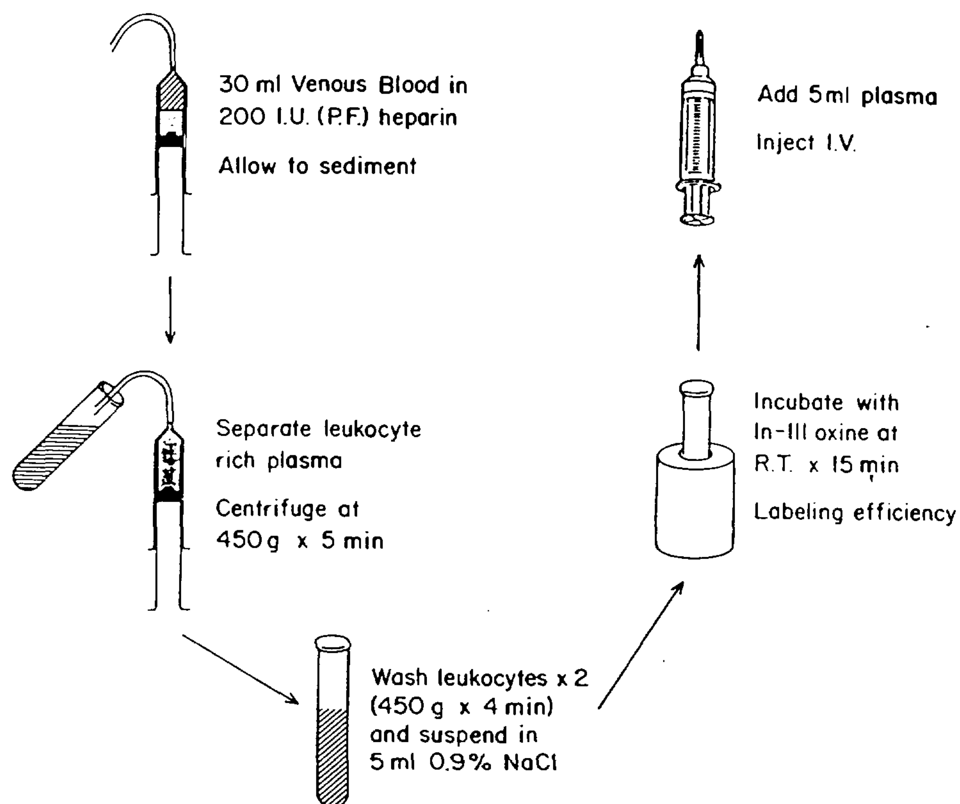

Fig. 27.2. A most commonly used leucocyte separation and labelling procedure. (i.v. = International units. P.F. = preservative free Heparin. P.F. heparin is not absolutely necessary).

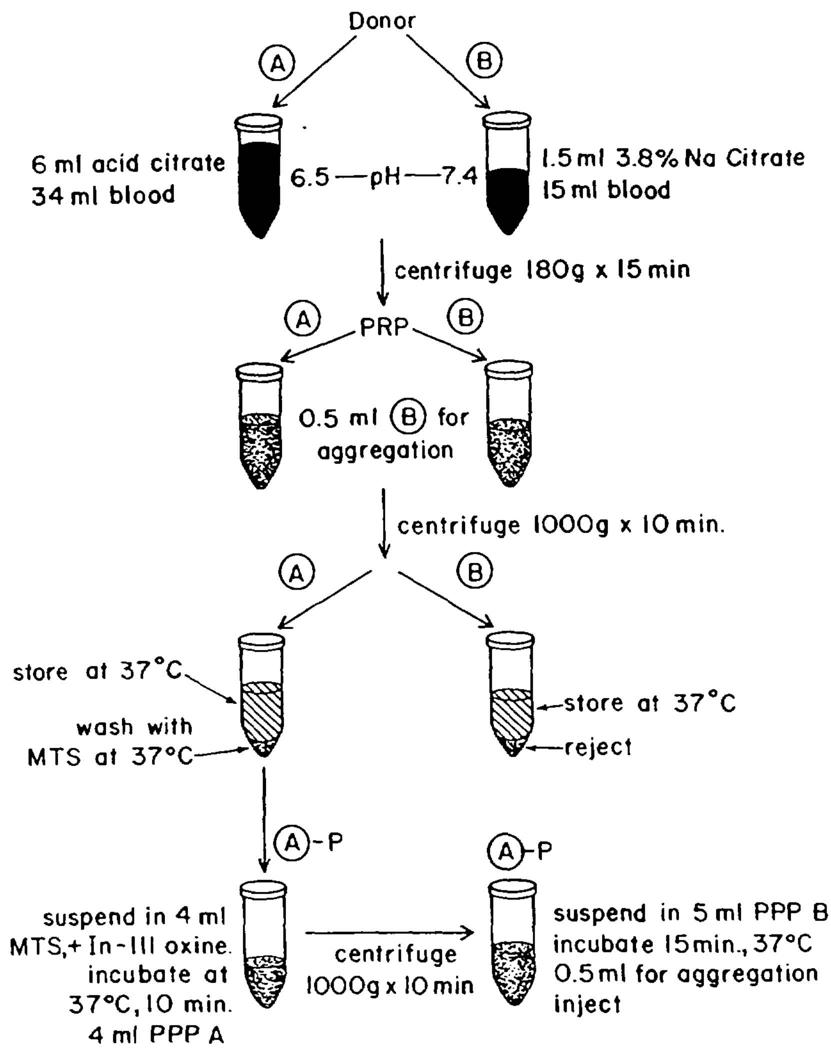

Fig. 27.3. A schematic presentation of a procedure for labelling human platelets with ^{111}In-oxine. (PRP = platelet poor plasma, MTS = modified Tyrode's solution).

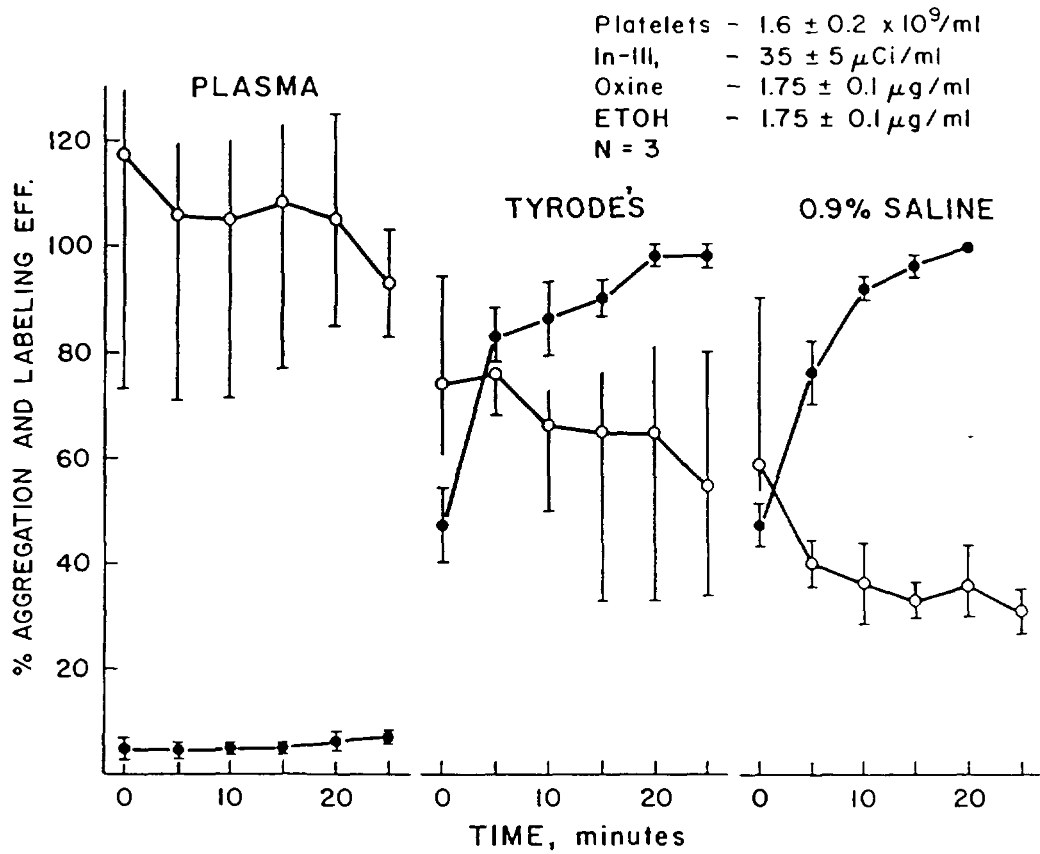

Fig. 27.4. Influence of suspending medium on labelling efficiency and aggregability of ^{111}In labelled human platelets. Open circles represent aggregation and close circles the labelling efficiency. Percentages are the mean values of three experiments.

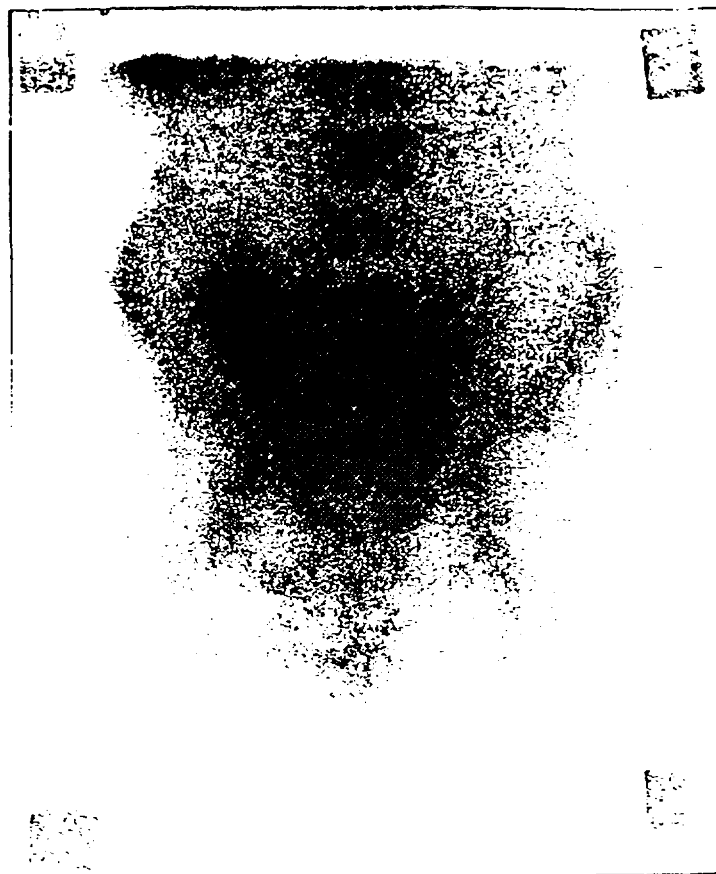

Fig. 27.5. An anterior gamma camera image of a patient with fever, right lower quadrant abdominal pain and elevated white blood cell count. The patient was given i.v., 100 μg of human neutrophil specific monoclonal antibody, MCA-480, labelled with approximately 5 mCi ^{99}Tc^{m} and imaged two hours later. At surgery the lesion was confirmed to be a perforated appendicitis. (From: Thakur, Marcus and Rhodes et al, JNM in press).

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Chapter 28

LIVER AND GASTROINTESTINAL TRACT

Maqbool Ahmed Shahid

Introduction

Liver is often a site of a variety of diseases. A palpable liver during a routine clinical examination is an important finding and requires further investigations. The availability of non-invasive liver imaging procedures using nuclear, ultrasound, C.T. (and now MRI) techniques have immensely enhanced diagnostic accuracy in liver diseases. In this Chapter, a detailed description of routinely practised nuclear medicine procedures related to liver is given. Brief reference is also made to other imaging techniques, particularly ultrasonography, only for the purposes of comparison. Most of the information is based on our own clinical experience of past 30 years.

While examining a radionuclide image of liver, it should be kept in mind that it is basically a functional image and illustrates only those areas which have retained their function in spite of disease. The unique advantage of this image is that at the same time it also shows anatomical parameters i.e.size, shape, displacements, distortions, etc. The disease pattern seen on the radionuclide liver image may be described as diffuse (as seen in hepatitis, cirrhosis, fatty infiltration etc.) or focal (as in abscess, cyst, tumour etc.).

The visualization of spleen in a radiocolloid liver image provides additional valuable information about the functional status of the liver. In addition to liver scintigraphy, other useful nuclear medicine techniques applicable to liver include blood pool imaging, hepatobiliary study, immunoscintigraphy, and radioimmunoassay for tumour markers.

A list of radiopharmaceuticals commonly employed for the diagnosis of liver diseases is shown in **Table I**.

TABLE I. RADIOPHARMACEUTICALS FOR LIVER IMAGING
Radiocolloids
$^{99}Tc^{m}$ Sulphur Colloid
$^{99}Tc^{m}$ Tin Colloid
$^{99}Tc^{m}$ Antimony Colloid
$^{99}Tc^{m}$ Phytate
Hepatobiliary agents
$^{99}Tc^{m}$ Iminodiacetic acid (HIDA)
$^{99}Tc^{m}$ Paraisopropyl iminodiacetic acid (PIPIDA)
$^{99}Tc^{m}$ Parabutyl iminodiacetic acid (BIDA)
$^{99}Tc^{m}$ Diisopropyl iminodiacetic acid (DISIDA)
$^{99}Tc^{m}$ Diethyl iminodiacetic acid (DEIDA)
Tumour/abscess localization
67-Gallium (Tumour & abscess)
111-Indium labelled WBCs (Abscess).
Blood Pool agents
$^{99}Tc^{m}$ 'in vivo' labelled RBCs.
$^{99}Tc^{m}$ 'in vitro' labelled RBCs.
$^{99}Tc^{m}$ labelled human serum albumin.

Anatomy

Liver is a bilobed, large solid organ which occupies almost the whole of the right hypochondrium. A part of its left lobe occupies the epigastrium and extends into the left hypochondrium. The liver is divided into two parts by the falciform ligament. This division is also very often visible on the scintigram, but does not correspond to the actual anatomical division of left and right lobes based on their blood supply and biliary drainage. The right lobe also includes two other small lobes called caudate and quadrate lobes. The caudate lobe is situated on the posterior surface while the quadrate lobe lies on the inferior surface of the right lobe immediately medial to the gall bladder fossa. The quadrate lobe, if prominent, may be visible on the radionuclide image. The confluence of the portal vein, bile ducts and hepatic artery is called porta hepatis and this may show prominently as a focal defect at the junction of the right and left lobes. Similarly the confluence of the hepatic veins at the cephalic attachment of falciform ligament sometimes appears as a wedge shaped defect along the upper border of the liver. The liver is surrounded from all sides by a large number of other organs which may affect the shape of liver image in a radionuclide study. The important organs include the diaphragm, right lung, heart, rib cage, gall bladder, right kidney, intestines, biliary tract, stomach and pancreas.

The knowledge of the anatomy of biliary tract is necessary to interpret a hepatobiliary study. The two main hepatic ducts emerge from right and left lobes and join in the region of porta hepatis to make common hepatic duct which travels inferiorly and is joined by the cystic duct from the gall bladder to make common bile duct. The common bile duct further descends downwards up to 10-15 cm and enters the duodenum at ampulla of Vater where it is also joined by the pancreatic duct.

Gall bladder is a sac-like, pear-shaped structure which fills and empties through cystic duct. It is about 10 x 4 cm in size and is attached to the inferior surface of the right lobe, lateral to the quadrate lobe. It stores and concentrates bile and contracts to pour bile into the lumen of the duodenum. In acute cholecystitis, following a blocked cystic duct, the gall bladder is not visible in a hepatobiliary study. Intrahepatic gall bladder may produce a defect in the lower lateral part of the right lobe. In case of congenital biliary atresia, the obstruction at the level of hepatic duct is sometimes clearly visible in a hepatobiliary study. Similarly the point of obstruction in the common bile duct due to a calculus or external pressure can be detected.

In a radionuclide scan of liver, the spleen is also imaged due to its reticulo-endothelial system which normally phagocytoses the colloid particles. A normal spleen measures about 12 x 7 cm in size and is situated posteriorly in the left hypochondrium. It can not be palpated below the costal margin unless it is about three times enlarged. Very occasionally an accessory spleen or a double spleen may be detected in the scintigram. In the developing countries, the large spleens are seen in cirrhosis of liver and chronic myeloid leukaemia. Large malarial spleens are now not common in many of the South-East Asian countries.

Physiology

The two main types of liver cells relevant to radionuclide imaging are the hepatocytes, which constitute the major bulk of the organ (80-90%) and the macrophages (Kupffer cells) which constitute only 2% of the total mass. The Kupffer cells are distributed in the lining of the vascular sinusoids and effectively remove colloid particles from the circulation. The ultimate clearance of the colloid from the circulation depends on the function of macrophages, perfusion of the hepatic lobules, and size of the colloid particles. Hepatocytes are capable of concentrating chemical substances like HIDA, Rose Bengal and other dyes from the circulation and excrete them with the bile. Diseases which affect the function of the hepatocytes e.g., inflammations, fatty infiltrations, toxic substances, malignancies, fibrosis etc. also affect the uptake and excretion of these substances.

RADIO-COLLOID LIVER-SPLEEN IMAGING

The function of phagocytosis is common to both liver and spleen due to the presence of reticulo-endothelial cells (macrophages) in both the organs. Similar cells are also present in the bone marrow and to a small extent in the lungs. The Kupffer cells make about 65% of all macrophages in the body but would concentrate 80 to 90% of the total colloid particles from the circulation. There is some correlation between the particle size and the extraction of the colloid by these organs. Smaller particles go the liver and smallest to the bone marrow. Spleen takes up the larger particles. Thus the variation in the particle size may alter the relative distribution of the radio-colloid injected.

Most commonly used radio-colloid for liver-spleen scintigraphy is $^{99}Tc^{m}$ sulphur colloid. The usual dose is 2 to 5 mCi (75-185 MBq) and the estimated particle size is 0.3 to 1 micron. The uptake of radio-colloid in the liver depends on the overall perfusion of the organ and the functional integrity of Kupffer cells. In conditions affecting these factors, the uptake of $^{99}Tc^{m}$ colloid by the liver is diminished with corresponding increase in the uptake by spleen and bone marrow and infrequently by the lungs. This phenomenon is sometimes described as 'spill over'.

Technical Procedure

The patient's abdomen is carefully palpated. Xiphisternum, costal margins, liver and spleen borders (if palpable) and outlines of any mass palpable in connection with the liver or in its vicinity are marked on the skin of the patient using an ordinary ink marker. These skin markings should be done in the same position in which the patient is going to be scanned on the imaging device. The marking of mid-axillary line for the Right Lateral view may also be useful for aspiration or biopsy if required later.

It takes about 10 to 15 minutes for the maximum accumulation of radioactivity after a radiocolloid injection, and this is the optimum time to commence imaging in most of the cases. In patients with impaired liver function or portal hypertension, the optimal

concentration may be reached as late as 30 minutes after injection. The scanning is usually done with patient in the lying position. If a rectilinear scanner is being used, this is the only possible posture. When using this machine, the markings already made on the skin of the patient are easily transferred to scan paper or X-ray film before starting the scan. A low energy, medium focus, five inches focal length collimator is used. The information density should be at least 800 counts/cm2. Background subtraction of 5 to 10% may be used. The 'hot spot' is taken over the highest count rate on the liver in the anterior, right lateral, and posterior views. If spleen is the organ of interest, 'hot spot' is taken over the spleen in the left lateral and posterior views. Anterior, posterior and Rt. lateral views are obtained routinely. If spleen is the organ of interest left lateral view should always be taken. Scan is started from the level of the fourth intercostal space downwards to below all liver or spleen activity.

While using a gamma camera both lying and standing positions can be utilized. The advantage of standing position is that the up and down movement of the liver due to diaphragmatic excursions is reduced. Such movements degrade the quality of the image and small focal lesions may be obscured. A low energy, high resolution, parallel hole collimator is used for the gamma camera and 500 000 to 1000 000 counts are collected for each image. Counts below 300 000 would be unsatisfactory. The views to be taken are the same as described for the rectilinear scanner. Skin markings can be transferred to the image by using radioactive point sources or thin lead strip. 2 cm^2 pieces of lead may also be used to serve as a reference marker for the size of focal lesions on the image.

In a gamma camera having a provision for taking counts over the region of interest (ROI), the square of the ROI can be adjusted to the minimum and marked on the image. With the help of a radioactive point source, skin markings can be transferred to the image in the form of small mini-squares. If a discrete focal lesion is seen in the liver, its position can be marked over the skin of the patient to facilitate biopsy or aspiration. A storage oscilloscope (persistent scope), if available on the gamma camera, is very useful in positioning the patient for different views.

Interpretation

The evaluation of a liver image should include:

(a) Size, shape and position of the liver. Upwards, downwards and lateral displacement should be noted if present. Any deformity in the outline due to external pressure caused by the pathology in the neighbouring organs like lungs, heart, stomach, pancreas, kidneys, gall bladder, biliary tract, intestines and ascitic fluid should be described. Degree of hypertrophy or atrophy of the liver should be noted.

(b) The homogeneity of activity in the liver is described as uniform, non-uniform, diffuse, mottled, decreased etc. referring to different anatomical parts of the organ.

(c) Presence of focal defects is noted and their number, size, and exact anatomical location in the liver is described.

(d) Splenic image is carefully studied for size, site, density, deformity, and presence of focal lesions. The presence of visibility of bone marrow and relative distribution of colloid among liver, spleen and bone marrow is noted. Occasionally the radiocolloid may also be visible in the lung fields.

(e) Artifacts due to pendulous right breast or metallic articles on the body of the patient such as coins should also be kept in mind while interpreting a liver scintigram. Hot spots produced by slight extravasation of radioactivity at the site of injection at the ante-cubitus and contamination of bed or patient's clothes by radioactive drops or spirit swab may be another source of artifacts.

The International Atomic Energy Agency (IAEA) in 1984, sponsored a three year regional coordinated research project on liver imaging in the South East Asian Countries, for which a standard liver scintigram report sheet was prescribed. This includes a comprehensive and convenient list of important points for the interpretation and description of a liver-spleen scintigram. This sheet can be requested from the Agency.

Normal liver scintigram

Radiocolloid image gives the best assessment of the size of the liver. It is difficult to give measurements for a standard 'normal' adult liver. The correct assessment of size comes with experience. Clinical examination of the patient improves this assessment but it should be always kept in mind that a palpable liver is not always enlarged. Conditions like chronic obstructive pulmonary disease, pleural effusion, tumours, subphrenic abscess, ascites, and laxity of the hepatic ligaments in the old age can make a normal liver palpable. On the other hand, an enlarged liver may not be palpable in cases of upward movement of right hemidiaphragm due to atelectasis or phrenic nerve paralysis.

The distribution of the radioactivity in a normal liver image is homogenous, gradually thinning out towards the left lobe. (**Fig. 28.1.**) Regarding the shape of the liver, various normal configurations have been described. A triangular outline due to less prominent left lobe; a prominent quadrate lobe; a tail-like projection extending downwards from the lower end of the right lobe (Riedal's lobe), which may sometimes appear 'detached' from the right lobe due to deep impression of the lower coastal margin; and so on. Impressions of porta hepatis and falciform ligament may be seen at the junction of right and left lobes. Gall bladder may indent the inferior surface of the right lobe. Hepatic veins can notch the upper border of the hepatic image. Similarly enlarged heart and pericardial effusion produce a deep concavity along the upper border. In the posterior view, the right kidney often produces an impression on the right lobe and there is some masking of radioactivity in the liver tissue lying in front of the vertebral column. Spleen appears more prominent in this view as it is

nearer to the detector of the imaging instrument. In cases of biliary obstruction, the dilated hepatic ducts may produce an appearance of focal defect at the junction of right and left lobes.

A normal liver may appear abnormal in the image due to extrahepatic causes e.g. displacement and compression by the abdominal masses, supra and subphrenic pathology and biliary disease.

Any deviation from the above description of a normal scan would indicate a strong possibility of a diseased liver. There are two main types of abnormalities which can be seen in the liver. These are (a) Diffuse or (b) Focal.

The diffuse abnormalities may manifest as hepatomegaly or atrophy of the liver. The image may show overall diminution of the uptake of the radioisotope irrespective of the size of the liver. The distribution of the radiopharmaceutical may be non-uniform with or without diminished uptake. A non-uniform uptake may be described, somewhat subjectively, in various ways, such as diffuse, patchy, mottled; occasionally a non-uniform uptake may mimic an appearance of multiple focal lesions, but such lesions do not show distinct boundaries.

The radiocolloid image is very sensitive for detecting the photon-deficient areas in the liver usually called focal defects. The smallest focal defect which can be detected in liver, using the modern gamma camera is about 1.0 cm in diameter. The detectibility also depends upon the location of the lesion. Rarely, focal hot spots may be visualized on the scan. This may happen in cases of Budd-Chiari syndrome (hepatic vein obstruction), focal nodular hyperplasia, and occasionally in superior vena caval obstruction where a bolus of activity injected into the basilic vein, can travel via collaterals and deliver a large amount of activity to the anterior mid-portion of liver.

Focal lesions can also be seen in the spleen, and one should not miss looking at the spleen critically in a radiocolloid liver image.

DIFFUSE DISEASES OF LIVER

Cirrhosis

Suspected cirrhosis of liver is a very important indication for radiocolloid liver scintigraphy. The typical picture in the advanced liver cirrhosis shows an atrophic, shrunken liver having markedly diminished radionuclide uptake with patchy, mottled appearance. The spleen is grossly enlarged and shows excessive radionuclide concentration. This phenomenon, where the normal liver-spleen ratio is reversed is called 'colloid shift' or 'spill-over', as the diseased liver is unable to accommodate sufficient quantity of colloid which is then shifted to or spilled over to spleen. (Fig. 28.2.) Additional confirmation of this condition is obtained by the visibility of spine, sternum and the pelvis in the scan and

occasionally the lungs as some colloid also spills over to the reticuloendothelial cells present in these organs.

In cases where the excessive intake of alcohol plays an etiological role, frequently the right lobe may be more damaged and appear smaller on the scan than the left lobe. The possible explanation for this being that the main stream of portal blood flow passes through the right lobe, and therefore more alcoholic damage to this lobe. In early cases of cirrhosis, the liver may appear enlarged or normal in size, although with diminished uptake, and spleen may be only moderately enlarged with positive 'spill-over' phenomenon. In long standing cases definite focal defects may appear indicating development of hepatocellular carcinoma for which cirrhosis is a predisposing condition.

The mechanism involved in producing diminished uptake of liver and spill-over to other organs seen on a radiocolloid scan involves:

(a) impairment of macrophage function

(b) diminished hepatic perfusion,

(c) replacement of liver parenchyma by fibrous tissue,

(d) shunting of blood from the liver to spleen and bone marrow,

(e) intrahepatic alteration in blood flow so that the blood is shunted away from the sinusoids which are lined by the macrophages.

In cirrhosis, widespread death of liver cells from many causes is accompanied and followed by progressive fibrosis, regenerative hyperplasia of surviving hepatocytes and distortion of liver architecture resulting in portal-systemic vascular shunts. Splenic enlargement is caused mainly by portal hypertension. The extreme degree of splenic enlargement is observed in advanced cirrhosis. The other important condition with comparable gross splenic enlargement is chronic myeloid leukaemia (CML). But in this disease, the most important finding on radiocolloid scan is normal relative uptake of radioactivity by liver and spleen and the 'spill-over' is therefore, not seen.

Using a gamma camera, we have done a short study of comparing the counts of small regions of interest (ROIs) of the same dimensions on the right lobe of liver and spleen. In normal individuals the average liver/spleen ratio was 4:1. Advanced cirrhotic cases showed exactly a reverse ratio. In cases of chronic hepatitis the liver/spleen ratio was 1:1.5. This technique can be applied in assessing the degree of impairment of liver function in quantitative terms to confirm the visual impression of diminished liver uptake.

Other Diffuse Liver disorders

Other diffuse liver disorders leading to liver enlargement with or without non-uniform distribution of radioactivity in the liver include:

(a) Congestive cardiac failure,
(b) Lymphomas,
(c) Leukaemia,
(d) Amyloidosis,
(e) Sarcoidosis,
(f) Kala Azar,
(g) Malaria,
(h) Schistosomiasis,
(i) Acute and Chronic Hepatitis,
(j) fatty degeneration in diabetes.

Lymphomas may show as diffuse disease or as definite focal lesions.

FOCAL DISEASES OF LIVER

Amoebic abscess

In the developing countries, the amoebic liver abscess is still one of the common causes resulting in the appearance of a focal defect in a radiocolloid liver scan. However, in my experience of about 30 years in nuclear medicine in a developing country, I feel that there has been a gradual decline in the number of liver abscess cases during this long period. The reason for this is probably the gradual improvement in the living conditions and standards of personal and community hygiene in some of the developing countries. It is also likely that less number of patients are referred to nuclear medicine department, as many of them are referred for ultrasound examinations.

Amoebic liver abscess usually appears as a solitary, well-defined rounded focal defect mostly situated in the right lobe (Fig. 28.3). Less commonly the left lobe may be involved. Multiple abscesses of different sizes may occasionally be seen. Sometimes the location of the abscess is such that it produces obstructive jaundice through pressure on the main hepatic ducts. The exact site of the abscess can be ascertained by taking multiple views. It must be remembered that a focal defect on radiocolloid image is a non-specific finding. More information would be required to confirm that the lesion is an abscess. Ultrasonography is a convenient procedure to differentiate between solid and cystic lesions. Cystic lesion in a developing country could be an abscess or an hydatid cyst, although the latter is not as common as an abscess. The abscess is characterized by the presence of abdominal pain, pyrexia, nocturnal sweating, malaise and liver tenderness. This clinical picture is not seen in the hydatid cyst where the main complaint is enlargement of liver which may feel hard in the area of cyst. Amoebic abscess is often so insidious in its progress that clinical history is

not very helpful in all the cases.It may also be remembered that in an early amoebic lesion where the disease is still in cellulitis phase and frank pus has not appeared the ultrasonography may give misleading information. The central area of the abscess (and cyst) is usually avascular. It is therefore, a routine at some centres to inject the radiocolloid when the patient is lying under the gamma camera and take sequential pictures after every 2-4 seconds for a dynamic blood flow study. A malignant lesion is usually hypovascular, but not completely devoid of perfusion as compared to the abscess or cyst.

Treatment with proper anti-amoebic drugs alleviates the patient's clinical symptoms within one or two weeks. However, the cold area on the scan persists for a long time, even for several months. There is no point in doing follow-up scans every week or so. In any case, ultrasound scans are better for follow-up evaluation.

Pyogenic Abscess

Pyogenic abscess is seen less commonly in the developing countries. A pyogenic abscess is accompanied by acute symptoms of fever, malaise, sweating and pain in the right hypochondrium and higher mortality. There may be a large single abscess or multiple small abscesses. Timely treatment with antibiotics and aspiration, if required, often cures the disease.

Subdiaphragmatic Abscess

This is an uncommon condition where there is an abscess in the potential space between right hemidiaphragm and right lobe of the liver. Clinically, the patient shows all signs and symptoms of pyogenic infection but it is difficult to find the location of the abscess. The patient may complain of pain in the lower part of the chest on the right side. The radiocolloid liver scan may appear normal or there may be slight flattening of the upper border of the right lobe. The condition can be diagnosed by doing a liver and lung radiocolloid scan simultaneously. Patient is injected with $^{99}Tc^{m}$ sulphur colloid and $^{99}Tc^{m}$ Albumin macroaggregates and liver-lung area imaged in anterior, right lateral and posterior views. In case of subphrenic abscess, the cold gap between liver and lung is clearly outlined.

Hydatid cyst

Hydatid cyst is an helminthic infestation caused by a tiny tapeworm called Echinococcus granulosum for which dog and certain wild canines act as definitive hosts. The worm resides in the gut of these hosts. Animals like sheep, camel and other cattle are infested by ingesting the eggs from the pastures or water contaminated by the faeces of dogs. Men who live in close contact with dogs have a risk of getting infested. The embryo is liberated from the egg in the small intestine and gains access to the liver through portal circulation. The resultant cyst which is the larval stage of the worm, grows very slowly for years. It may ultimately calcify or rupture giving rise to multiple cysts. When the cyst has grown to a large size, it may produce pressure symptoms where it is located.

In nearly 75% of patients with hydatid disease, the right lobe of liver contains a single cyst. Multiple cysts are not very common. Hydatid cyst may also be found in lungs, brain or elsewhere. The treatment is essentially surgical. Cases of recurrence may be seen if some larvae escape to other parts of the liver. On clinical examination, the cyst feels hard and may be mistaken for a malignant growth. Calcification if present can be seen on radiological examination.

On a radiocolloid scan, the hydatid cyst appears as a 'cold' area **(Fig. 28.4)**. The margins of the defect may be sharp and the shape more rounded as compared to an abscess or a tumour. Ultrasonography is a simple procedure to illustrate the cystic nature of the hydatid disease. Further confirmation may be possible through identification of daughter foci in the main cyst in the ultrasonogram. A blood pool or a perfusion study will exhibit a largely avascular nature of the defect. Intradermal (Casoni's) test, complement fixation and immunofluorescent tests give support to the diagnosis in the presence of a long history and absence of signs and symptoms of an abscess. No attempt of confirming diagnosis with needle biopsy should be made in suspected hydatid cyst because of the risk of dissemination within and outside the liver.

Primary liver tumours

Hepatocellular carcinoma is a fairly common finding in some of the developing countries. It is the first or second commonest malignancy in the male population.

Cirrhosis of liver, a predisposing condition, increases the risk of primary liver cancer manifold. In a study done in Pakistan 12% of the cases of cirrhosis of Liver were found to be positive for cancer on radiocolloid scan and histological examination (needle biopsy). Liver cancer can, however, occur in an otherwise normal liver at any age. Regular use of oral contraceptives, which may produce hepatic adenomas, may be another causative factor. HBsAg positive sera helps in detecting high risk groups. On a radiocolloid scan, the liver cancer appears as a 'cold' area which is solitary in most of the cases but multiple primary lesions are also found. ^{67}Ga study may produce a positive image of the cancerous area but it is not always outlined well and the appearance is non-specific. It is, therefore, not advised as a routine test. The better alternative is to do ultrasonography and make sure that the lesion is solid and proceed with needle biopsy for the exact diagnosis. High blood level of alpha-fetoprotein is a common finding but for diagnosis, it is still non-specific. Sequential estimations at regular intervals are supposed to be of prognostic value.

The use of contraceptive pills on a regular basis, although uncommon in developing countries, may be seen in young women in urban areas. This may give rise to asymptomatic hepatic cell adenomas incidently detected on a radiocolloid image as a focal defect due to lack of Kupffer cells. The tumour tends to regress with the discontinuation of pills. The hepatic cell adenoma should be distinguished from focal nodular hyperplasia of liver which is also common in women and appears as normal liver tissue in a radiocolloid image.

Metastatic liver disease

Primary malignancies of the gastro-intestinal tract are likely to give rise to early secondary deposits in the liver via portal circulation. Direct local extension from the gall bladder is also a common finding. Other more common primary malignancies Likely to produce metastatic liver disease are those of breast and lungs (Fig. 28.5). Lymphomas also involve liver at an advanced stage. The hepatic metastases in most of the cases appear as multiple lesions which may be discrete or infiltrative in type. However, a solitary lesion in the presence of a known primary malignancy indicates a strong possibility of a secondary deposit. Ultrasound examination may be done to ascertain the solid nature of the lesion.

Massive infiltration of liver by metastatic tissue may produce impaired reticulo-endothelial function causing colloid shift from liver to spleen. The sensitivity of radio-colloid scan to detect metastatic disease in the liver has been mentioned to be in the range of 75-80% as the small deep seated lesions are likely to be missed. The sensitivity will probably improve with the use of single photon emission tomography (SPECT) but its use in the developing countries is still limited.

Spleen Image

Reference to enlargement of spleen in cases of portal hypertension has already been made while discussing cirrhosis of the liver. In the tropical countries, repeated malarial infestation may be an important cause of splenomegaly but is not as frequently seen as before. A number of haematological disorders also produce splenic enlargement. A grossly enlarged spleen with moderate liver enlargement is quite a frequent finding in cases of chronic myeloid leukaemia. Hepatosplenomegaly may also be seen in patients suffering from lymphoma. It may be emphasized that the normal liver/spleen ratio of radiocolloid accumulation is not altered in splenomegaly due to reasons other than portal hypertension unless the liver is also damaged.

Focal defects in spleen is a rare finding which may be seen in cases of lymphoma. Haematoma as a result of injury to an enlarged spleen also appears as a cold area on a radiocolloid scan. We have not seen any case of cyst or abscess in the spleen.

Like liver, the spleen can also be displaced by mass lesions and other abnormalities in the neighbouring structures like stomach, pancreas, left lung, pleura, left kidney and masses in the abdomen.

PATTERN OF LIVER DISEASE IN PATIENTS IMAGED WITH RADIOCOLLOIDS

The information given below was presented in a meeting of Experts Group for the IAEA-RCA Regional Cooperative project on **"Imaging procedures for the diagnosis of liver diseases"** held at Seoul on 22 - 24 August, 1984. It is derived by analyzing the results of 1000 consecutive patients in whom radiocolloid liver imaging was done during July to October, 1983 at the Mayo Hospital, Lahore, Pakistan. Out of these 1000 patients 514 were imaged with a gamma camera and 486 with a rectilinear scanner.

Table II shows the age and sex distribution of these 1000 cases. About half of the patients (45%) are between the age 41 and 60, and the male to female ratio is almost same. Table III shows the disease-spectrum in these patients. Definite focal defects were found in 11.3% cases. A majority of the patients (46.9%) were those with Hepatomegaly of diverse origin. 18.6% cases had no abnormality. In the patients with definite focal defects malignancy was the most common (6.8%). Abscess was found in 3% and the cyst in only 1.4%. Spleen was enlarged in 31.3% of the images. Cirrhosis of liver is still fairly common. In 3.8% cases, it was not possible to say whether the abnormality seen is a true focal defect or is a diffuse low activity area.

This information describes a pattern of hepatic diseases in patients referred for imaging to a nuclear medicine department in one of the developing countries. It may not represent the pattern in all the developing countries of South East Asia but it still gives an overall picture of the types of liver diseases with their relative frequencies as seen in one of them.

TABLE II. AGE AND SEX DISTRIBUTION OF 1000 PATIENTS REFERRED FOR LIVER IMAGING. AEMC, LAHORE, PAKISTAN.

AGE	MALE	FEMALE	TOTAL	%
0 - 10	20	9	29	2.9
11 - 20	45	33	78	7.8
21 - 30	63	67	130	13.0
31 - 40	75	111	186	18.6
41 - 50	100	142	242	24.2
51 - 60	102	120	222	22.2
61 - 70	50	27	77	7.7
> 70	28	8	36	3.6
TOTAL	**482**	**518**	**1000**	**100**

TABLE III. DISEASE SPECTRUM OF PATIENTS FOR LIVER IMAGING.

DISEASE	MALE	FEMALE	TOTAL	%
Cirrhosis	51	51	102	10.2
Abscess	19	11	30	3.0
Cyst	08	06	14	1.4
Subphrenic Abscess	01	0	01	0.1
Malignancy	34	34	68	6.8
Hepatomegaly; unif. upt.	212	257	469	46.9
Diffuse low upt. areas	44	48	92	9.2
Uncertain	18	20	38	3.8
Normal	95	91	186	18.6
TOTAL	482	518	1000	100
Spleen enlargem.	156	156	312	31.2

COMPARISON OF NUCLEAR HEPATIC IMAGING WITH ULTRASONOGRAPHY

In most of the developing countries, the nuclear medicine facilities are scarce and work load immense. A considerable number of routine referral is for radiocolloid liver imaging. At the same time, there is a feeling that the information obtained by a nuclear liver image can also be obtained from ultrasound examination which is less expensive and also time-saving. In the developing countries therefore, if most of the load of liver imaging is shifted from nuclear to sonography, the nuclear facility can be spared for tests like detection of skeletal metastases, cardiac studies etc. which can not be done by non-nuclear techniques. The question to be answered is, how far an ultrasonographic examination can replace the nuclear radiocolloid liver imaging? The following **Table IV** summarizes the differences between these two imaging modalities:

TABLE IV. COMPARISON OF NUCLEAR WITH ULTRASOUND IMAGING OF THE LIVER.

	Nuclear	Ultrasound
Cost	more expensive	less expensive
Spatial resolution	2 cm or larger	can detect smaller lesions
Differentiation between cystic vs solid	not possible	possible
Skills and training required	modest	highly skilled and experienced doctor required
Liver size estimation	excellent	less satisfactory
Radiation exposure	within permissible limits	nil
Imaging in the presence of dressings, drains, plasters, open wounds etc.	possible	Impossible

Previously the ultrasound examination of the liver suffered from the presence of 'rib-shadowing' rendering a part of the liver 'invisible' to the transducer, but now with the advances in the design of transducers and availability of sector scanners the entire liver can now be 'seen'. One can even scan through the intercostal spaces.

The International Atomic Energy Agency under its Regional Cooperation Agreement (RCA) program for Asia Pacific Region has started a regional cooperative research project in 1989 comparing the efficacy of ultrasonography with nuclear imaging for the detection of focal and diffuse liver disease. Our institute (INMOL Lahore) is also one of the participants in this study.

Although it will take another two or more years for the final results of the IAEA's project to come but in the meantime, we have been collecting information on patients who have undergone both radiocolloid and ultrasound imaging and have analyzed results in 196 patients up to now which is as follows.

	Total number of Patients	=	**196**
	A) Focal lesion positive cases (combining nuclear and ultrasound images)	=	**142**
Nuclear image	detected	=	126
	missed	=	16
	Total	=	**142**
		=	
	Sensitivity	=	**88%**
		=	
Ultrasound	detected	=	134
	missed	=	8
	Total		**142**
	Sensitivity		**94%**

This means that nuclear imaging missed 16 out of 142 cases of focal defects which were detected by ultrasound imaging while ultrasound missed 8 out of 142 cases of focal defects which were detected by the nuclear image.

	Diffuse liver disease positive	=	**44**
Nuclear scan	detected	=	32
	missed	=	12
	Total	=	**44**
	Sensitivity	=	**72.7%**
Ultrasound	detected	=	40
	missed	=	4
	Total	=	**44**
	Sensitivity	=	**90%**

This means that 12 out of 44 cases missed by the nuclear imaging are detected by the ultrasound and 4 out of 44 missed by the ultrasound were detected by the nuclear imaging.

(a) Cases normal both on nuclear and ultrasound imaging = 10

(b) Ultrasound examination revealed that 26 cases who were positive for focal defect both on ultrasound and nuclear imaging were cystic in nature.

(c) In 12 cases, where nuclear scan showed only solitary lesion, the ultrasound revealed multiple lesions.

(d) The cases of diffuse disease which were missed by the nuclear image were mainly those with congestive cardiac failure and biliary obstruction in which the ultrasound scan showed congested vessels and dilated hepatic ducts.

From the above observations, it appears that ultrasonography is superior to nuclear imaging for the detection of focal defects and diffuse liver disease **(Figs. 28.6 A and B and 28.7 A and B)**. There is, however, a small percentage of cases missed by the ultrasound and detected by nuclear imaging. It may, however, be prudent to see the final results and recommendations of the above mentioned multicentric trial initiated by IAEA.

HEPATOBILIARY IMAGING.

The chemical substances which follow the route of bilirubin in liver and biliary passages, can be used with a suitable radioactive label to study the function of hepatocyte and the biliary system. In fact, the first compound recognized as a possible liver imaging radiopharmaceutical was a hepatobiliary agent, the ^{131}I labelled Rose Bengal, a fluorescent halogenated dye. The other dye used for this purpose was Bromosulphthalein (BSP). As compared to the colloids, these dyes had the disadvantage of rapid excretion and constantly changing levels of activity in different parts of the liver even during the course of examination, especially with the rectilinear scanner, resulting in poor images. Later on, with the development of gamma cameras ^{123}I labelled Rose Bengal and BSP were revived for studying hepatobiliary function but by that time $^{99}Tc^{m}$ labelled compounds had established their superiority for use with the gamma camera.

These new imaging agents are **iminodiacetic acid (IDA)** analogues which provide high quality images of the biliary system. The first IDA derivative used widely was the dimethyl IDA also called hepatobiliary IDA or HIDA. Approximately 85% of this compound is excreted by the liver and 15% by the kidneys. In cases of liver damage, a larger fraction is excreted through the kidneys. However, acceptable biliary images can be obtained with serum bilirubin levels as high as 5-7 mg/dl. The other derivatives of IDA devised later include paraisopropyl IDA (PIPIDA), parabutyl IDA (BIDA), diisopropyl IDA (DISIDA) and

diethyl IDA (DEIDA). DISIDA is probably more suitable because of its high biliary excretion.

Procedure

About two hours fasting before the test is usually advised. Prolonged fasting renders the gall bladder akinetic and filled with viscous, static bile which inhibits the entry of HIDA in the gall bladder and therefore poor visualization of gall bladder. Similar thing happens in patients on total parenteral nutrition or patients of chronic alcoholism.

The patient is given 3 - 5 mCi (100 - 200 MBq) of $^{99}Tc^{m}$ labelled IDA intravenously. Sequential images of anterior abdomen are taken with a gamma camera, at 10 minutes intervals for one hour. The first image taken five minutes after injection may be useful to outline the liver as the radioactivity is passing through it.

As the study proceeds, the radioactivity passes from the liver towards porta hepatis and hepatic ducts may be **(Fig. 28.8)** visualized. Simultaneously the common bile duct and the cystic duct become visible. The gall bladder, normally, fills up within half an hour after injection. At about the same time, the loops of duodenum are also visualized. Clearance of activity from the liver starts within 10-15 minutes and at the end of the study the liver is hardly visible and the gall bladder still shows prominently. At this point, the patient may be given a standard fatty meal as in cholecystography to demonstrate contraction and emptying of the gall bladder.

In those cases, where the overall liver function is poor the study can be continued for a longer period as there may be a delayed visualization of various structures. If the liver function is grossly impaired the radiopharmaceutical is excreted mainly through the renal system and both kidneys and urinary bladder show up prominently. This also happens in cases of biliary atresia. In such situations, the activity in the right kidney may be mistaken for the gall bladder, but presence of the left kidney image in the scan picture should avoid such a confusion.

The IDA hepatobiliary study gives useful information in a number of clinical conditions like acute cholecystitis, chronic cholecystitis, gall stones, biliary tract obstruction, jaundice, bile leak, congenital biliary atresia **(Fig. 28.9)** and choledochal cysts.

Acute cholecystitis

Acute cholecystitis is almost always associated with obstruction of the gall bladder neck or cystic duct by a gall stone or an intestinal parasite like a round worm. Occasionally, obstruction may be by mucus or rarely by a neoplasm. HIDA hepatobiliary study is ideal for **(Fig. 28.10)** investigating the patency of the cystic duct. If the gall bladder is outlined in the study, the patency of the cystic duct is proved beyond doubt. HIDA is so sensitive for the diagnosis of this condition that intravenous cholangiogram for this purpose is now considered unnecessary.

Although the non-visualization of gall bladder in a HIDA study in the presence of clinical signs and symptoms of acute cholecystitis establishes the diagnosis, some rare situations should be kept in mind where the non-visualization could also be due to few other reasons. As already mentioned a patient fasting for more than 12 hours has his gall bladder filled with bile and therefore the radioactive bile after HIDA injection cannot enter unless the gall bladder contracts and expels the stored bile. This can be achieved by giving an injection of Cholecystokinin (CCK) along with HIDA. However two other situations still remain in which CCK fails to contract gall bladder. These include cases with chronic alcoholism and/or pancreatitis and those who are on total parenteral nutrition. In these situations the ultrasonography can help in diagnosis by demonstrating a full gall bladder corresponding to the clinically tender area in the right upper abdomen. Sonography is also capable of detecting calculi, but the presence of calculi alone is not sufficient to prove the diagnosis of acute cholecystitis.

Although there are no specific findings in a HIDA study characteristic of chronic cholecystitis, a few important points can be briefly mentioned which are very often associated with chronic gall bladder disease:

(a) Delayed visualization of the gall bladder, i.e. one hour or more after injection of HIDA.

(b) Delayed biliary to bowel transit time.

(c) Filling defects within the gall bladder due to stones.

(d) Suboptimal contractile response of a filled gall bladder to cholecystokinin.

Biliary Atresia

In jaundiced neonates, it is important to differentiate between congenital biliary atresia and neonatal hepatitis, so that biliary atresia could be corrected by timely surgical intervention. If HIDA hepatobiliary study shows a patent biliary passage, biliary atresia is ruled out. In cases of atresia, the exact site of obstruction can be seen sometimes at the level of hepatic duct, as it emerges at the Porta hepatis. In such cases, the radioactivity is ultimately excreted through the urinary system and both kidneys and the urinary bladder are outlined in the scan.

The hepatobiliary study is the most convenient, sensitive, and non-invasive test for detection of bile leakage after trauma or a surgical procedure. After an intravenous injection of the radiopharmaceutical (5 mCi or 200 MBq), the abdomen is imaged for at least two hours. Delayed pictures may be taken at 12 hrs and 24 hrs. Presence of radioactivity outside liver and biliary system would confirm pathological extravasation. Ultrasonography and CT imaging may reveal abnormal fluid collection in the area but cannot detect the exact nature of the fluid.

Blood Pool Imaging of Liver

It may be useful to find out the state of perfusion of a focal defect seen on a radiocolloid liver scan. While cysts and abscesses are devoid of blood supply, the neoplastic focal defects may show varying degree of perfusion. However, the absence of perfusion does not necessarily prove that the lesion is non-neoplastic because a neoplastic focal defects may also appear avascular in a blood pool scan due to central necrosis. Hemangiomas, on the other hand, show as hypervascular areas in the blood pool scan.

If a computerized gamma camera is available for the study, information on perfusion of different parts of the liver can be obtained as a part of the routine radiocolloid imaging. For this purpose, the patient is positioned under the gamma camera before injecting the radiocolloid. The radioactivity over the liver is recorded continuously, immediately after the injection as the blood carrying the bolus of radiocolloid appears in the liver. Later on, the static radiocolloid uptake images are taken as usual. Drawing the regions of interest (ROIs) over the focal defects in the perfusion phase and radiocolloid uptake phase it can be assessed whether the focal defect appearing in the radiocolloid phase is vascular or avascular. Time-activity curve can also be generated on these ROIs to get better understanding of the perfusion of the lesion.

Alternatively, a blood pool agent can be used to study the vascularity of the area corresponding to the focal defect in the radiocolloid image. For this purpose, the most convenient technique is the 'in vivo' of red blood cells using $^{99}Tc^{m}$ as a label.

A sterile solution of 1.2 mg stannous chloride and 1.8 mg of pyrophosphate stabilized at a pH around six is injected intravenously. $^{99}Tc^{m}$, at least 10 mCi (400 MBq), is injected i.v. half an hour later. This results in 'in vivo' of RBCs. We have used this technique successfully for investigating focal defects in the liver. The other possibilities are to use $^{99}Tc^{m}$ labelled human serum albumin or in vitro labelled red blood cells.

^{67}Gallium imaging

Gallium localizes non-specifically in soft tissue tumours and inflammatory lesions. It is expensive and difficult to obtain in the developing countries. Its physical characteristics are not ideal for in vivo imaging; low energy photons (91 keV) not suitable for optimal intrinsic resolution and the high energy photons (394 keV) difficult to collimate. Its relatively slow excretion through the intestine, often interferes with accurate Imaging of abdominal and pelvic lesions.

^{67}Ga scintigraphy is useful in detecting sites of acute infections as well as other inflammatory and granulomatous processes. It is useful especially in detecting pulmonary inflammatory diseases, abdominal and pelvic inflammations and in inflammatory diseases of the skeleton.

^{67}Ga scintigraphy is also useful as a diagnostic procedure for tumour detection, staging and monitoring the effect of therapy. The tumours usually detected with ^{67}Ga are lymphomas, lung carcinoma, primary hepatic carcinoma and malignant melanoma. Gallium scintigraphy is neither very sensitive nor very specific but occasionally it might provide some useful information. However, it has no significant role in routine diagnostic work up of a patient with a space occupying lesion in the liver.

A more specific and sensitive method for imaging inflammatory disease is by radiolabelled white blood cells, which is described extensively in Chapter 27.

SPECT vs conventional imaging of the liver

With the developments in ultrasonography and computerized axial tomography the value of radionuclide liver imaging is significantly reduced as the sensitivity of the detection of liver lesions by isotopic methods has been reported to be 75% and 85% whereas these other imaging modalities claim accuracies of 90% or better. Single photon emission tomography has been found to improve the detectability of liver lesions **(Fig. 28.11)**.

Liver is a large solid organ with complex internal scatter patterns. Liver imaging with SPECT needs attenuation correction and a high resolution collimator. Sensitivity of SPECT for liver focal defects is as high as 92% in some of the reported series.

Since the size of a liver lesion is a useful parameter to a clinical oncologist, the system that provides this information is advantageous. Lesion reduction is a volumetric phenomenon, not necessarily measured accurately in only two dimensions. It is possible to determine the volume of liver lesions from SPECT data. This type of study is very helpful in monitoring established liver lesions in patients on chemotherapy. SPECT, not only provides improvement in the detectability of liver lesions, but also gives exact measurement of the volume of such lesions.

GASTROINTESTINAL TRACT

Gastro-oesophageal Function

Radionuclide techniques can be employed for studying the function of oesophagus, stomach and any abnormalities at the gastro-oesophageal junction. Using an appropriate radiopharmaceutical and a computerized gamma camera a number of useful tests can be performed quite conveniently. The commonly performed tests include: gastric emptying rate, oesophageal transit time, and detection and quantification or gastro-oesophageal reflux. In performing these studies non-absorbable radiopharmaceuticals such as $^{99}Tc^{m}$ sulphur colloid or $^{99}Tc^{m}$DTPA (diethylene triamine penta-acetic acid) are used.

Gastrointestinal Bleeding

Since 1960, we have been using ^{51}Cr labelled red blood cells for measuring the amount of blood loss in the stools in suspected gastro-intestinal bleeding by a simple technique. Using an activity of 100 μCi ^{51}Cr, 5 to 10 ml of red cells of the patient are labelled in vitro, and re-injected immediately. The patient is asked to take two identical empty dry milk tins of about half a litre capacity and collect 24 hours stools in one of these tins. Next day the tin containing the stools, closed with a tight lid, along with the empty tin is handed over by the patient to the hospital staff.

Two ml of patient's blood is taken and added to the empty tin with some saponin to haemolyze the blood. The tin containing stools is weighed on a small balance. The other tin, now containing two ml blood is placed on the same balance and water added to it slowly till it weighs the same as the other tin containing the stools. The lid is tightly closed and tin is shaken few times to make a homogeneous solution of the haemolyzed blood. The radioactivity of the two tins is measured by a scintillation counter in identical geometrical conditions. Counts of the stools compared with the blood counts. The exact volume of blood present in the 24 hours stools can be calculated by a simple equation.

$$\textbf{Blood loss in mls.} = \frac{\textbf{CPM of stools x 2}}{\textbf{CPM of blood}}$$

Two ml blood sample is taken as this volume gives reasonable counts on the scintillation counter. Thus simply by looking at the counts obtained from the 24 hours stools one can find out at a glance whether there is any excessive blood loss present. The exact volume is then calculated.

This simple test, although quite useful for measuring total blood loss in stools in 24 hours, does not give any information about the exact site of bleeding in the gut for which an imaging technique is needed.

It is important to locate the site of gastrointestinal bleeding before a decision for an active intervention. The radiopharmaceutical generally used for detecting lower G.I. bleeding is $^{99}Tc^{m}$ sulphur colloid. It is routinely available in the nuclear medicine departments because of its use for radiocolloid liver imaging. The other important advantage which it has over the intravascular blood pool agents (e.g. $^{99}Tc^{m}$ labelled RBCs) is its rapid clearance from circulation by the reticuloendothelial system of liver and spleen resulting in very low tissue background activity providing excellent contrast between background and extravasated radioisotope at the bleeding site.

The radioactivity in the liver makes it difficult to detect any bleeding sites in the upper abdomen, but even with the blood pool agents the liver activity is quite high because of the vascularity of liver. The procedure requires a dose of 10 mCi (400 MBq) of $^{99}Tc^{m}$ sulphur colloid to be injected intravenously with the patient placed under the gamma camera. A large

field-of-view camera which can cover the whole abdomen at a time and a low energy collimator are necessary. 300 000 to 500 000 count images are obtained every two minutes for 15 minutes. A bleeding site is seen as a hot spot in the image. If the study is continued, the activity may be seen travelling distally along the intestine with peristaltic movements. A carefully performed study has a high degree of sensitivity with a low false negative rate. Because the sulphur colloid is cleared from the circulation within 15 minutes, only that bleeding can be detected which occurs within 15 minutes of injection.

For detecting intermittent bleeding, the intravascular blood pool agents should be used and patient imaged intermittently for 24 hours after the injection. As the sensitivity of this procedure is low, larger amounts of blood should extravasate for detection as compared to the radiocolloid technique.

Meckel's diverticulum

Meckel's diverticulum is a developmental anomaly which is in the form of a small sac like protrusion from anterior mesenteric border of the ileum about three feet proximal to the ileocecal valve. This diverticulum is lined with ectopic gastric mucosa. This occurs in about 2% of the population and in most of the cases remains symptomless. Occasionally haemorrhage, intussusception and volvulus may occur in this abnormal sac. These complications are accompanied by bleeding. The bleeding can be demonstrated by the radionuclide tests already described. The presence of ectopic gastric mucosa can be demonstrated by the $^{99}Tc^{m}$ pertechnetate imaging. About 1-2 mCi (37- 54 MBq) of the radiopharmaceutical is injected intravenously and abdominal images are obtained sequentially every five minutes. Any spot of radioactivity appearing in the intestinal area should be interpreted with reference to gastric activity. A positive scan usually shows a focal area of increased activity in the right lower quadrant of the abdomen. Barret's disease (gastric mucosa at the distal end of the oesophagus) can also be demonstrated by this technique.

Gastric Emptying

Gastric emptying can be studied either with liquids or with solids. The liquids leave the stomach faster than the solids. For the study of liquid phase about 500 μCi of $^{99}Tc^{m}$ sulphur colloid or $^{99}Tc^{m}$ DTPA is dissolved in half a glass of milk and given to the patient to drink after overnight fasting. For solid phase study, the same amount of radio-pharmaceutical is mixed with about 150 grams of solid diet like mashed potatoes and given to the patient to eat. Radioactivity is recorded over the stomach for one hour with the patient lying supine under a computerized gamma camera. A region of interest over the stomach is fixed and time activity curve generated. The results are usually expressed as "T½ emptying" or the time taken by the gastric radioactivity to reduce to half the original value.

The technique is frequently useful in finding out the state of gastric mobility in systemic diseases like diabetes mellitus and scleroderma and following surgical procedures such as vagotomy.

Gastro-oesophageal reflux

The same dose of radiopharmaceutical is used in liquid form as prescribed for the gastric emptying procedure. The radioactivity is recorded with the help of a gamma camera over the gastro-oesophageal junction, with the patient lying supine. The study may continue for half an hour. Continuous recording on a computerized gamma camera or repeat images at fixed intervals may be obtained. The simplest procedure would be to see the appearance of radioactivity in the distal part of the oesophagus in the images taken. Pressure may be applied over the stomach to provoke reflux. Using regions of interest over the lower oesophagus and stomach the amount of reflux can be expressed in quantitative terms as percentage of the gastric contents observed in the oesophagus. (Fig. 28.12).

Oesophageal transit.

The oesophageal transit time may be prolonged in diseases like achalasia, scleroderma, diffuse idiopathic oesophageal spasm and other non- specific motor disorders of the oesophagus. This parameter can be measured by using $^{99}Tc^{m}$ sulphur colloid or $^{99}Tc^{m}$ DTPA in liquid form in the same dose as for gastric reflux. The patient drinks, in one swallow, the radioactive liquid while lying supine under a computerized gamma camera. In normal subjects the radioactivity rapidly traverses the oesophagus and is not visualized by 4-10 seconds after deglutition. In patients with motor disorders the activity can be seen in the oesophagus for a long time In spite of repeated attempts to swallow (successive dry swallows). In such cases, the study should be prolonged and actual transit time determined, which may be as long as 15-30 seconds.

Immunoscintigraphy for colorectal cancer

The presence of carcinoembryonic antigen (CEA) in the colorectal cancer makes it possible to localize such tumours with immunoscintigraphic technique by using $^{99}Tc^{m}$ labelled anti-CEA antibodies. This procedure has been found useful in the following situations.

(a) In cases of apparently curative resection of the colorectal cancer, the rise in the serum levels of CEA precedes clinically evident relapse by several months. Scintigraphy using labelled anti CEA antibodies may detect the recurrent sites at an early stage.

(b) In cases of recurrence, the immunoscintigraphy may reveal an extensive disease and save the patient from second laparotomy.

(c) Recurrence in the scar may be detected and removed.

(d) An apparently benign rectal polyp may give a positive image and resected extensively like a malignant lesion.

During the last few years, the anti-CEA colorectal scintigraphy has been fairly standardized and kits are now commercially available.

RADIOIMMUNOASSAYS

Tumour markers

Tumour markers are chemical substances mostly proteins, which are associated with the presence of certain specific tumours in the body. These substances are usually present in the serum of normal subjects in low quantities. With the appearance of specific tumours, and secretion of these substances in large quantities by the tumour cells, the serum levels of these tumour markers rise above the normal values. Regression of the tumours results in their return to normal values. Any increase in the level of a particular tumour marker after the patient has been successfully treated would indicate relapse. There are two important tumour markers related to gastro-intestinal tract and the liver malignancies which can be measured by RIA technique. These include the carcinoembryonic antigen and Alpha-fetoprotein.

Carcinoembryonic antigen (CEA)

This antigen is a glycoprotein with a molecular weight of approximately 180 000, which is produced in appreciable amounts by the fetal large intestine. In adults, the CEA may be present in serum in small quantities usually not exceeding 4 ng/ml, but in the presence of certain malignancies the serum concentration may rise to high levels. The malignant tumours of gastro-intestinal tract and other sites such as pancreas, ovary, breast, lung and uterus may be associated with excessive serum levels or CEA. Very high levels of CEA are often an indication of spread of tumour to the liver.

CEA levels in serum may be raised in a number of non-malignant conditions. For instance about 14% of chronic cigarette smokers have elevated serum levels of CEA. Patients with some non-malignant tumours may also have raised levels. 15 - 20% of subjects with inflammatory disorders such as ulcerative colitis, Crohn's disease, pancreatitis, acute and chronic liver diseases and lung infections show elevated serum levels of CEA. In spite of the fact that raised serum CEA is not specific to malignant tumours, very high concentrations e.g. above 20 ng/ml are highly suggestive of malignancy.

The CEA test is not useful as a screening procedure to detect cancer in general population or in an otherwise asymptomatic person. However, the CEA test is now well-accepted as the best non-invasive test which may be used to assist in the management of patients with colorectal, breast, lung and other cancers.

The CEA serum assays by radioimmunometric techniques are being widely used even in the developing countries by using commercially available kits. Whenever possible the patients must be given the benefit of this useful investigation. It is possible to reduce the cost of these assays by using bulk reagents.

Alpha-fetoprotein (AFP)

Alpha-fetoprotein is a specific fetal serum alpha-globulin which consists of a single polypeptide chain and is composed of 96% protein and 4% carbohydrate. Initially during the fetal life the AFP is produced by the yolk sac and the fetal liver. By 13 weeks of gestation, the fetal plasma concentrations of AFP reach peak level of approximately 3000 μg/ml which is totally derived from the hepatic origin. Subsequently the AFP levels decline and reach a level of approximately 80 μg/ml at birth, and 0 - 20 ng/ml at the age of two years. This level is maintained for the rest of the life.

Elevated levels of AFP are found in many patients of hepatocellular carcinoma and teratoma of the testis or the ovary. Measurement of the serum AFP levels can be useful in the diagnosis and as a baseline information in these patients in following the response to treatment, which if successful results in decrease in AFP levels in serum. Similarly any rise of the AFP levels during follow-up would indicate a relapse or an uncontrolled disease.

AFP is not entirely specific to hepatocellular carcinoma and teratoma as elevated AFP levels have been seen in liver cirrhosis and viral hepatitis probably associated with liver regeneration. However, persistently elevated level in excess of 1000 ng/ml are strongly suggestive of presence of hepatocellular carcinoma or teratoma.

Hepatitis B surface antigen

Hepatitis B surface antigen (HBsAg) is a 22 nm particle, which can be produced by the virus in the human body in large amounts. It is the outer coat of Hepatitis B virus (HBV) and is probably not infectious as such. Nevertheless, finding of HBsAg in the blood is regarded as an indicator of the presence HBV. All patients, who receive blood containing HBsAg, do not develop hepatitis, but the risk that they will develop hepatitis is very high. This close association between detection of HBsAg in the blood and the transmission of hepatitis B led to the realization that all blood to be transfused should be screened for HBsAg. Thus a lot of work was done to develop the assay procedures for the detection of HBsAg. Since the use of screening procedures, the incidence or transfusion induced hepatitis has dropped sharply. Moreover it has been established that a sensitive screening test like Radioimmunoassay (RIA) can result in a very significant decrease of transfusion hepatitis.

There are several methods, which are employed for screening HBsAg from blood. Most commonly used are:

(1) Latex agglutination

(2) Red Cells agglutination

(3) Enzyme Immunoassay

(4) Radioimmunoassay

Radioimmunoassay is considered to be the most sensitive assay for the detection of HBsAg, and is based on the principle that ^{125}I conjugated to anti-HBs can act as a good indicator for the presence of HBsAg in the antigen - antibody reaction. In this method anti-HBs is coated on to a solid phase e.g. polystyrene beads. This is done by dipping the beads overnight in anti-HBs solution buffered at pH 9.5. The specimen to be tested for HBsAg is poured on to the anti-HBs coated beads in a tube and incubated overnight at room temperature. After the incubation the beads are washed and ^{125}I labelled anti-HBs is added and incubated again for one to two hours depending upon the method used. At the end of this, the beads are washed again and binding of ^{125}I is measured by a gamma counter. The number of counts are directly proportional to the amount of HBsAg present in the sample tested. This is known as Sandwich method.

Routine screening of the patients with HBsAg resulted in the discovery that many people have HBs in their blood. These people are healthy and are asymptomatic carriers of HBsAg. Test should be repeated to confirm the presence of HBsAg in their blood. If the antigenemia lasts for more than six months and the persons are found positive for HBsAg in two serum samples obtained six months or more apart, they should be considered as HBsAg chronic carriers.

It has been found that the numbers of HBsAg chronic carriers are high among the following groups:

(1) Drug Abusers

(2) Homosexuals

(3) Patients in renal dialysis and oncology units

(4) Recipients of blood transfusions

(5) Recipients of organ transplants

(6) Persons in lower socioeconomic areas.

Though the male population has been found to be positive for HBsAg twice as often as female population, it is essential that if the pregnant female is found positive for HBsAg, her neonate should be vaccinated within seven days after delivery so that the infant be protected.

In dialysis centres, separate machine should be used for HBsAg positive patients, so that cross contamination be avoided.

HBsAg chronic carriers should be given the following instructions:

(1) The blood of the person should be checked every 6-12 months.

(2) The person should be told NOT to donate blood.

(3) The person should not handle blood or blood products and his blood should be considered infectious and all precautions should be taken to prevent the spread of the infection.

Although HBV (Dane Particles) has been shown to have other serological markers such as anti-HBs, anti-HBc, HBeAg and anti-HBe, and the tests for these markers are also available, these markers are not used in the routine screening of the blood for hepatitis B.

It is worth mentioning here that an association exists between the presence of HBsAg carrier state and cirrhosis of liver and to the eventual development of Primary Hepatocellular Carcinoma. It has been found that the rate of development of Primary Hepatocellular carcinoma is much higher among the people, who are HBsAg chronic carriers than those who are negative for HBsAg.

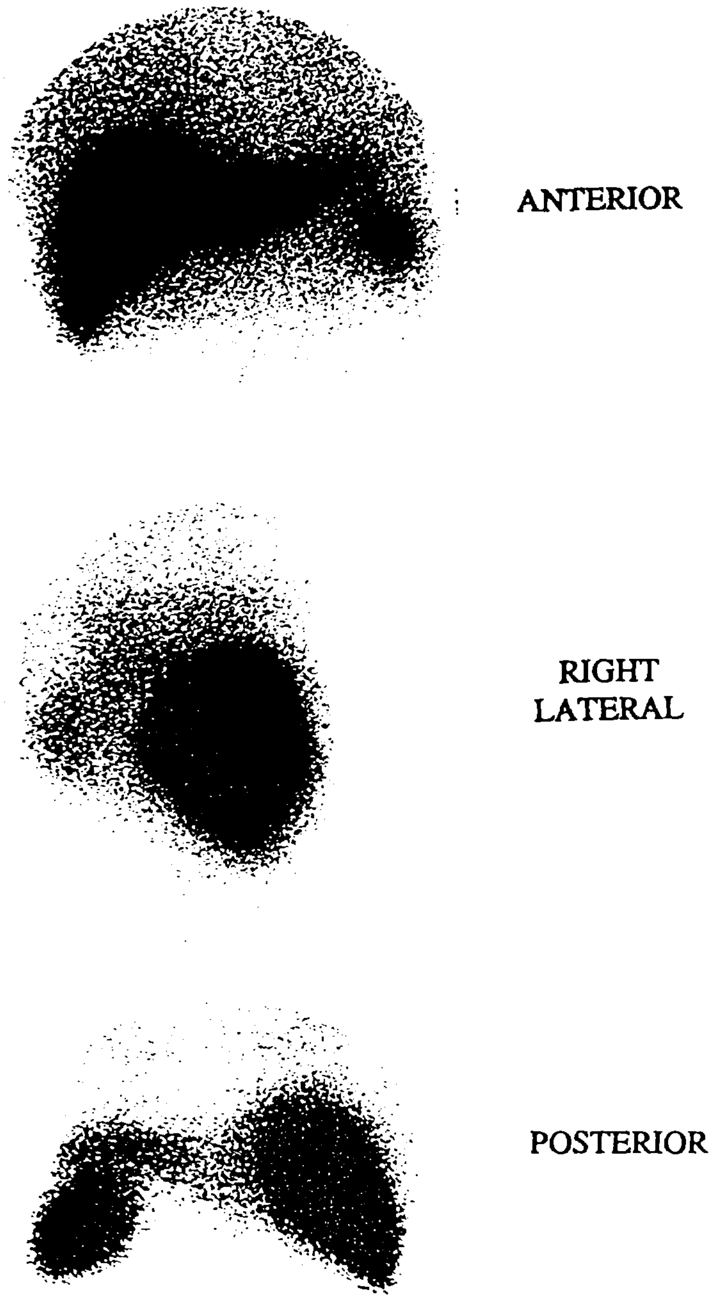


Fig. 28.1 Normal radiocolloid spleen / liver scan. Anterior, right lateral and posterior views.

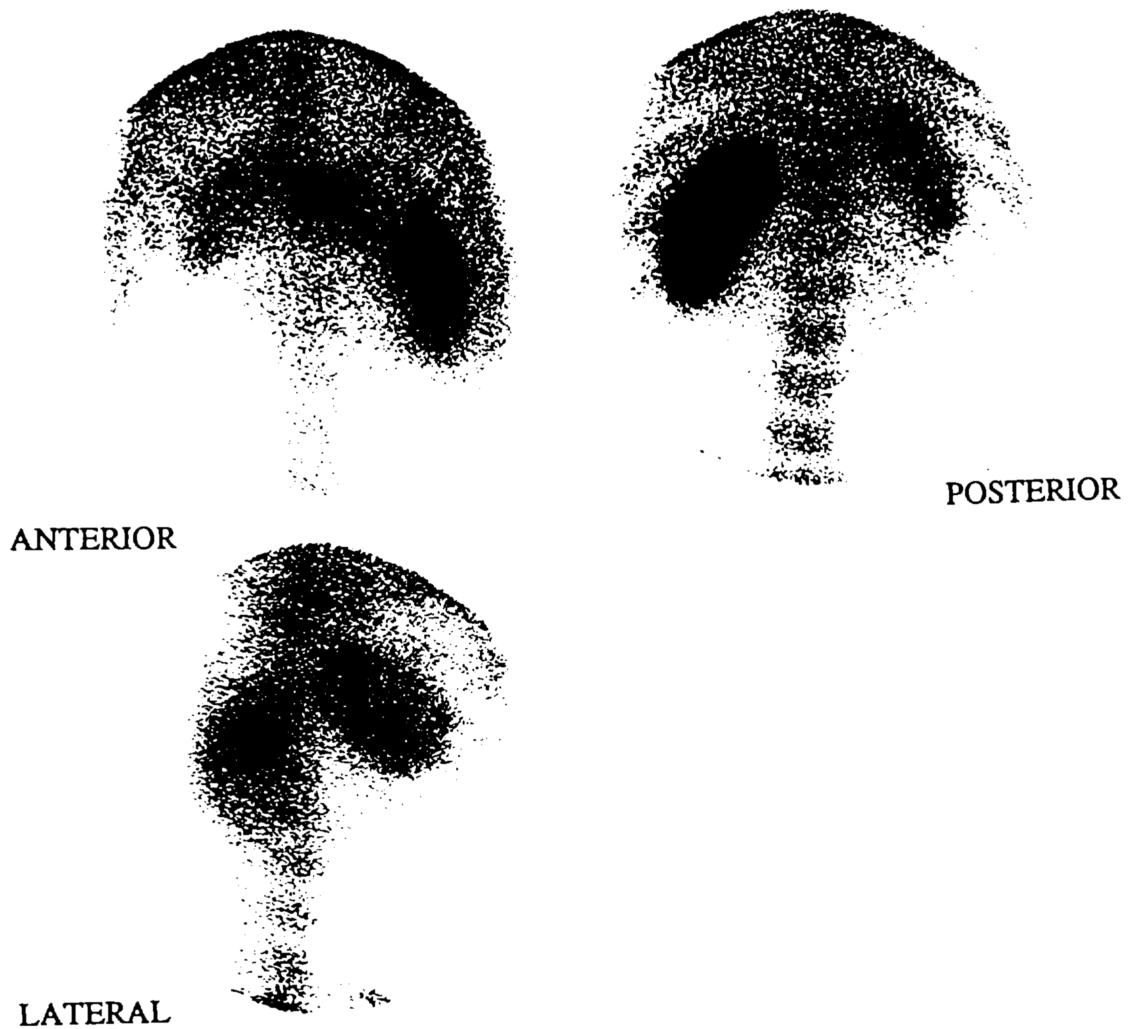


Fig. 28.2 Radiocolloid liver scan showing Cirrhosis of the liver. Note the shrunken liver and marked 'spill-over' to the spleen and visualization of the bone marrow.

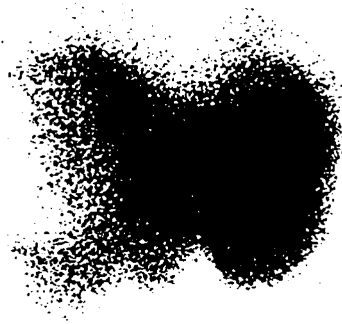


Fig. 28.3 Radiocolloid liver scan of a patient with large liver abscess.

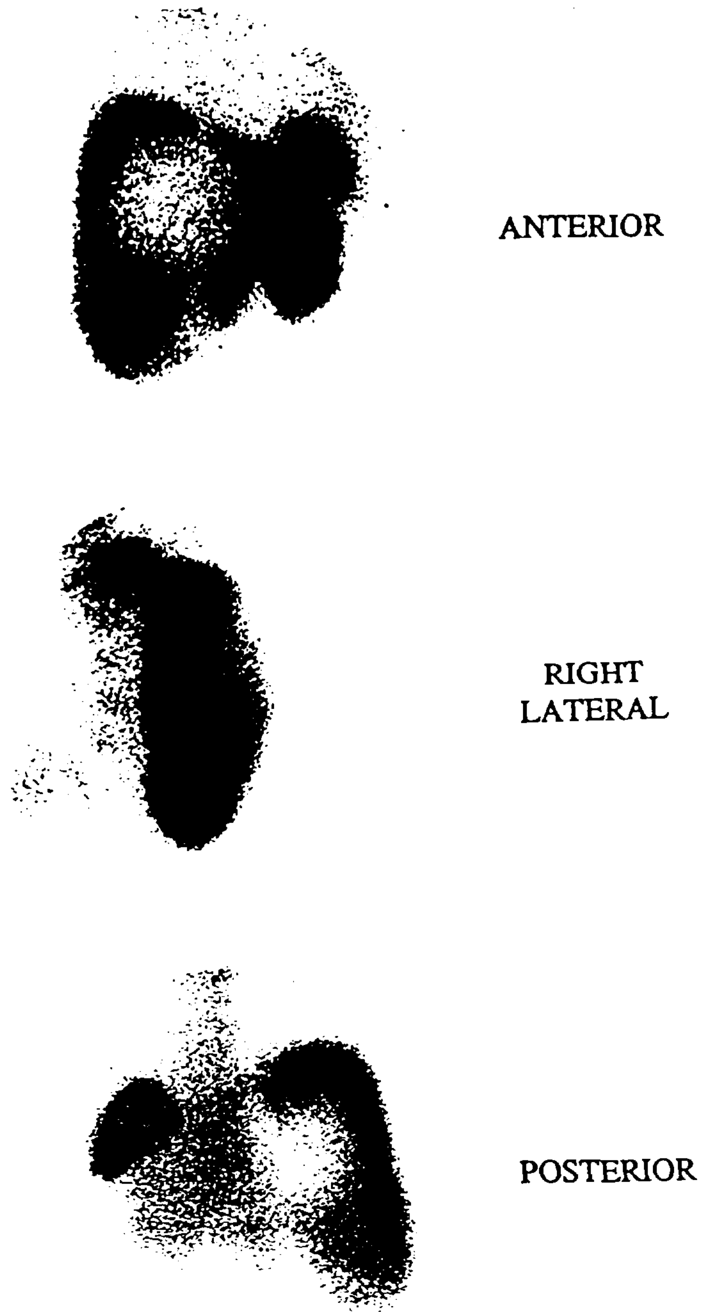
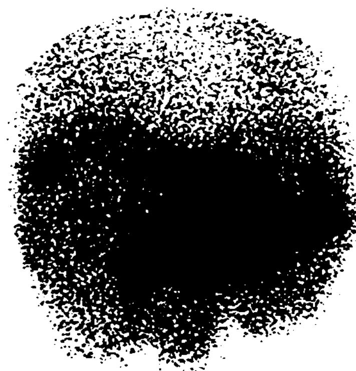


Fig. 28.4 Radiocolloid liver scan of a large hydatid cyst.



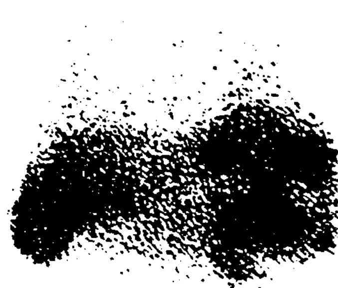


Fig. 28.5 Radiocolloid liver scan showing multiple metastases from primary lung cancer.

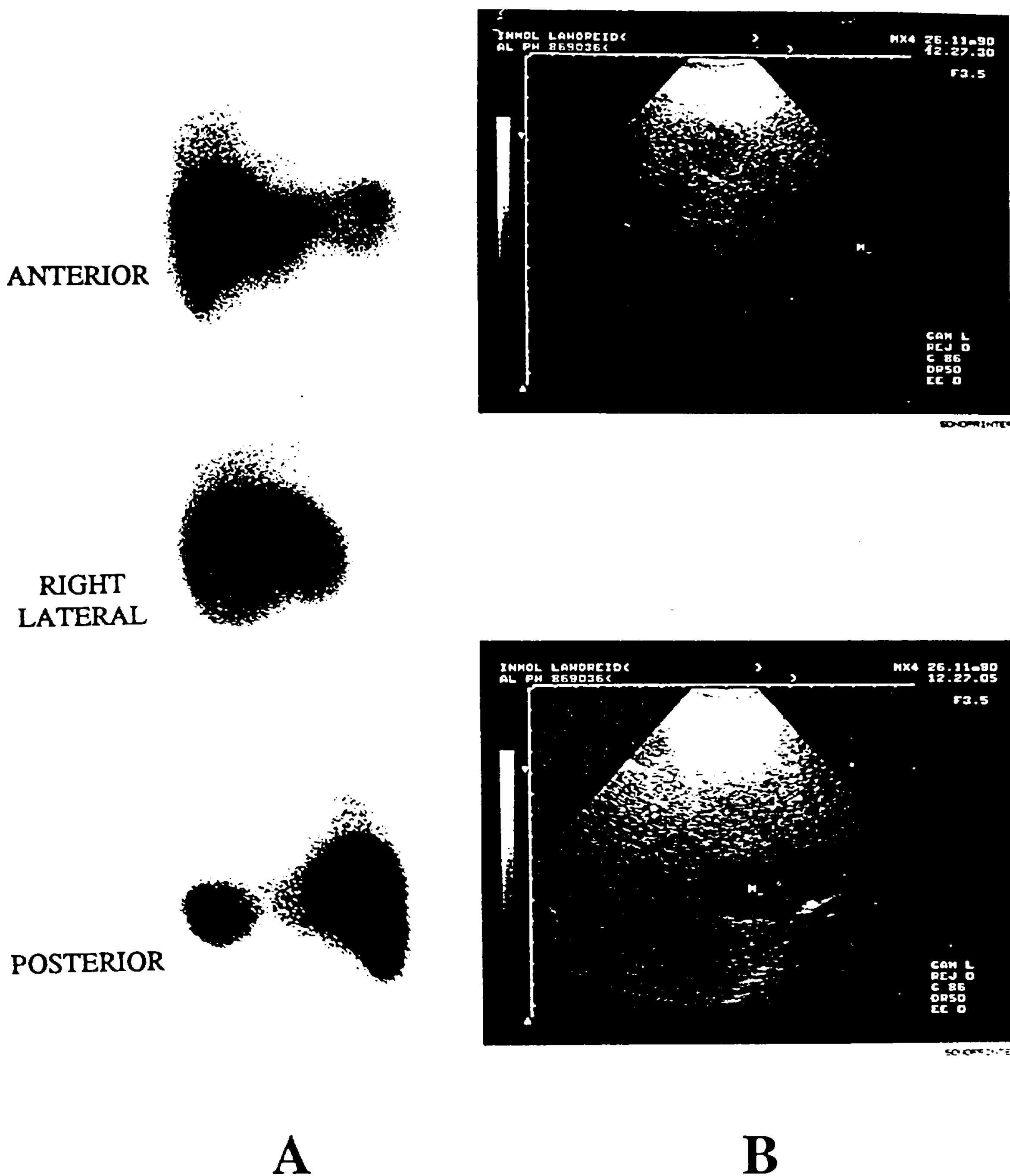
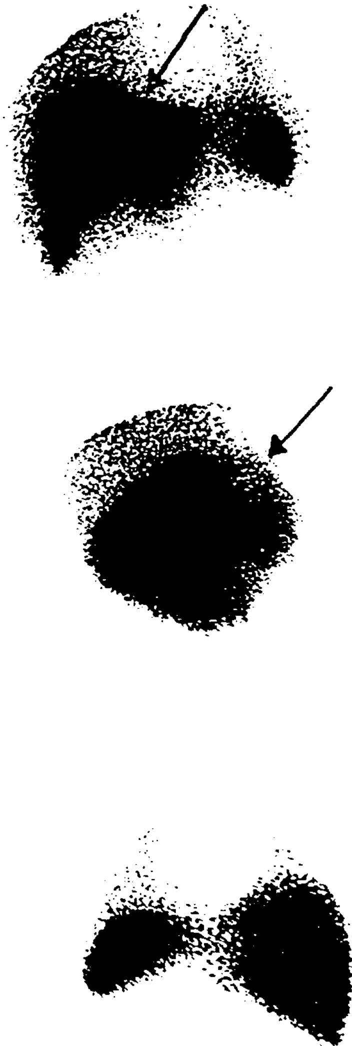
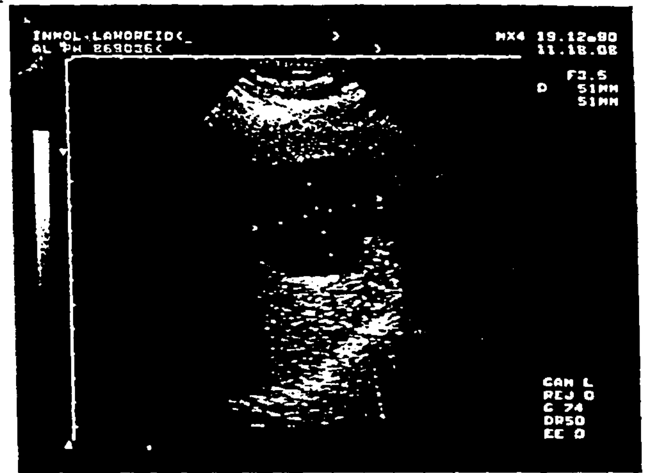


Fig. 28.6 A. Radiocolloid liver scan shows no evidence of a focal defect.

Fig. 28.6 B. Ultrasound examination of the same liver showing a focal lesion.





A **B**

Fig. 28.7 A. Radiocolloid liver scan of a case of breast cancer showing a focal defect in the upper medial part of the right lobe which was considered to be a metastatic deposit.

Fig. 28.7 B. Same patient. Ultrasound scan shows the lesion to be cystic.

Fig. 28.8 A normal hepatobiliary study using $^{99}Tc^{m}$ - HIDA.

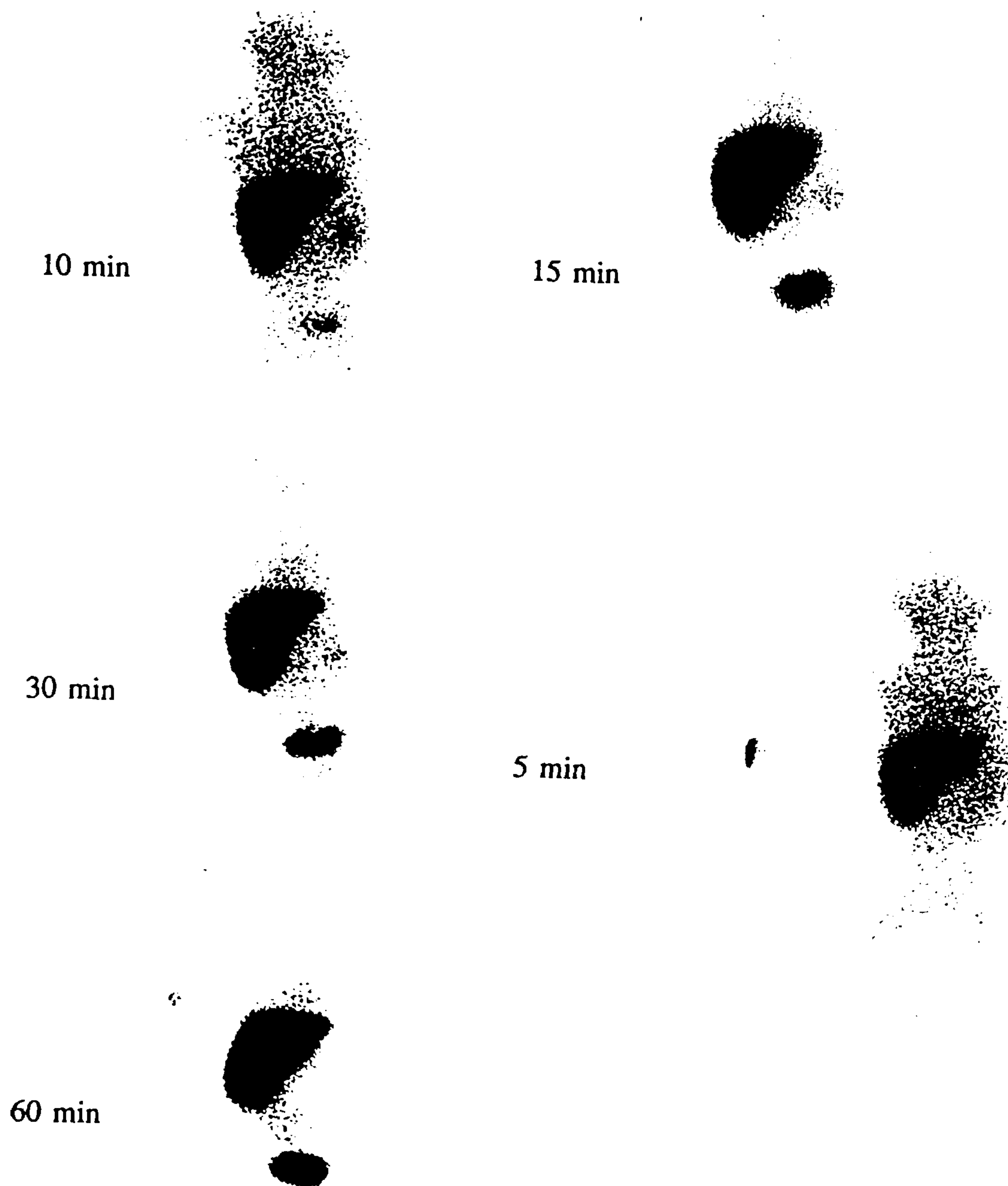
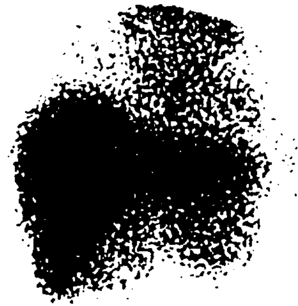


Fig. 28.9 Hepatobiliary study in an infant with biliary atresia. Note satisfactory liver uptake and non-visualization of the gall bladder, bile ducts and duodenal loops. The entire activity is excreted through the kidneys.

ANTERIOR
10 min



ANTERIOR
30 min

ANTERIOR
60 min

Fig. 28.10 Hepatobiliary study showing a non-functioning gall bladder in a case of acute cholecystitis.

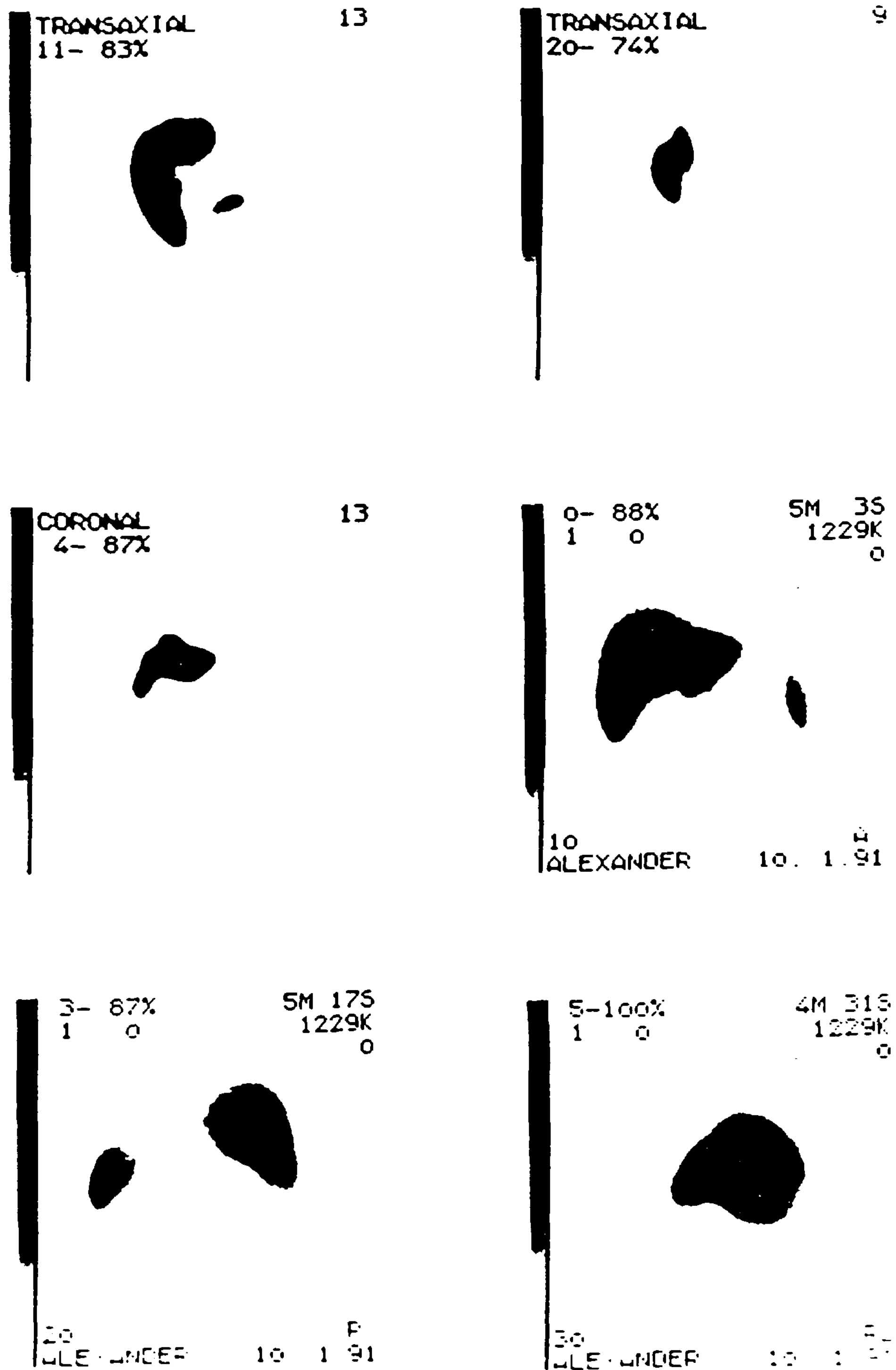


Fig. 28.11: SPECT imaging of liver The small lesion seen in the Coronal section was not detected in the planar images. Biopsy of lesion shows hepato cellular carcinoma.

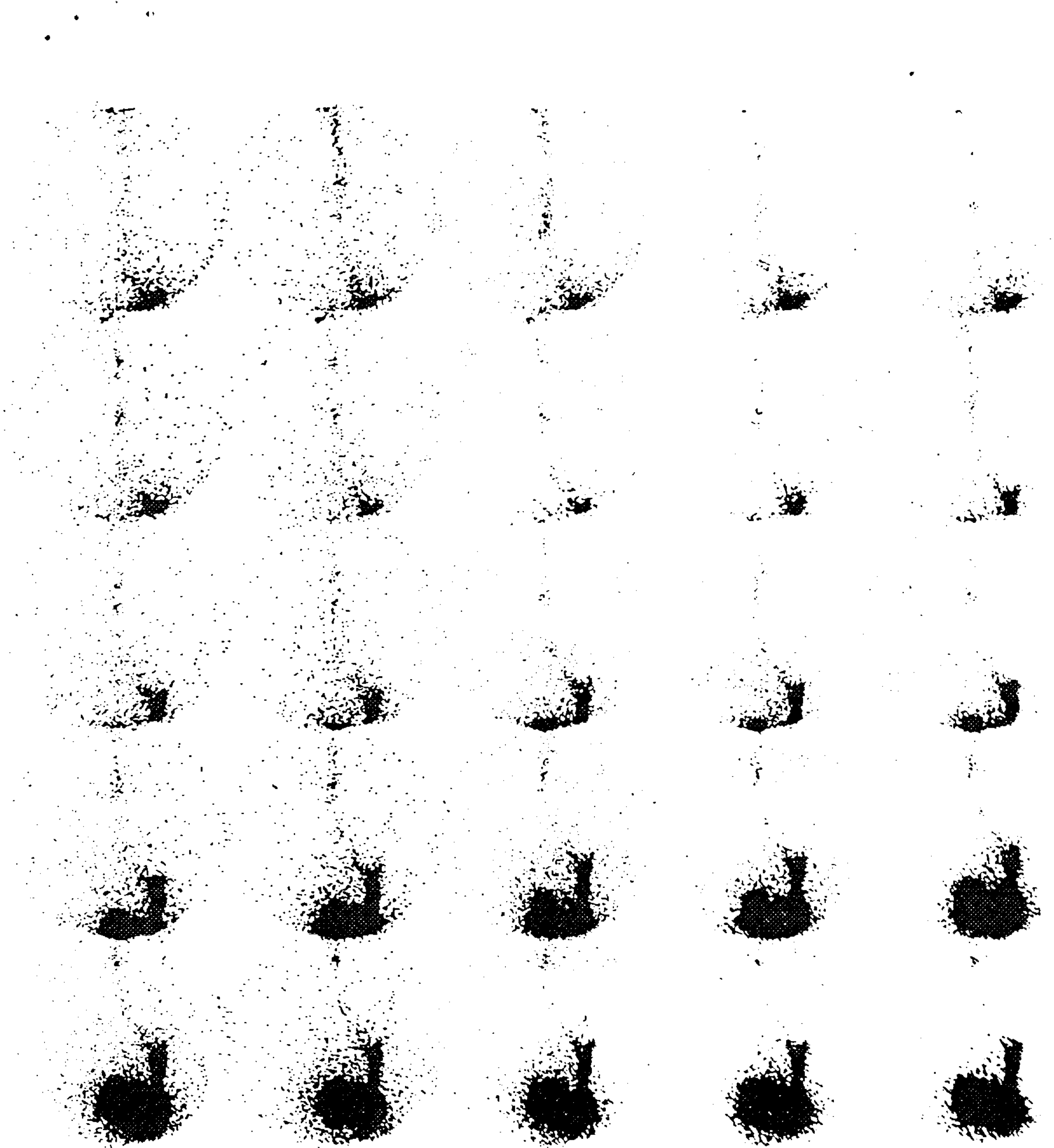


Fig. 28.12 Gastro-oesophageal reflux study showing the filling of the lower half of the oesophagus with the regurgitant radioactive meal.

Chapter 29

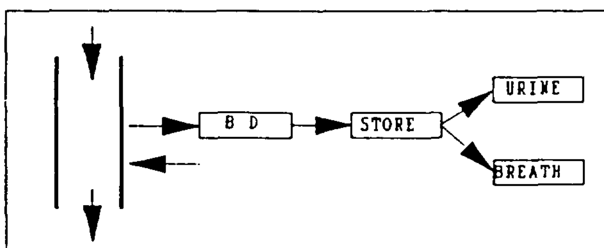
ABSORPTION STUDIES

R.D. Ganatra

Introduction

Absorption studies were once quite popular but hardly anyone does them these days. It is easier to estimate the blood level of the nutrient directly by Radioimmunoassay (RIA). However, the information obtained by estimating the blood levels of the nutrients is not the same that can be obtained from the absorption studies. Absorption studies are primarily done to find out whether some of the essential nutrients are absorbed from the gut or not and if they are absorbed, to determine how much is being absorbed. In the advanced countries, these tests were mostly done to detect pernicious anaemia where vitamin B_{12} is not absorbed because of the lack of the intrinsic factor in the stomach. In the tropical countries, "malabsorption syndrome" is quite common. In this condition, several nutrients like fat, folic acid and vitamin B_{12} are not absorbed. It is possible to study absorption of these nutrients by radioisotopic absorption studies.

The primary requirement of an absorption study is a labelled nutrient. The radionuclide label should be stable enough so that it is not dissociated in the gut. The basic principle of the tests is quite simple. Labelled nutrient is administered orally to a fasting subject and for quantitative determination total collection of faeces is required for several days. Most of the technology has evolved around finding an appropriate method for reliably counting the collected faeces and counting the administered dose as a standard in an identical geometry. Total collection of the faeces is always unreliable; particularly so in the developing countries where suitable toilet facilities are not usually available to the patient. This difficulty undermines the diagnostic usefulness of these procedures. In addition, counting of faeces is always an unpleasant task and that also limits their popularity.



Characteristics of the tracer used in absorption studies:

(a) Radioactive label should be stable. It should not be broken down in the gut, nor should it be re-excreted in the gut.

(b) The carrier associated with the tracer should be low and constant from one study to the other. For example, in case of vitamin B_{12} it should be 1 μc per 1 μg.

(c) Natural label is better than an artificial label because it is less likely to break down. Cobalt radionuclide is a natural label for B_{12} but ^{131}I is an artificial label for

triolein, used for fat absorption studies. In the latter case, the label might alter the metabolic characteristics of the nutrient.

Methods of counting faeces:

(a) liquify and then homogenize. Definitely not favoured by any one.

(b) large crystal counter with sample in front or on top of the detector.

(c) sample between two crystals.

(d) annular counter; GM, plastic, or liquid scintillation counter.

All these methods have the problem of counting the standard in a comparable way. Unless the patient referral for absorption studies is large, none of the above methods is cost effective.

Schilling's test:

This has been the most widely used test for B_{12} absorption, especially for the diagnosis of pernicious anaemia, where a qualitative answer is acceptable. Protocol for the test is described in the chapter on Haematology. About 1 μc of the labelled B_{12} is given orally. After 30 minutes, a dose of 1 mg of stable B_{12} is given parenterally, which doesn't allow the labelled B_{12} to settle down in the stores but flushes it out in the urine. Limitations of the test are that it provides a semi-quantitative answer only, 24 hours urine collection is also not likely to be reliable and the flushing dose is in the therapeutic range, likely to vitiate all other related investigations.

Plasma counting:

Counting of the radioactivity in the plasma sample¦weight various times after the administration of the labelled nutrient is also not favoured. Because of the dilution, the counts from an aliquot of blood sample are usually very low.

External counting.

Organ counting for some substances is possible when the organ is conveniently located for external counting (e.g. liver for B_{12} and iron) and where it is known that the nutrient after absorption is primarily stored.

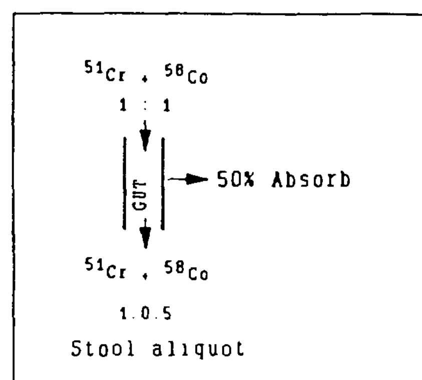
Whole body counting:

This is reliable but possible only in case of those nutrients where they are not rapidly excreted from the body e.g. B_{12} or iron. The counting will have to be carried out for several days till a stable count rate is obtained. The counts in the patient immediately after the administration of the oral dose are taken as standard counts but many investigators feel that the ideal time for this would be after the distribution of the substance in the body which is, unfortunately, not a precise point and varying from patient to patient. The method is not

cost-effective unless the counter is used for other purposes also. If the counter is used for health physics monitoring, the physicists will not tolerate the presence of the patient with administered radioactivity in their counters.

Double tracer technique:

This is an ingenious method to get a quantitative answer by faecal counting without total collection of the faeces. In the method for B_{12} absorption along with the labelled B_{12}, a non-absorbable marker like radioactive chromic chloride is administered to the patient. Ratio of the two substances in the standard and in an aliquot of the faecal sample obtained at 24 hours allows computation of the amount absorbed. The method has been tried extensively for B_{12} but can be used for any labelled substance where the energy characteristics of the radionuclide are such that it can be differentiated from that of the unabsorbable marker. The method requires careful spectrometry and a good understanding of the energy spectra of the radioisotopes. The method has been applied for Iron absorption by simultaneous administration of ^{55}Fe and ^{59}Fe, one by the oral route and the other by the intravenous route and by differential counting of a blood sample.



Radiorespirometry:

The most common application is breath analysis after administration of a ^{14}C labelled nutrient like fat. The breath is collected in a liquid scintillation vial which traps a constant amount of $^{14}CO2$. There is a general reluctance to use ^{14}C in human subjects and this method has never been popular as a clinical diagnostic tool.

Absorption of Bile Acid from the gut:

This is homocholic acid, an analogue of naturally occurring taurocholic acid, labelled with 75Se (commonly known as SeHCAT). Normally it is absorbed from the terminal ileum, passes through the liver, excreted in the bile and remains confined to the enterohepatic circulation. If there is no absorption in the ileum, it will be excreted in the faeces. It is usually administered in 10 μc doses and the patient measurements done in a whole body counter. The first count is for the 100% value and the second count is after seven days. It traces bile acid metabolism and is useful in assessment of ileal function in chronic diarrhoea, Crohn's disease, and after intestinal surgery. In the absence of a whole body counter, attempts have been made at quantitation by placing a gamma camera on the abdominal region. The test is mostly of research interest and the requisite radiopharmaceutical is available from only one commercial firm.

Protein losing enteropathy

This condition is also supposed to be quite common in the developing countries. The total faecal collection is unavoidable in the studies. The radioactive label should be stable and it should not get reabsorbed in the gut. For a quantitative study, it is essential to collect faeces for several days. It is therefore, necessary that the label has a long half life. Macromolecules which resemble protein are usually chosen, for example ^{131}I albumin, ^{131}I PVP, ^{51}Cr albumin, ^{67}Cu ceruloplasmin etc.

As the total collection of faeces is never a favourite with any one these tests are seldom done for routine diagnostic studies. From a plasma disappearance curve of the protein kinetics it is also possible to surmise if there is an abnormal leak from the plasma compartment.

For "malabsorption syndrome" and for the "protein losing enteropathy" no etiology is yet established and there is no specific therapy. This also might be a reason for these tests not being utilized to a great extent in a clinical nuclear medicine department. However, these tests are few of the non-imaging applications of nuclear medicine still surviving.

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Chapter 30

NUCLEAR IMAGING OF THE SKELETAL SYSTEM

Yong Whee Bahk

Introduction

Bone may well present as a mere inert weight-bearing scaffold of the human body to those who acquired the anatomical knowledge of the skeleton with the aid of dried bone specimens. Nevertheless, like all other active organ systems, the bone changes dynamically as it undergoes incessant turnover with modelling and remodelling through the physiological and metabolic activities of osteoblasts and osteoclasts. The principal roles played by these bone cells are the maintenance of the skeletal and calcium homeostasis by balancing between the ratio of bone collagen production and its resorption or destruction, and governing the mineralization processes. Collagen production is common to various connective tissues but mineralization is unique to bone cells.

Bone scintigraphy is one of the most valuable nuclear imaging procedure, especially remarkable for its high sensitivity in disclosing bone metastasis of cancer long before radiographic demonstration. Bone scintigraphy is also useful in the diagnosis of covert fracture, occult trauma, bone contusion, early acute osteomyelitis, acute pyogenic arthritis and avascular bone necrosis. Measurements of bone clearance of radiopharmaceuticals, absorptiometry and quantitative bone scintigraphy are applied to the study of metabolic bone disorders such as osteoporosis and osteomalacia.

Physiology of bone

The living bone undergoes ceaseless turnover that is mediated by bone production and absorption through the activities of osteoblasts and osteoclasts, respectively. Bone turnover is well balanced and in a state of equilibrium unless disturbed by disease. When bone production is superseded by bone destruction (absorption), as in acute pyogenic infection or invasive malignant neoplasm, osteolysis ensues. In a reverse situation, osteoblastic reaction in the form of bony sclerosis or increased bony density may result.

There are five types of bone cells in the skeletal tissue. They are osteoprogenitor cells, osteoblasts, osteocytes, osteoclasts and bone-lining cells. All the bone cells are contained in bone matrix. Bone formation or osteogenesis is accomplished by the mineralization of organic matrix or osteoid tissue, which is composed of collagen (90%) and surrounding mucopolysaccharides. Bone formation is stimulated by physical stress and strain to the skeletal system, calcium regulatory hormones (parathormone, calcitonin), growth hormone, vitamins A and C and calcium and phosphate ions.

Bone resorption sets forth as the bone matrix has been denatured by the proteolytic action of collagenase which is secreted by osteoclasts. Then phagocytic ingestion and clearing of disintegrated organic material and freed inorganic mineral constituents come into play.

Factors that stimulate osteoclastic activity include physical disuse, hyperaemia, parathormone, active metabolites of vitamin D, thyroid hormone, heparin, interleukin-1 and prostaglandin E.

Bone scanning agents

The first radionuclide successfully used for the photoscanning of bone lesions was ^{85}Sr. Strontium acted as a substitute for calcium; hence its easy incorporation into the hydroxyapatite crystal rendered bone scanning possible. Inconveniently, however, ^{85}Sr had a long physical half-life of 65 days. Moreover, disturbing excretion of the agent after intravenous injection by the gut and kidneys during the first 24-48 hours distracted considerably the quality of scan image. Strontium-87m had a much shorter physical half-life (2.8 hours), but this was too slowly cleared from the blood resulting in prolonged high background activities with consequent low bone to background activity ratio. Fluorine-18 was a useful alternative to strontium but for its limited availability in ordinary nuclear medicine laboratories which were not located within easy reach of medical cyclotron or nuclear reactor.

It was not until the introduction of $^{99}Tc^{m}$ labelled stannous triphosphate complex that bone scanning became widely accepted. Within a short period of time, $^{99}Tc^{m}$ polyphosphate, pyrophosphate and diphosphonate were developed for general use. Chemically, the phosphate compounds contain as many as 46 phosphate residues (P-O-P), and its simplest form is pyrophosphate with two residues. The phosphonate is a compound with P-C-P bonds instead of P-O-P bonds. Of these the diphosphonates are the most widely used. They are now available in the form of $^{99}Tc^{m}$ hydroxy ethylene diphosphonate (HEDP) and $^{99}Tc^{m}$ methylene diphosphonate (MDP). The phosphonate compounds have strong avidity for hydroxyapatite crystals in the mineral phase of bone, especially at the sites where new bone is actively formed as in epiphyseal plates of growing long bones.

Following intravenous administration, $^{99}Tc^{m}$ labelled phosphates and diphosphonates become rapidly distributed in the extracellular fluid space throughout the body, approximately half of the injected radiopharmaceuticals are then fixed by bone while the rest excreted into the urine by glomerular filtration. The amount of the radiopharmaceuticals accumulated in bone at one hour after injection is 58% with MDP, 48% with HEDP and 47% with pyrophosphate.

Mechanism of bone adsorption

The mechanism of $^{99}Tc^{m}$ phosphate complex deposition in bone has not been fully clarified. However, it is known that the uptake and retention of $^{99}Tc^{m}$ diphosphonate complex are strongly influenced by such factors as metabolic activity, blood flow, surface bone area available to extracellular fluid and calcium content of bone. It has been demonstrated by autoradiography that the deposition of diphosphonate takes place almost exclusively on the surface of the inorganic calcium phosphate.

Imaging instruments

Gamma camera is now the most commonly utilized scintillation detection system for nuclear imaging of bones and joints. Normally for a whole body skeletal survey, parallel hole collimators are used but for amplifying the details of the suspected area, pinhole and low energy converging collimators are indispensable adjuncts. The pinhole collimator with 3-mm aperture seems the collimator of choice for image quality with high resolution for this purpose.

Before the gamma-camera era, the rectilinear scanner was used for radionuclide imaging in the diagnosis of bone and joint diseases. This instrument is still used in many nuclear medicine departments of the developing countries but unless one has a fast moving whole body version, it is almost impossible to do a skeletal survey with a rectilinear scanner. **Fig. 30.1** is a typical example of the black-and-white photoscan image made with a rectilinear scanner in 1960s. The rectilinear scanner is now nearly extinct. On the other end of development, is SPECT, which permits the separation of a selected plane or a small volume of tissue from other overlapping structures both in foreground and background. The separation of tissue in a thin slice can reveal detailed information regarding the distribution of radiopharmaceutical in bone and joint. SPECT is now commonly used in the study of complex structures of the skull, face, spine, pelvis and hip.

Bone scan technique

For static views, it is better to wait for three to four hours to let the non-target radioactivity get excreted in the urine. During this period, the patient is encouraged to drink plenty of fluids to promote rapid clearance of the radiopharmaceutical. The patient is instructed to void the bladder just before starting the scanning, otherwise the bladder activity will be very prominent in the pelvic view. Scrupulous attention should be given to avoid contamination of clothes and the limbs while passing urine.

The usual practice is to obtain first a single-pass or double-pass view of the whole skeletal system **(Fig. 30.2)**. Upon inspection of the whole-body scintigraph, spot views using low energy converging collimator or pinhole collimator are to be taken to detail the region of interest or equivocal findings. In most instances, anterior and posterior views, supplemented by a lateral or oblique view in special situations, are sufficient. It should be emphasized that pinhole collimator scintigraph is not only geometrically magnified but also provides high resolution of anatomy. **(Figs. 30.3A and 30.3B)**.

The three-phase dynamic scintigraphy is another important technique. By this, vascularity of a bone lesion can be quantitatively evaluated. Its typical application is in the differential diagnosis of infectious and non-infectious bone lesions. A recommended protocol includes an immediate nuclear angiography (16 consecutive frames of 2-4 second image), a blood-pool image at two minutes and a delayed static image at 2-4 and 24 hours after intravenous administration of 30 mCi(1.1 GBq) of $^{99}Tc^{m}$-MDP.

CHAPTER 30

NORMAL BONE SCAN

Logically, abnormality is an antithesis of normality. It is, therefore, essential to be thoroughly familiar with normal findings and the factors that affect normal findings to recognize true abnormalities. Abnormality in a bone scan is seen either as increase or decrease in radionuclide accumulation (RA), euphemistically described as "hot" or "cold" respectively. Of the many factors that distort scan findings, asymmetry about the mid-sagittal plane of the body with tilting of the image to one side is probably the most important. Even a slight difference in the target-detector distance produced by such a tilt can result in significant alteration of the scan image giving fallacious impression. **(Figs. 30.4A and 30.4B)**.

In normal subjects, greater RA occurs in the cranial vault, facial bone around nasal cavity, sternum, spine and around pelvis and hip. Prominent RA is also observed in the large joints of the extremities.

The sacroiliac joints show the greatest RA and this is due to constant, strenuous weight bearing.

Skull and Neck

Prominent RA can be seen in the cranium along the cranial tables and suture lines, around orbits and paranasal sinuses and in the unfused occipitosphenoid synchondrosis. Often the maxilla and mandible with a denture show increased RA. Various parts of the vertebra lie on the same transverse plane in the cervix (in the thoracic and lumbar regions, the vertebral body and posterior structures are on the different transverse plane) making their separation difficult on bone scintigraphy. The thyroid cartilage is not infrequently visible due to $^{99}Tc^{m}$-MDP uptake simulating the thyroid gland that has concentrated unbound $^{99}Tc^{m}$-pertechnetate. Occasionally, the hyoid bone can be also seen.

Thoracic Cage and Shoulders

The sternum is regularly visible. Greater RA can be noted in the sternoclavicular joint, manubriosternal junction and calcified costochondral junctions. Prominent accumulation can also occur in the coracoid process, acromioclavicular joint, glenoid process and inferior angle of the scapula. The clavicles are visible on the anterior view, whereas the rib cage and scapulae are more clearly visible on the posterior view.

Vertebral Column

Individual vertebra along with its small parts such as the pedicles, laminae, apophysial joints and spinous process can be visualized only by pinhole collimator. The kyphotic thoracic spine is more clearly seen in the posterior view, whereas the lordotic lumbosacral spine is well visualized in the anterior view.

Pelvis and Hips

The sacrum and iliac bone overlap at the sacroiliac joints, but they can be portrayed as separate structures along the joint space in the butterfly view. The iliac bone accumulates more radionuclide than the sacral part. RA in the pelvis and hips is symmetrical along the mid-sagittal line. The adult hip joints also present a problem of overlapping. The acetabular roof and femoral head superimpose each other. The RA is highest along the acetabular roof.

Extremities

The long bones are visible in scintigraph unless the subject is very old with advanced osteopaenia. RA is comparatively great in the bones around the shoulders, elbows, hips, knees and ankles. Pinhole collimator scintigraphy can image the small bones of hands and feet. The patella often shows greater RA. The growing bones of infancy and adolescence accumulate more radionuclides, particularly along epiphyseal lines. The greatest accumulation is seen in the long bones of the late teens before epiphyseal fusion takes place.

ABNORMAL BONE SCAN

Basically, the scintigraphic manifestations of bone abnormality can be described from three different view points viz. anatomy, RA patterns and vascularity. The anatomical changes are expressed in terms of size, shape and position, and RA or vascularities is either increased, unaltered or decreased. The majority of bone lesions show increased RA or "hot spot" and only a small fraction presents as photopaenic or "cold spot". Typical lesions that manifest as photopaenic area are avascular bone necrosis, photopaenic cancer metastases and multiple myeloma.

Altered biodistribution of radiopharmaceuticals

Significant dehydration, ascites, anasarca and renal and/or hepatic failure cause increased RA in the soft tissues of the body resulting in low bone-to-background ratio and poor bone image (**Fig. 30.5**). Unlabelled free $^{99}Tc^{m}$-pertechnetate and oxidation of the $^{99}Tc^{m}$-phosphate complex may increase background activities, and cause thyroid or liver uptake and alimentary tract excretion. Anticancer chemotherapeutic drugs, steroid and iron have been shown to suppress RA in bones. It is interesting to note that chemotherapy makes some bone lesions in the healing stage "flare up".

It should not be forgotten that normally kidneys and breasts show some concentration of the radiopharmaceutical. Abnormal accumulation in the soft tissues may also be seen in contused muscles, brain infarct, myocardial infarct, calcified lymph nodes and metastatic calcification of soft tissues in hypercalcemic states.

CHAPTER 30

CLINICAL APPLICATIONS

General Considerations

Nuclear bone imaging was initially utilized for the demonstration of metastatic cancer and fracture of bone. Since then, the scope of bone scintigraphy has enormously expanded, and the imaging techniques have become highly refined and versatile, both qualitatively and quantitatively. This is now the method of choice for screening, localizing and specifying many bone and joint diseases. The sensitivity and specificity can be significantly increased when scintigraphy is supplemented with nuclear angiography (three-phase test), pinhole collimator imaging and SPECT. Bone scintigraphy is particularly indicated in the diagnosis of cancer metastases, septic conditions of bones and joints, multiple trauma, occult fractures, diseases involving multiple bones such as fibrous dysplasia, enchondromatosis, Paget's disease, myelomas and patients with bone pain or fever of unknown origin. The arthritis that affect more than a single joint also constitute an important indication. The scintigraphic examination in combination with augmenting techniques is useful in assessing the extent and activity of rheumatoid or rheumatoid-related arthritis, osteoarthritis and spondylosis. The pinhole collimator scintigraphy has been shown to be of great value in the differential diagnosis of metastases, fractures, infections and degenerative diseases of the spine (**Fig. 30.6**).

Metastatic Bone Tumours

Bone metastasis of malignant neoplasms of both extra- and intra-osseous origin is the most widely accepted indication of bone scintigraphy. The purposes are the early diagnosis, evaluation of disseminated area, staging of the primary disease, prognostication and for follow-up of patients on therapy.

The majority of metastatic bone lesions are multiple, around 7% being solitary. Regarding the site of predilection, approximately 80% of cases involve the axial bones and the remaining 20% involve the skull and long bones in equal frequency. It has been known that cancers of the breast and prostate tend to disseminate via the vertebral veins to the spine, while lung cancer and thyroid spread haematogeneously to random sites in the skeleton. Some metastatic bone lesions from anaplastic carcinomas of the kidney, breast and lung produce photopaenic or "cold spot". The incidence of cold-spot metastases was reported to be around 2% but with application of pinhole techniques, this incidence may be found to be higher. (**Figs. 30.8A and 30.8B**). Extensive skeletal metastases with diffuse increased RA give rise to the "superscan" sign (**Fig. 30.9**).

Bone scintigraphy is highly sensitive in detecting bone metastases. A positive bone scan predates negative radiograph by months. Nevertheless, there are cases with false negative scan. A painful focus with radiographically visible osteolytic change may not be visible in bone scan. Bone is reported to be normal in approximately 5% of skeletal metastases with radiographically evident osteolysis.

Certain scintigraphic features are helpful in distinguishing cancer metastases from benign conditions. Multiple vertical band-like hot spots in the ribs lined in a row represent fractures **(Fig. 30.10)**, while an elongated transverse accumulation of RA in the rib is highly suggestive of cancer metastasis **(Fig. 30.11)**. A solitary hot spot in a rib is malignant only in 1.4%, and 90% of solitary lesions have a benign etiology such as trauma or postoperative irradiation. In contrast, 68% of solitary lesions in the axial skeleton are malignant. A solitary hot spot in the sternum in patients with known primary malignant neoplasm indicates metastasis when trauma can be excluded. A short segmental RA in the vertebral end plate or diffuse homogeneous uptake throughout the body of the vertebra suggests metastases **(Figs. 30.6 and 30.12)**, while a long arcuate uptake along the whole length of the end plate is rather characteristic of compression fracture **(Fig. 30.7)**.

Rationale for Bone Scintigraphy in Metastases

Ever since its initial clinical applications, bone scanning has been utilized for the diagnosis, evaluation of therapeutic effects and for prognosis of both extraosseous and intraosseous malignancies. There are still many unanswerable questions regarding the appropriate utilization and reasonable indications of this diagnostic modality in cancer patients. Of these questions, indication for bone scintigraphy in patients with known primary malignancy in the non-skeletal organs is probably the central issue. In spite of innumerable publications, there exist still much confusion and controversy over whether or not bone scintigraphy should be a routine procedure in cancer patients and how often a follow-up study should be performed. In particular, the utilization of bone scan for the detection of bone metastases in an early cancer is a subject of diverse opinion. These questions are of particular importance for the developing countries. Bone imaging is a time consuming procedure. Most of the nuclear medicine units in these countries have a single gamma camera and the perpetual question is how much time should be spared for bone scintigraphy on this one instrument. Whatever prescriptions arrived at on the basis of the Western data are not going to be practical for the developing countries.

Nonetheless, there are a few useful guidelines. The most important indication is bone pain in patients with known malignancy, especially when the tumour has a high propensity to metastasize to bone. Such tumours include cancers of the breast, lung, kidney and prostate. Probably the next most important indication is a baseline documentation before starting an anticancer regimen.

Bone scintigraphy as a part of tumour staging constitutes another important indication. This indication, however, is valid only for tumours with a tendency to metastasize to the bones.

A follow-up period after the initial negative bone scan has been variously prescribed from one year to five years. The follow-up period should be shorter for cancer that metastasize rapidly, haphazardly and with high frequency.

Primary Malignant Bone Tumours

As far as specific and differential diagnosis of primary bone tumours is concerned, the yield of bone scintigraphy is much lower than that of radiography. This is simply due to the difference between the resolution in imaging achieved with these two modalities. Scintigram can not replace or substitute radiography particularly when definition of fine structures,such as trabeculae and periosteum, is required. Nevertheless, bone scintigraphy can demonstrate altered bone turnover which is not evident on radiograph permitting early diagnosis of metastasis. This may also help distinguish some bone tumours from inflammatory processes. Another advantage is that bone scintigraphy can detect bone-to-bone metastasis and assess local extent of primary neoplastic focus. Osteogenic sarcoma and Ewing's tumour are well known indications in this respect.

The use of bone scintigraphy may be encouraged in the study of suspected multiple myeloma since a negative bone scan in the presence of multiple punched-out osteolytic lesions in bone radiograph strongly suggest myeloma.

Benign Bone Tumours

Bone scintigraphy can not play a decisive role in the diagnosis of bone tumours because of its low specificity. However, it may have helpful findings in some benign tumours. One important indication is detection of suspicious pathological fracture.

Among the bone tumours that may be indications of bone scintigraphy are bone cyst, enchondroma, osteoid osteoma and giant cell tumour (osteoclastoma). In general, there are no pathognomonic findings of benign bone tumours or tumorous conditions in scintigraphy. Nevertheless, the diagnosis may be suggested by bone scintigraphy in some diseases. For example, ring-like RA along the periphery of photon deficient area in the end of a long tubular bone is highly suggestive of bone cyst. Hot spots within hot area is pathognomonic of Osteoid osteoma. This pattern can indeed be well demonstrated in pinhole scintigraphy (Fig. 30.13) Bone islands or pacchionian depressions in the skull may sometimes impose the problem of differential diagnosis from metastatic bone lesions. Neither of them concentrates radiopharmaceutical. Eosinophilic granuloma, the most common and benign variety of histiocytosis X, shows considerable RA.

Traumatic Bone Lesions

It may be justified to state that one of the most widely accepted indication of bone scintigraphy is traumatic and sports injuries of the musculoskeletal system. This statement is particularly valid for stress fracture, bone contusion, covert fracture of the ribs, sternum, lumbosacral spine and acute skeletal muscular injuries. Most of these lesions are either elusive or invisible in a radiograph.

The ordinary spot scintigraphy made with general purpose low-energy converging collimator is sufficient for the detection of stress fracture or covert fracture. For the

demonstration of more specific features of injuries, however, pinhole collimator scintigraphy is necessary. **Fig. 30.14** is a case of stress fracture accompanied by periosteal reaction in the tibia. Acute bone contusion may be defined as blunt trauma to bone without fracture so that condition will not be diagnosed by radiography. Bone scintigraph is of great value in this condition since it reveals intense RA uptake in the contused bone **(Figs. 30.15A and 30.15B).**

OSTEONECROSIS AND RELATED DISEASES

Avascular Bone Necrosis

This is one of the frequent indications of bone scintigraphy. Bone necrosis is a state in which the bone cell is devitalized along with its neighbouring bone marrow cells resulting from deprivation of blood supply. Hence, the process is termed avascular or aseptic. The single most important mechanism of avascular bone necrosis is vascular injuries, causes of which are either direct or indirect. Among the direct causes are trauma, embolism, thrombosis, elevated bone marrow pressure and vasculitis. Caisson disease, sickle cell anaemia and radiation necrosis are well known clinical entities. Indirect or probable associations have been related to steroid therapy, minor trauma, collagen disease, alcoholism and pancreatitis. The third category is osteochondrosis and ischemic necrosis, the aetiology of which is obscure.

Scan findings are rather specific when the dead bone is visualized as a photopaenic area within normal or reactive bone of, for example, the femoral head in Legg-Perthes disease **(Fig. 30.16)**. Pinhole collimator image is particularly suited for the study of bone necrosis as there will be a greater possibility of demonstrating even a small cold spot. As bone necrosis recovers and is revascularised, photopaenic area will begin to concentrate the radionuclide. Thus, the bone scintigraphy is a valuable tool in assessing recovery in bone necrosis.

Avascular bone necrosis is an annoying and disabling complication of kidney transplantation. The incidence has been reported to range from 2% to 17%. It is presumed to result from exogenous use of corticosteroids. Bone necrosis in transplant patient affects most frequently the femoral head. Rarely are the bones of the knee involved.

METABOLIC AND RELATED BONE DISEASES

Metabolic diseases of bone result from vitamin deficiency or excess, endocrinopathy, disturbed calcium and phosphate metabolism and malnutrition. Osteomalacia and osteoporosis are two examples of metabolic disorders which are of special relevance to the developing countries. The former is a failure of calcium deposition in bone matrix, whereas the latter represents deficient matrix formation. Rickets is a special form of osteomalacia that affects

growing bones of infants and children due to vitamin-D deficiency. Osteoporosis is either generalized or regional. There is a third form of osteoporosis which is associated with endocrine disorders like hyperparathyroidism, hyperthyroidism and acromegaly.

Bone scintigraphy is significantly more sensitive than radiography in detecting osteomalacia, renal osteodystrophy and primary hyperparathyroidism. The relative sensitivity is, however, reverse in case of osteoporosis. On the whole, the high specificity and accuracy of radiography in the diagnosis of metabolic bone diseases are usually not achieved by bone scintigraphy.

Osteomalacia.

Osteomalacia is marked by softening of bone due to poor mineralization and excessive osteoid accumulation. The causes of osteomalacia include vitamin D deficiency or impaired intestinal absorption of calcium and phosphorus. The deficiency of vitamin D and minerals leads to inadequate calcification of osteoid, the net effect of which is demineralization and consequent bone fragility. In essence, osteomalacia is a disease of mature bone of adults, and its counterpart in infancy is rickets. Unlike rickets that occurs in unfused growing bones, osteomalacia affects the bones which have already been formed. In osteomalacia, uncalcified osteoid accumulates within fused bone resulting in diffuse osteopaenia. Thus, malacic bone easily sustains infraction or pseudofracture.

Bone scan shows diffuse increase in RA in skeletal system along with occasional focal hot areas indicating generalized osteopaenia and infarction OR pseudofractures respectively (Fig. 30.17).

Rickets

Histologically, uncalcified osteoid accumulates luxuriously in the metaphyseal end of actively growing long bones. The affected bone becomes cupped, widened and flared due to weight bearing.

At this stage, bone scan shows characteristic "chicken bone" appearance due to intense RA both in the epiphyseal ossification centre and widened metaphysis. As the condition improves, scan changes return slowly to normal over a period of months.

Osteoporosis

In osteoporosis the bone mass is reduced. It may be classified clinically into senile (postmenopausal) form, idiopathic form in males and idiopathic juvenile form. Senile osteoporosis in women is clearly related with the loss of stimulation by oestrogen, but definite hormonal relationship has not been established in men. Idiopathic osteoporosis of males has probable association with alcoholism and liver cirrhosis, which are commonly present. Idiopathic juvenile osteoporosis is an uncommon disease of self-limited nature. In osteoporosis, the spine and limb bones are easily fractured. Regional osteoporosis includes

disuse osteoporosis, reflex sympathetic dystrophy syndrome, transient regional osteoporosis and regional migratory osteoporosis. Other forms of osteoporosis are osteoporosis due to hyperparathyroidism, hyperthyroidism, acromegaly and multiple myeloma.

In general, bone scan is insensitive and nonspecific in the diagnosis of osteoporosis. Occasionally, however,"washed out" pattern of generalized osteoporosis can be seen. This pattern is encountered in patients with little or no osteoblastic activity. For quantitative analysis, radiograph or more sophisticated methods like single or dual photon absorptiometry are to be resorted to.

Bones are brittle and prone to fracture in osteoporosis. In bone scintigraph, fractured bone shows marked RA. Osteoporotic vertebra is particularly liable to compression fracture, which produces characteristic pathognomonic sign of arcuate "hot end plate" in pinhole collimator scintigraph (Fig. 30.7).

Disuse Atrophy of Bone

When a limb is immobilised for a certain period of time, bone mass becomes reduced due to increased endosteal bone resorption from the loss of stimulation. Disuse atrophy is a type of osteoporosis, in which remodelling is accelerated. Disuse atrophy of bone is a frequent occurrence in hemiplegia, paralysis or immobilization. Bone scan shows increased RA in the bones around large joints, which have been in disuse.

Reflex Sympathetic Dystrophy Syndrome

The syndrome is known also as Sudeck's atrophy or causalgia. Aetiology is usually trauma with or without bone fracture. Infection and peripheral or central nervous system abnormalities may precipitate the disorder. Clinical symptoms include pain, often severe and incapacitating, and skin atrophy with glistening appearance. In 69% of cases, radiograph demonstrates patchy and mottled pattern of deossification or osteopaenia which is similar to the bone changes seen in severe disuse atrophy. Bone scan is characterized by marked RA in the affected joint representing hypervascularity. Radionuclide angiography reveals increased blood flow to the lesion.

Bone Irradiation

Irradiation can induce vasculitis and vascular stenosis, which, in turn, leads to osteitis and osteonecrosis, The ischemic bone becomes photopaenic in scintigraph, as early as few months after radiation therapy, and after a cumulative dose as low as 2000 rad.

Another possible effect of irradiation in bone are on osteoblasts. Damaged bone cells may die causing osteonecrosis. Radiation osteitis is considered to be secondary to a combination of irradiation, infection and trauma. The mandible, clavicle, humeral head, rib and femur are usual sites of such osteitis. Scintigraph reveals intense RA in the afflicted bone.

CHAPTER 30

INFLAMMATORY BONE DISEASES

Osteomyelitis, cortical bone abscess, osteitis and bone tuberculosis constitute major inflammatory bone diseases. Osteomyelitis is an acute febrile bone disorder of infancy and childhood. Inadequate or even properly treated acute osteomyelitis may persist or recur as chronic form. In some patients, acute osteomyelitis is walled off by reactive bone to form abscess. Cortical bone abscess is a special type of pyogenic bone infection in which infectious focus is harboured within the bone cortex. Osteitis is either infective or noninfective. Infective osteitis is usually concomitant to osteomyelitis. Noninfective osteitis includes condensing osteitis, radiation osteitis and others. Tuberculous osteomyelitis is predominantly a problem of the developing countries. It usually affects the spine and joints. Fungi, parasites and viruses also produce osteomyelitis. In drug addicts, pseudomonas, Klebsiella and enterobacters are prevailing offenders.

Acute Osteomyelitis

This is primarily a pyogenic infection of bone marrow with subsequent spread to periosteum, cortex and neighbouring soft tissue. The lesion is associated with frank osteolysis. The unmatched value of bone scintigraph in early detection of acute osteomyelitis has been well documented. Nuclear angiography demonstrates increased blood flow and blood pool (**Fig. 30.18A**). Static bone image reveals prominent RA in the affected bone (**Fig. 30.18B**). Such triple-phase bone scintigraphy can be utilized as an excellent discriminator between osteomyelitis and cellulitis which is not attended by bone infection. In the latter condition, blood flow and blood pool are increased but there is no significant RA in the bone. Chronic osteomyelitis has been related to the persistence of infective organism in the haversian system. In adults, acute haematogenous osteomyelitis frequently affects the spine, and pyogenic spondylodiscitis ensues. Typically, pyogenic spondylodiscitis involves the end plates of two opposing vertebrae with the disc sandwiched in between. Although infrequent, acute haematogenous osteomyelitis can involve multiple bones.

The sensitivity of bone scintigraph in the diagnosis of acute osteomyelitis approaches 100%. However, the examination is not so much sensitive in early infancy or neonatal period probably because suppuration progresses so rapidly and aggressively that there is not enough time for bone to resist and react. Another problem is associated with the fact that the predilected site of acute osteomyelitis viz. the metaphysis is closely placed to the epiphyseal line. Thus, in unmagnified view, the two bands of intense RA may often be seen as a single band. This problem can be resolved when pinhole collimator image is obtained.

Labelled Leucocytes and ^{67}Ga Scan in Osteomyelitis

Leucocytes labelled with ^{111}In or ^{99}Tc^{m} or ^{67}Ga have been used in the diagnosis of osteomyelitis. The rationale of this examination is that leucocytes are taken up by inflammatory tissues. When granulocytes and lymphocytes are selectively labelled, the former can be localized by acute inflammatory focus having granulocytic infiltration, whereas the latter by chronic inflammatory focus having lymphocytic infiltration. The sensitivity and

specificity of ^{111}In leucocyte scan have been reported to range between 50%-100% and 69%-100%, respectively.

Recently, ^{99}Tc^{m}-HMPAO (hexamethyl propylene amine oxime) was introduced to label leucocytes. The quality of the ^{99}Tc^{m} image in terms of detail was found to be comparable or superior to that of ^{111}In image. The sensitivity is reported to be 100% and the specificity 95%.

The usefulness of ^{67}Ga scan in the study of acute bone infections has been questionable because ^{67}Ga is localized not only by infectious bone diseases but by neoplastic conditions, making the test nonspecific. However, it has been demonstrated that ^{67}Ga uptake is rather intense in acute bone infection compared to neoplasm. ^{67}Ga uptake is greater than ^{99}Tc^{m}-MDP uptake in acute bone infection.

Bone Tuberculosis

Clinically, bone tuberculosis consists of spondylitis (spine tuberculosis) and tuberculous osteomyelitis of other bones. Spondylitis is far more common than other osseous tuberculosis. Joints are also frequently affected with tuberculosis.

Bone tuberculosis is characterized by a destructive process with minimal reactive bone formation. In long tubular bone, destruction takes place typically at the metaphysis as in acute pyogenic osteomyelitis. As tuberculous process advances, a cystic lesion accompanied by marginal osteosclerosis and periosteal reaction can be produced.

Bone scan findings of tuberculosis are not specific. Affected bone simply shows increased RA. Occasionally, photopaenic area can be portrayed within intense RA due to necrosis.

Tuberculous spondylitis is still not a rare disease in many developing countries. It is characteristic of spine tuberculosis to involve simultaneously two neighbouring vertebrae and the intervertebral disc interposed between them. This is due to the presence of freely anastomosing rich venous network within two vertebrae. In established spondylitis, the affected vertebrae and disc are destroyed as a block.

Bone scan shows diffuse irregular RA within the vertebral body. Disc space becomes narrowed and obliterated. Pinhole image is valuable in differentiating spine tuberculosis from pyogenic spondylitis, compression fracture and cancer metastases.

ARTHRITIS

With accumulation of knowledge in nuclear imaging of various arthritides, the categorization of arthritis into two groups viz. inflammatory and non-inflammatory has become warranted. The inflammatory type is attended by synovitis so that the RA is diffuse

within the articular confinement, whereas the non-inflammatory type is characterized by degeneration of articular cartilage and associated subchondral bone reaction with focal or compartmental RA.

Among the common inflammatory arthritides are rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, infectious arthritis and Reiter's syndrome. Osteoarthritis is probably the most common non-inflammatory arthritis. For the nuclear imaging of arthritis, $^{99}Tc^{m}$-MDP, ^{67}Ga citrate and radiolabelled leucocytes can be utilized. Nuclear angiography is of great value in septic arthritis.

Acute Pyogenic Arthritis

This is one of the excellent indications for nuclear imaging. Frequent offenders are staphylococci, streptococci, pneumococci and gonococci. As is the case with acute osteomyelitis, acute pyogenic arthritis is difficult to detect in its early phase by radiography. However, bone scan demonstrates increased blood pool and intense RA in the septic joint already in its early stage (**Figs. 30.19A and 30.19B**). Pinhole scintigraph may disclose diffuse RA in periarticular bones.

Synovitis

Highly vascular synovial membrane becomes markedly congested when inflamed, and shows marked concentration of radionuclide especially in the vascular phase of the scintigram. In acute synovitis, periarticular bones also concentrate radionuclide due to concomitant bone inflammation. Moreover, nuclear angiograph and blood-pool image may well reveal hypervascular state of the lesion. Pinhole image may prove that periarticular bones are not the site of primary infection. Thus, the discrepancy between a "hot joint" in standard scintigraph and normal or only slight RA by periarticular bone in pinhole image can be used as a differential finding.

Osteoarthritis

Osteoarthritis is essentially a degenerative disease of joints characterized by histological derangement of cartilage and subchondral bone without obvious inflammation. Synovitis is not a prominent feature.

Bone scan shows RA which is typically focal or compartmental in distribution. Compartmental, segmental or focal nature of RA can be clearly depicted in pinhole image (**Fig. 30.20**). This finding may be confused with spontaneous osteonecrosis when it occurs in the knee joint. Distinction is, however, possible because the hot spots in the latter condition tend to localize within the osseous portion (**Fig. 30.21**).

Rheumatoid Arthritis

Bone scan has been shown to be useful in the diagnosis of rheumatoid arthritis and assessment of its activity. Multiarticular involvement can be clearly portrayed by whole body bone imaging. In magnified view, inflammatory changes in small bones and joints of hands and feet can be detailed. Nuclear angiography may provide information regarding the lesional vascularity, and, in turn, the activity of the pathologic process. In remission, vascularity reverts to normal. Pinhole scintigraph shows obliteration of joint space due to ankylosing process which is characteristic of rheumatoid arthritis.

Ankylosing Spondylitis

It is well known that ankylosing process of the spine is usually preceded by sacroiliitis. Bone scan reveals characteristic features of the obliteration and bridging of vertebrae giving rise to the appearance of "square vertebrae in unsegmented spinal column". Pinhole image permits to recognize that ankylosis involves not only the longitudinal ligaments and interspinous ligaments, but small apophysial joints.

CONCLUSION

Skeletal imaging is one area where nuclear medicine has an edge over other imaging modalities. Its importance in the early diagnosis of metastases and its unequivocal role in the overall management of cancer are unquestionable. Gradually this mode of imaging is establishing its usefulness in other non-malignant conditions of bones and joints. This Chapter also emphasizes the extensive information that can be obtained with the use of pinhole collimator, which is bound to be available in a nuclear medicine department of the developing countries because of their traditional obsession with thyroid imaging. What is not available is time to do many bone scans in a day in these monogamous (mono-gamma camera) units of the developing countries. Ideally, and rationally from the point of view of health care benefits, each nuclear medicine centre should have one gamma camera exclusively devoted to bone imaging.

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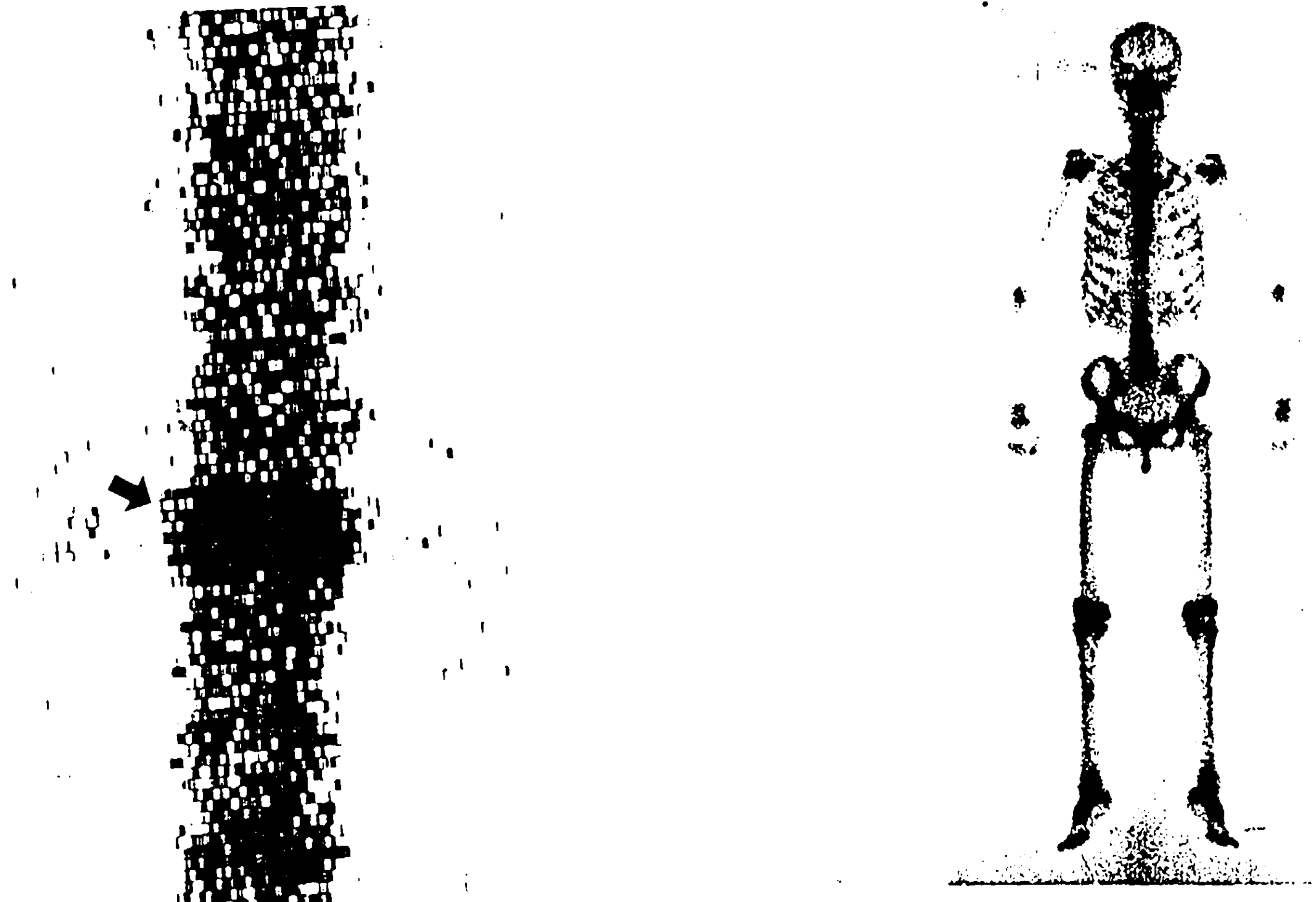

Fig. 30.1 An anterior black-and-white photoscan of the thoracolumbar spine made with a rectilinear scanner. Intense radionuclide accumulation is seen in the body of D12 representing gastric cancer metastasis (arrow).

Fig. 30.2 Anterior single-pass view of the whole skeletal system.

Fig. 30.3 A. Anterior low-energy converging collimator view of the chest showing a questionable hot area in the right suprasternal region (?).

Fig. 30.3 B. Anterior pinhole view of the area in question clearly localizing the hot spot in the costovertebral junction of D2 vertebra (arrow).

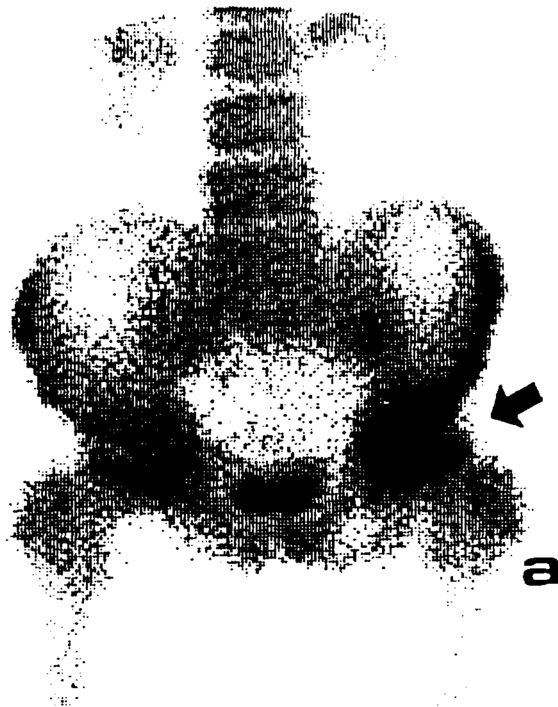

Fig. 30.4 A. Anterior view of the pelvis, tilted left anteriorly, showing fallacious increase in radionuclide accumulation in the left hip (arrow).

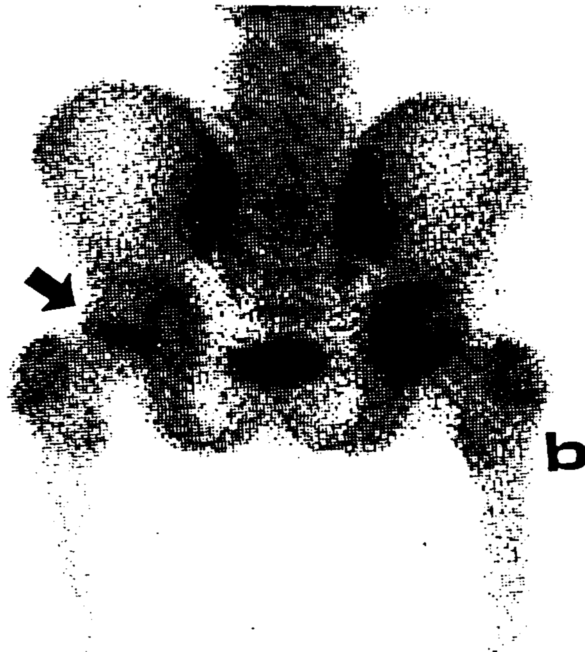

Fig. 30.4 B. Posterior view of the same hip showing normal left hip (arrow).

Fig. 30.5 Generalised increase in soft tissue radionuclide accumulation due to anasarca from advanced cancer.

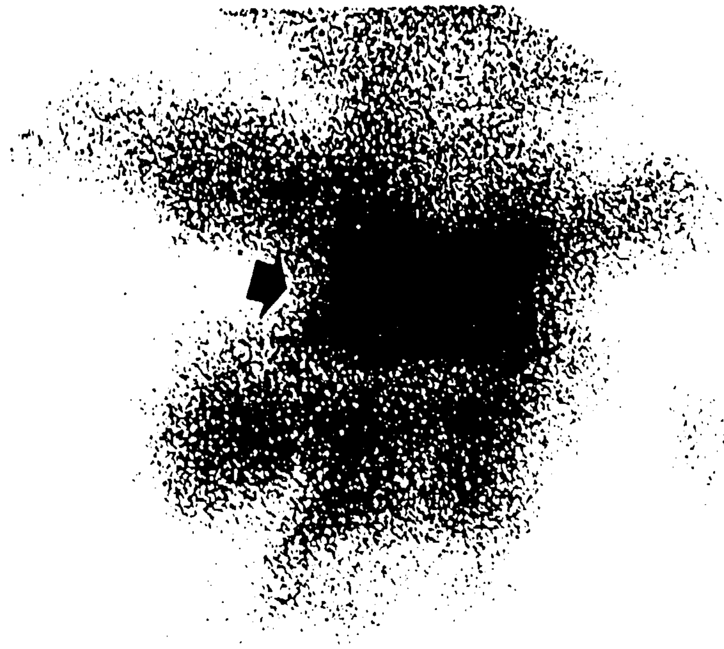

Fig. 30.6 Posterior pinhole view of D12 vertebra showing diffuse radionuclide accumulation due to metastasis from nasopharyngeal carcinoma (arrow).

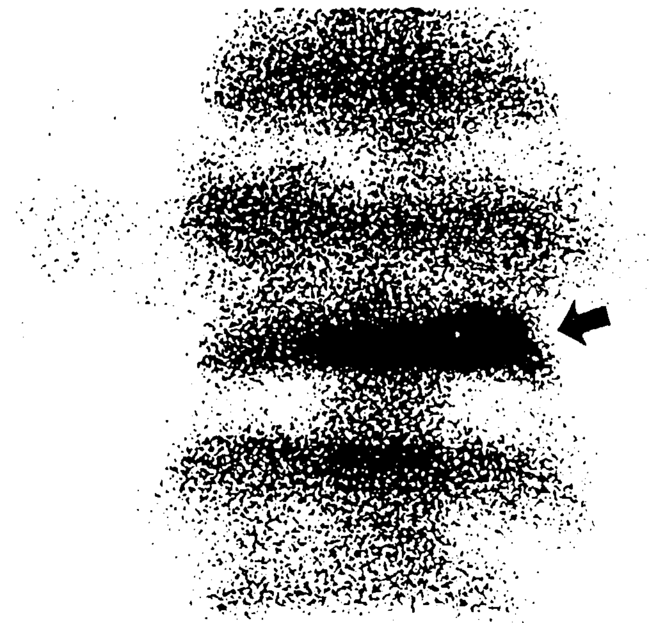

Fig. 30.12 Posterior pinhole view of L2 vertebra showing characteristic segmental radionuclide accumulation in the lower end plate representing breast cancer metastases (arrow).

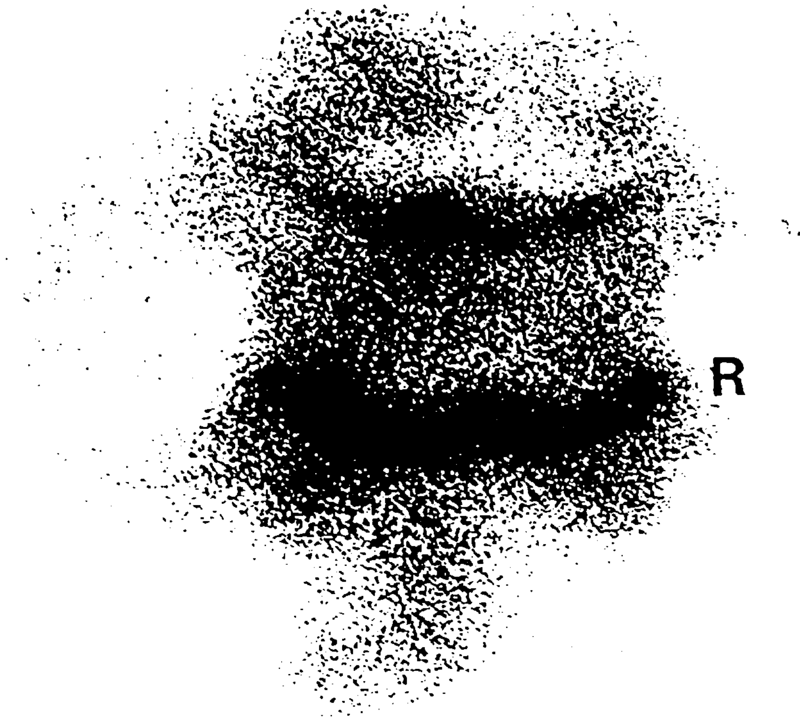

Fig. 30.7 Posterior pinhole view of D12 and L1 vertebrae showing characteristic arcuate radionuclide accumulation along fractured upper plates.

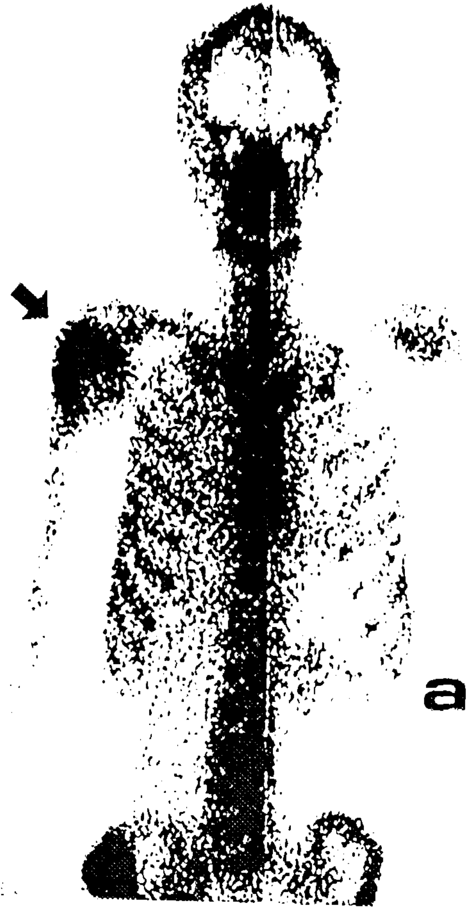

Fig. 30.8 A. Anterior single-pass view of the chest showing an ill defined hot area in the head of the right humerus (arrow). The lesion, does not appear to be photopaenic in this view.

Fig. 30.8 B. Pinhole view of the humeral head showing the lesion to be predominantly osteopaenic with areas of increased radionuclide accumulation indicating pathological fracture.

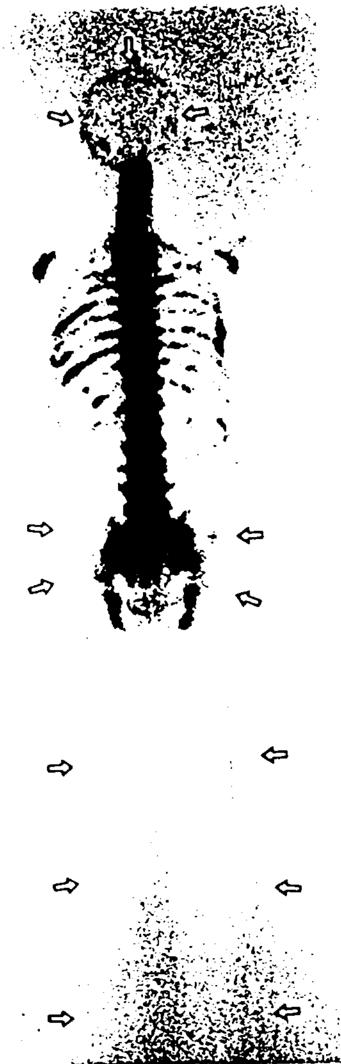

Fig. 30.9 Posterior single-pass view of the whole skeletal system showing diffuse radionuclide accumulation in the spine and sacroiliac joint showing "super scan" appearance. Open arrows indicate markedly diminished radionuclide accumulation in the skull and limb bones. This is the case of gastric cancer metastasis.

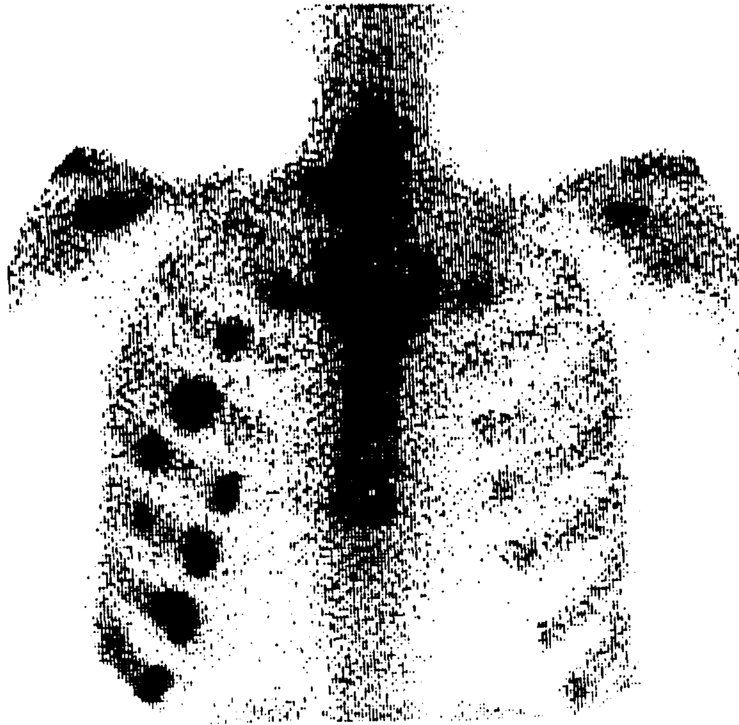

Fig. 30.10 Anterior view of the chest showing multiple rib fractures involving the right hemithorax. Band-like hot spots lined in a row are characteristic of fractures.

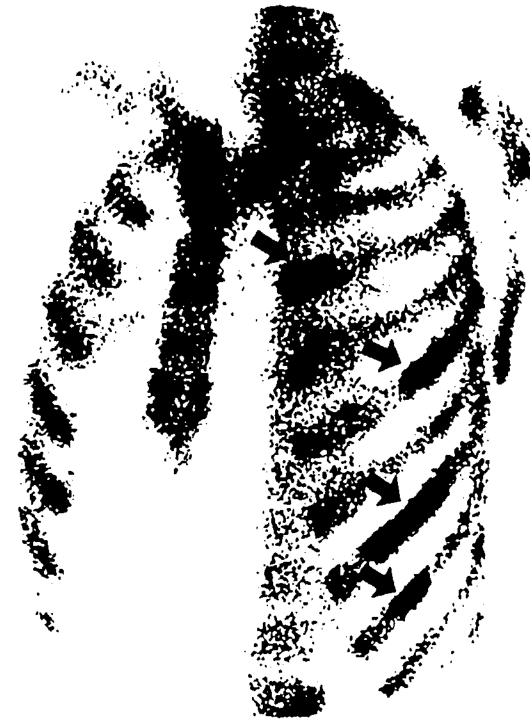

Fig. 30.11 Left anterior oblique view of the chest showing multiple elongated transverse hot areas involving many ribs representing breast cancer metastases (arrows).

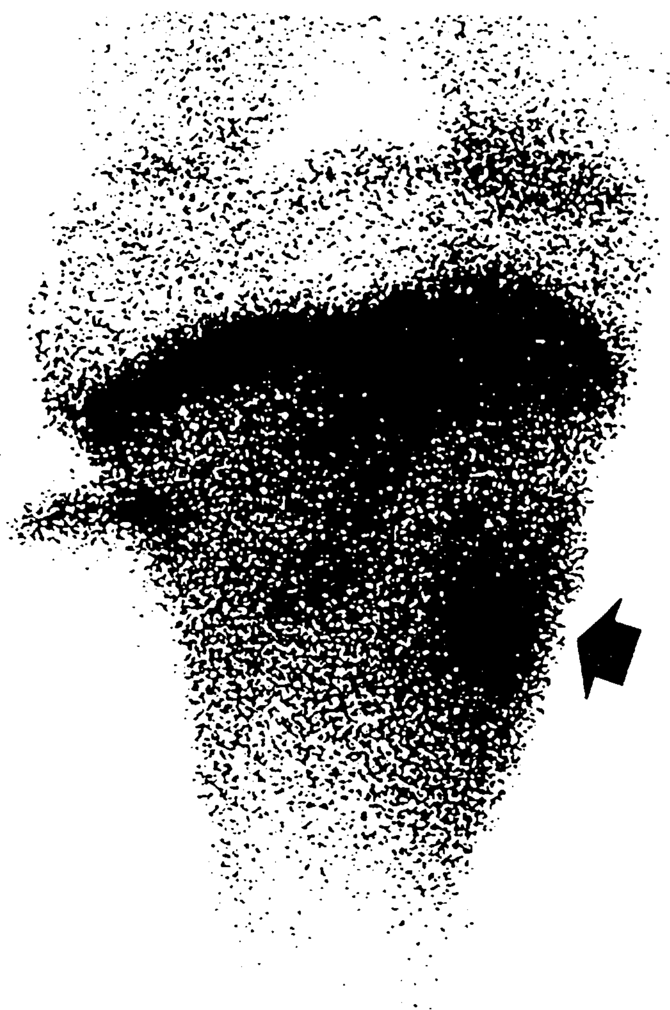

Fig. 38.13 Pinhole scintigraph of the right proximal tibia showing a hot spot within hot area indicating the nidus surrounded by reactive osteosclerosis in osteoid osteoma.

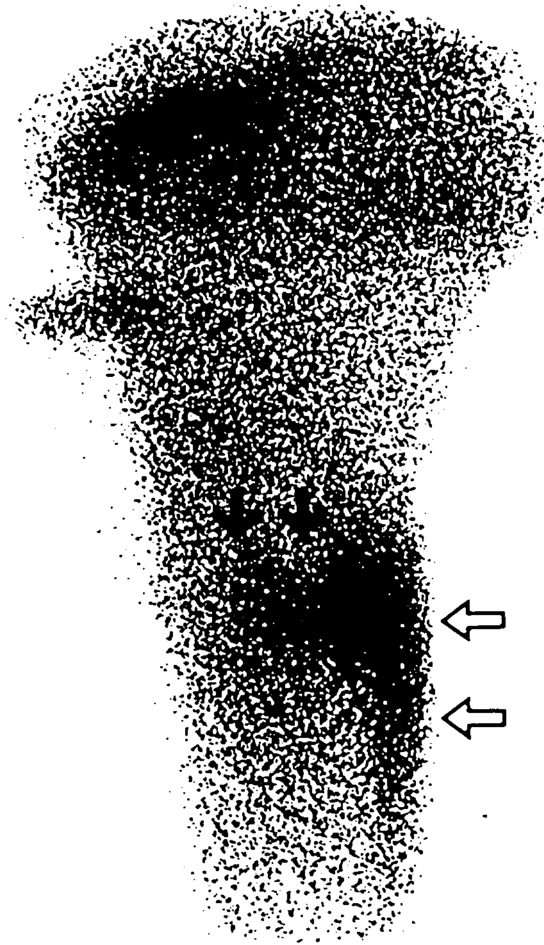

Fig. 30.14 Anterior pinhole view of the right proximal tibia showing radionuclide accumulation along stress fracture (solid arrows) and associated periosteal reaction (open arrows).

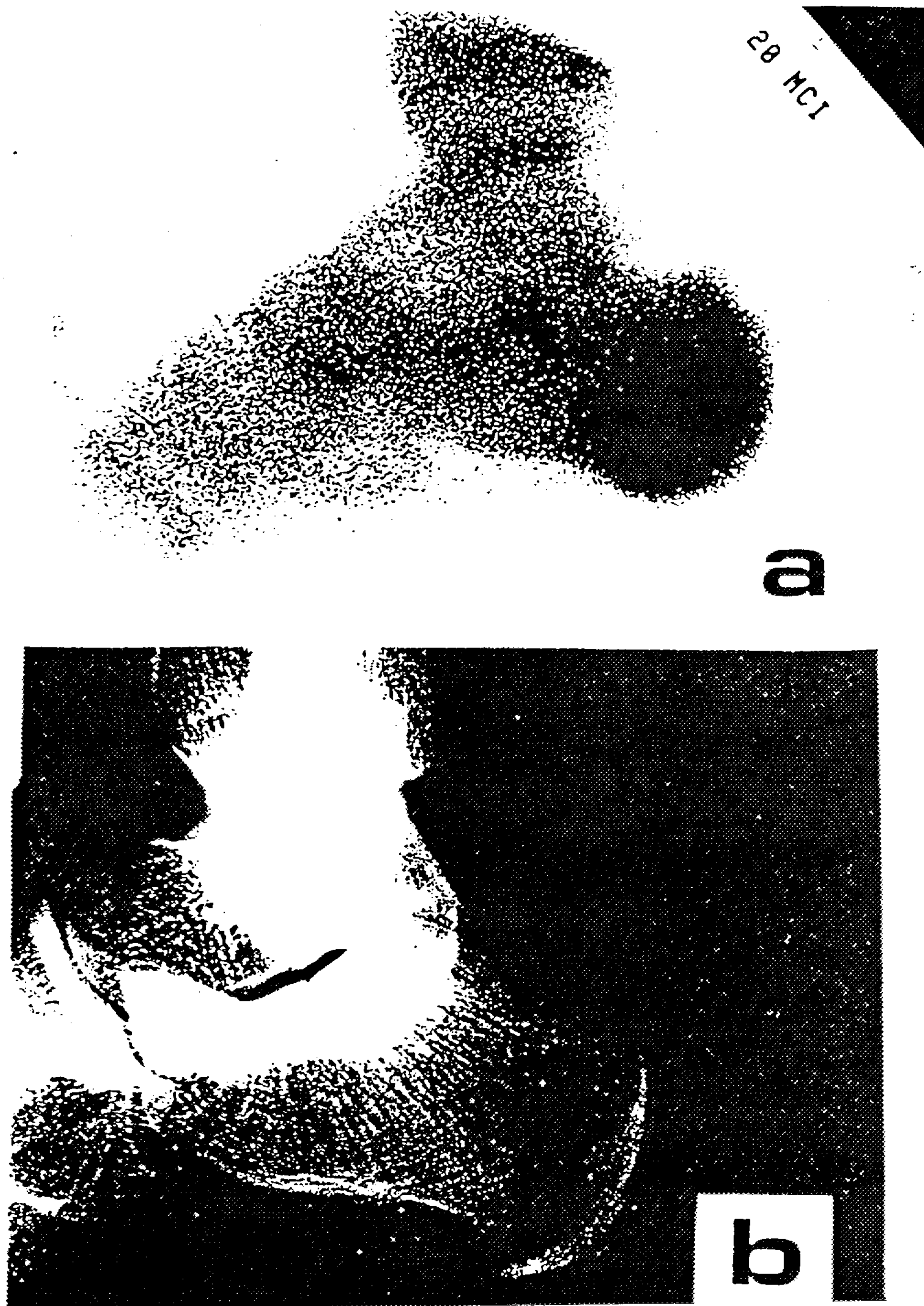

Fig. 30.15 A. Lateral pinhole view of the left os calcis showing diffuse increased radionuclide accumulation in the posterior aspect indicating bone contusion.

Fig. 30.15 B. Lateral X-ray of the same os calcis showing no bone abnormality.

Fig. 30.16 Anterior pinhole view of the pelvis showing photopaenic area in the left capital femoral epiphysis representing aseptic bone necrosis (Leg-Perthes Disease), (open arrows).

Fig. 30.17 Anterior single-pass view of the whole skeletal system showing generalized increase in radionuclide accumulation and several hot areas (arrows) representing osteomalacia and pseudofractures, respectively.

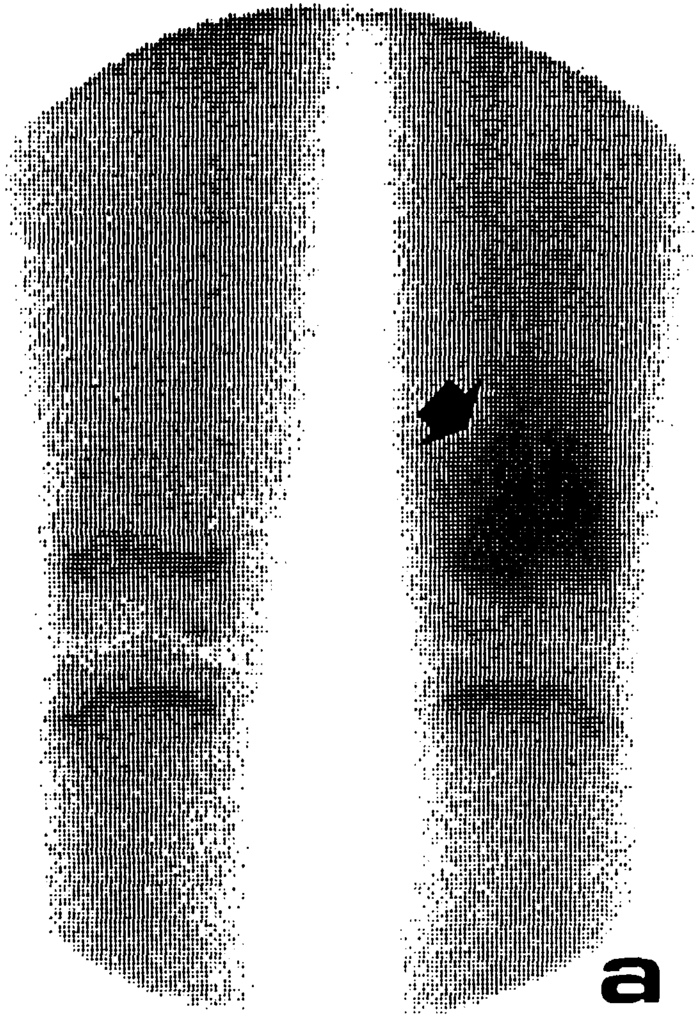

Fig. 30.18 A. Nuclear angiograph of the left lower extremity showing increased blood flow and blood pool in the distal femur in the site of acute osteomyelitis (arrow).

Fig. 30.18 B. Static view showing prominent radionuclide accumulation in the affected left distal femur (arrow).

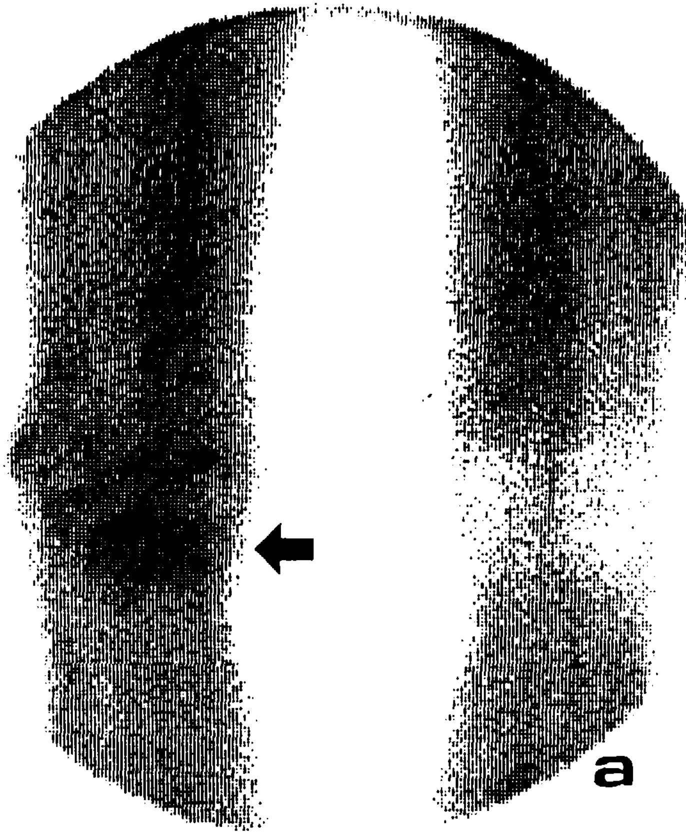

Fig. 30.19 A. Nuclear angiograph of the right lower extremity showing increased blood flow and blood pool in the septic right knee (arrow).

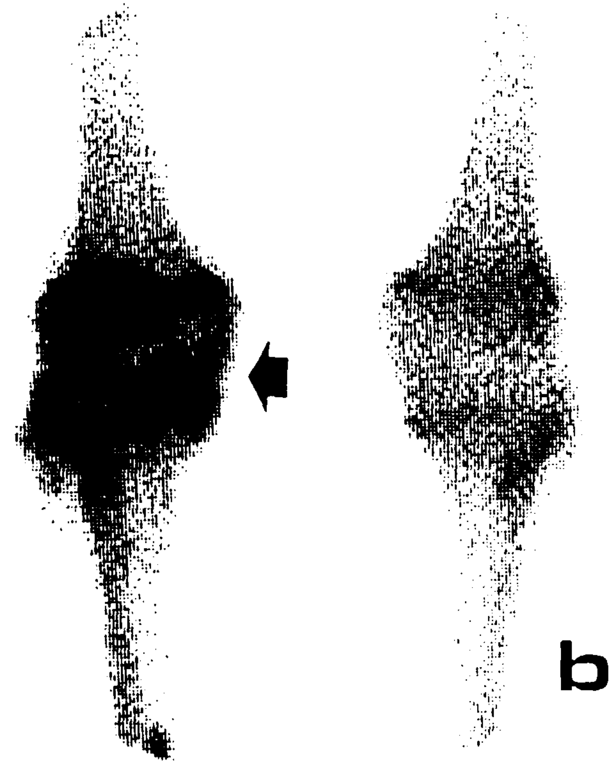

Fig. 30.19 B. Static view showing prominent radionuclide accumulation within the confinement of the joint capsule and periarticular bones (arrow).

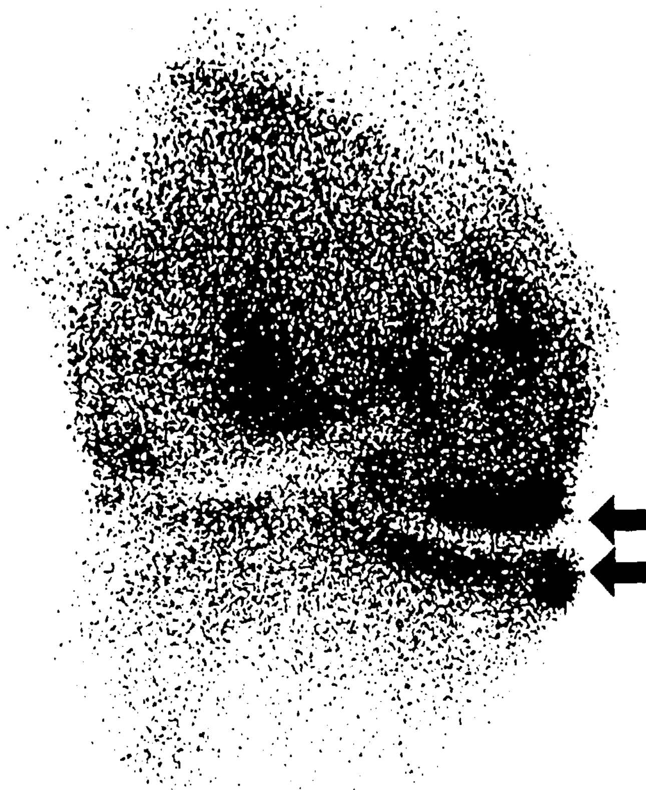

Fig. 3 .20 Anterior pinhole view of the right knee showing characteristic segmental localization of the radionuclide accumulation in subchondral bones of the medial compartment representing osteoarthritis (arrows).

Fig. 3 ..21 Anterior pinhole view of the right knee showing intraosseous localization of radionuclide in the distal femur around the condylar fossa representing spontaneous osteonecrosis (arrows).

CHAPTER 30

Chapter 31

KIDNEYS AND URINARY SYSTEM

Gopinathan Nair

Anatomy and physiology

Nuclear medicine studies, though primarily concerned with the functional aspects of the organ, can also provide useful information about the anatomy. An understanding of the anatomy and physiology of the kidneys and urinary system greatly helps in the interpretation of data from radionuclide studies.

The various anatomical regions are shown in (Fig. 31.1). The outermost region, the renal cortex, contains most of the glomeruli and the proximal convoluted tubules. The inner region, the renal medulla contains all the collecting tubules which open into the renal papillae. Urine flows from the ducts in the papillary tips into a minor calyx. Minor calyces unite to form a superior and inferior major calyx which join to form the renal pelvis. The pelvis is continuous with the ureter. In a good renal scintigraphic image, it will be possible to identify the cortex and medulla - essentially the urine-forming and urine-draining system - and draw regions of interest to separate these functionally different compartments. The renal hilus marks the region where the major renal vessels enter or leave the kidney.

The ureters enter the bladder through the orifices situated at the two corners of the triangle called the trigone. The ureters pierce the muscle and mucosal walls obliquely. This arrangement helps in the prevention of reflux of urine as the intravesical pressure increases. As the bladder contracts, the urine leaves through the orifice into the urethra.

The functional unit of the kidney is the nephron. There are approximately 100 000 nephrons in each kidney. A nephron is composed of a glomerulus, and in succession, a number of tubular segments. Collecting tubules from several nephrons progressively unite to form collecting ducts which join other collecting ducts and ultimately empty their contents into a minor calyx through the duct in the papillae.

The glomerulus is composed of a plexus of capillaries and a covering of epithelial cells. Glomerular capillaries arise from the afferent arteriole and reunite to form the efferent arteriole. The capillaries are covered on their external surface by a thin layer of epithelial cells, the visceral layer of Bowman's capsule. This layer of Bowman's capsule is continuous with the epithelium of the proximal convoluted tubule via the parietal layer of Bowman's capsule. The Bowman's space is continuous with the lumen of the proximal convoluted tubule. The process of urine formation begins with the filtration of plasma across the glomerular capillaries into Bowman's space and progresses through complex mechanisms of transtubular absorption and secretion.

The blood supply to the kidney is through the renal arteries. The main renal artery undergoes a series of branching within the kidney until the afferent arterioles are formed.

These give rise to the glomerular capillaries which in turn unite to form the efferent arteriole. The efferent arteriole then breaks up to form a peritubular capillary network. These join to form small veins which progressively unite to form the renal vein.

The glomerular filtration rate (GFR) for both the kidneys is the sum of the individual filtration rates of all the nephrons. GFR in a healthy adult is approximately 100-125 millilitres per minute. GFR can be accurately and conveniently measured by radionuclide methods.

Tubular function modifies the composition of the glomerular filtrate by processes of selective absorption and secretion, leading to the formation of urine. A great deal is now known about the resorptive and secretory functions of the various renal tubular segments which have important bearing on maintaining the electrolyte, acid base and fluid balance of the body. Established radionuclide methods are not yet available to study tubular function selectively; because a suitable radiopharmaceutical has not been developed for this purpose.

The collecting system, the pelvis, the ureters and the bladder are essentially concerned with the reservoir and transit functions for the urine formed in the kidney. Using suitable radionuclide techniques, it is possible to obtain qualitative and quantitative information regarding these functions.

Basis of scintigraphic study.

The radiopharmaceutical, when injected intravenously, is transported to the kidney through the renal artery and its branches. This phase of the transport of the radiopharmaceutical across the renal artery which may be termed the "arterial phase" lends itself very conveniently for imaging the renal arterial flow using a scintillation gamma camera. Significant obstruction to the blood flow through the renal artery or any of its major branches will be manifested as delayed appearance and diminished accumulation of the radioactivity in the whole or part of the kidney.

The functional unit of the kidney which is responsible for the discriminate handling of the injected radiopharmaceutical is the nephron. The radiopharmaceutical is passed into the tubular lumen through the glomerular filtration route. The factors which determine the route include:

(a) degree of protein binding

(b) molecular size and

(c) modecular charge.

On this basis, the kidney seeking radiopharmaceuticals are classified as glomerular agents (e.g. $^{99}Tc^{m}$ DTPA), tubular agents (e.g. ^{131}I Hippuran, $^{99}Tc^{m}$ MAG3, $^{99}Tc^{m}$ DMSA) and mixed type (e.g. $^{99}Tc^{m}$ GHA) (Table I).

TABLE I. COMMONLY-USED RADIOPHARMACEUTICALS FOR KIDNEY DISORDERS

Radiopharmaceuticals	Biokinetics	Clinical application
Tc^{99m}-labelled compounds		
1. Diethylene triamine Pentaacetic acid (DTPA)	Negligible protein binding. Major renal route is glomerular filtration. Plasma clearance is slow. Renal clearance reflects GFR. Renal excretion is not fast.	Quantitation of GFR. Evaluation of urinary drainage, vesico-ureteric reflux, bladder function and urethral patency.
2. Dimercapto succinic acid (DMSA)	Significant protein binding. Major renal route is tubular excretion, part by glomerular filtration,. Renal excretion is slow.	Overall assessment of kidney function. Imaging of kidney for focal renal abnormality such as scarring, infarction. etc.
3. Glucoheptonate (GH)	Some protein binding. Excretion is through glomerular filtration and tubular excretion. Renal excretion is slow.	Same as for DMSA
4. Mercapto acetyl triglycine (MAG3)	Significant protein binding. Major renal route is tubular. Behaves like orthoidodo-hippurate. Renal excretion fast.	Overall assessment of kidney functions. Evaluation of morphology of collecting system by imaging. May be used for assessment of renal tubular disorders.

Tc^{99m} Principal photon energy is 140 keV, good matching with gamma camera, physical half-life of six hours. Radiation dose to patients comparatively low, easily available, not expensive. so the most popular radiolabel.

TABLE I. (cont.)

Radiopharmaceuticals	Biokinetics	Clinical application
Radioiodine-labelled compounds		
1. ^{131}I orthoiodo hippurate	Significant protein binding. Major renal route is tubular excretion (80%) part by glomerular filtration (20%). Renal clearance reflects ERPF (effective renal plasma flow). Renal excretion is fast	Quantitation of renal plasma flow. Evaluation of upper urinary tract drainage.
2. ^{123}I orthoiodo hippurate	Significant protein binding. Major renal route is tubular excretion (80%) part by glomerular filtration (20%). Renal clearance reflects ERPF (effective renal plasma flow). Renal excretion is fast.	Quantitation of renal plasma flow. Evaluation of upper urinary tract drainage. Renal imaging for morphological evaluation.

^{131}I Principal photon energy is 360 keV, not a good match for gamma camera, poor quality images. Physical half-life is eight days, patient radiation dose is high. Easily available, not expensive. Mostly used for non-imaging probe studies.

^{123}I Principal photon energy is 160 keV, good match for gamma camera, good images. Physical half-life is 13 hours. Not easily available, very expensive.

Once the radiopharmaceutical arrives in the tubular lumen, it flows along with the contents of tubular fluid and follows the flow of fluid within it, i.e. via the proximal convoluted and straight tubules, the descending and the ascending loops of Henle, the distal convoluted tubules to reach the collecting tubule. From there it flows on to papillary collecting ducts and to the minor and major calyces, where usually free mixing of radioactivity occurs with the urine contained in it. The sojourn of the radiopharmaceutical from the beginning to the end of the nephron can be referred to as the nephronal phase. This phase helps in obtaining the image of the kidney to study its gross morphology such as size, shape and position and presence of any intrarenal space occupying lesions. The image of the kidney obtained during this phase is the result of the collective function of all the nephrons. The image of the kidney defining the cortical part of the nephron can be termed "the cortical image". Occasionally, in the study of the kidney, it may be necessary to identify and separate these phases of the radiopharmaceutical transport in the kidney images. This can be useful in determining the transit time in different parts of the kidney, e.g. cortical transit time versus whole kidney transit time.

Renal transit time truly represents the time that it takes for the radiopharmaceutical, after its pulsed input into the renal artery, to travel through the tubules and collecting channels to reach the renal calyces. The factors which influence the transit time are:

(a) urine flow rate and

(b) the chemical nature of the radiopharmaceutical.

For a given flow rate, different renal radiopharmaceuticals can have different transit times. The amount of radiopharmaceutical present in a given kidney at the end of this transit time or during a fraction of this period is proportional to the function of the kidney. This fact is used in determining the relative function of the kidneys. The renal uptake of $^{99}Tc^{m}$ DTPA and ^{131}I OIH reflect the well defined renal functions like GFR and effective renal plasma flow (ERPF) respectively. The renal uptake of $^{99}Tc^{m}$ DMSA and $^{99}Tc^{m}$ GH reflect the function of the kidney, but do so without addressing any specific physiologic function.

As the urine, along with the radioactivity, collects in the renal pelvis, the pressure in the pelvis increases and initiates a peristaltic contraction beginning in the pelvis and spreading down along the ureter to force urine towards the bladder. A peristaltic wave occurs once every ten seconds to once every two or three minutes.

Once the radioactivity reaches the urinary bladder, it is usually so well and uniformly mixed with the urine in it, that bladder imaging is possible at this stage. The act of micturition forces the urine out through the urethra. The mucosal arrangement at the urethral orifices and the oblique course of the ureters through the wall of the bladder prevent the reflux of urine back into the ureters. The rate of disappearance of radioactivity in the bladder during the act of micturition provides valuable information regarding the detrusor function and patency of urethra, provided that there is no significant vesico-ureteral reflux. The radionuclide cystography and urine flowmetry have thus useful applications in clinical

urology. The dynamics of different renal radiopharmaceuticals, the renal functions which lend them for study, possible investigations and their indications are shown in **(Fig. 31.2)**.

Common disorders of kidney and urinary tract

There are quite a few disorders of kidney and urinary tract which are commonly encountered in routine clinical practice. The frequency of occurrence of these disorders is more or less similar in many parts of the world with minor variations depending on environmental, hereditary and local factors.

The following is a list of these disorders wherein radionuclide investigations have proven value in diagnosis of the disease and management of the patient.

1. Obstructive uropathy/nephropathy
2. Urinary tract infection
3. Renal failure
4. Space occupying lesions
5. Renal/renovascular hypertension
6. Urinary tract injury
7. Congenital abnormalities
8. Renal transplant dysfunction (rejection, acute tubular necrosis, etc.)

It is quite possible, and indeed is the case in many patients, that two or more of these conditions co-exist and aggravate the harmful effects on the kidney.

1. Obstructive nephropathy/uropathy

This is perhaps the commonest disorder affecting the urinary tract and kidneys. The importance of this disorder lies in the fact that it is totally reversible when detected early and managed properly. It is in this context of early detection and proper management that the radionuclide tests have the greatest value. This is due to the very high sensitivity and specificity of the tests in identifying the obstruction. The obstruction can occur at any point in the urinary tract starting from the calyces of the kidney to the external urethral meatus. Such obstruction may be acute, subacute or chronic; unilateral or bilateral; and total or subtotal. The resultant rise in back-pressure may eventually lead to anatomical changes such as distension and dilatation of the collecting system proximal to the obstruction, and functional impairment of the nephrons leading to nephropathy and renal failure. It is

important to note that the very same investigations which are very useful in the early stages of the disease may prove least useful in the late stages of nephropathy and renal failure, as these tests basically study the function of the kidney. **Table II** gives the common causes leading to urinary tract obstruction.

TABLE II. COMMON CAUSES OF URINARY TRACT OBSTRUCTION

Intrinsic causes	Extrinsic causes
Pelviureteric junction (PUJ) dysfunction Vesico-ureteral dysfunction Calculus disease Neurogenic bladder Bladder neck dysfunction Infection Urethral valves Tumor Trauma	Ureteral ligation during pelvic surgery Tumour Idiopathic fibrosis Lymphocoele

While there can be definite mechanical (organic) obstruction in many of the conditions listed in the table, it may be only functional in some such as vesico-ureteral reflux and neurogenic bladder. Dilatation of the renal calyces, pelvis, and ureter are common consequences of obstruction regardless of the cause and type of obstruction, i.e. mechanical or functional. In the case of the former, corrective measures are undertaken to remove the physical cause such as stone, stricture or tumour, while in the case of the later the attempt should be to correct the functional abnormality such as reflux or bladder dysfunction. Thus it is obvious that the proper management of the patient depends very much in distinguishing the type of obstruction and also in determining the extent of renal damage. Carefully selected radionuclide investigations can provide accurate information on both these aspects. Most other investigations such as plain X-ray, ultrasound and even computerised X-ray tomography give only anatomical information. Intravenous pyelography, rarely used in many centres after the advent of ultrasonography and radionuclide studies, also provides mainly anatomical information. But none of these non-invasive investigations can clearly distinguish the obstructed dilated urinary tract from the non-obstructed, but dilated tract. Radionuclide studies score over the others in this respect. The simplicity and safely inherent in radionuclide tests make them eminently suitable for repeat studies which form an important part in the management of patients with obstructive disorders prior to and following the corrective intervention.

Selection of radiopharmaceutical, while investigating obstruction, depends on the information required. The first question to answer is whether there is any actual obstruction or not, and the second question relates to the functional damage to the kidneys. The presence of obstruction is demonstrated by the continuously up-going second phase of the renogram curve and prolonged retention of radioactivity in the renal images. For this, it is better to use radiopharmaceuticals with relatively fast excretion such as ^{131}I OIH or ^{99}Tc^{m} DTPA. The function of the kidneys is better evaluated by the absolute uptake of ^{99}Tc^{m} DMSA. Deterioration of function as seen from serial studies is a definite indication for surgery to remove the obstruction.

Radionuclide studies should, therefore, be considered as a front-line investigation in the management of patients with urinary tract obstruction. Use of these studies as a stand-alone tests in certain clinical circumstances or as a complimentary test to others can be highly cost-effective in patient management.

2. Urinary tract infection

Urinary tract infection is a common problem in clinical practice. The group at risk for urinary tract infection (UTI) includes children, and patients having urinary obstruction, vesico-ureteral reflux, neurogenic bladder and diabetes mellitus. In one of the western countries, it is estimated that 1% of schoolgirls have asymptomatic bacteriuria and 5% of schoolgirls have proven UTI before they are ten years old. Around 10% of children with UTI will develop renal scarring at the time of first infection. Around 10-20% of children with scarring will subsequently develop hypertension. Between 50 and 60 adults per million develop renal failure every year and 15% of these are due to chronic pyelonephritis. Similar figures are not available for developing countries; if any, it will only show a higher incidence of renal damage due to UTI as a result of lack of awareness, late detection of disease and half-hearted or inadequate treatment of infection. If morbidity is to be prevented, it is necessary to keep a high index of suspicion, particularly in the high risk group, to diagnose the condition in time and to treat it adequately and vigorously.

Currently, out of the imaging modalities like intravenous pyelogram (IVP), ultrasonogram and radionuclide scan, it is the radionuclide scan using ^{99}Tc^{m} DMSA which is considered most sensitive in detecting renal scarring. There is a definite morbidity and mortality associated with the use of contrast media even with the newer, and more expensive non-ionic forms. The renal outlines may be difficult to see due to overlying bowel gas and scanty perinephric fat. Ultrasound in the child, even more than in the adult, is extremely operator-dependent. In principle, ultrasound does not involve ionising radiation, and should be used as the first investigation. But in view of the relatively high false negative rate of ultrasound, a negative examination should be followed by scintigraphy with DMSA. In any case the quantitative information on kidney function obtainable with DMSA is so unique that there is a strong case for performing this in every case of UTI, more so in the recurrent and chronic variety. Combined with other radionuclide methods such as renography, and radionuclide cystography it is possible to make a complete evaluation of the whole urinary tract to detect predisposing and correctable factors like obstruction, and vesicoureteral reflux, thus giving the

scintigraphic package a high index of cost effectiveness which is particularly relevant for developing countries.

3. Renal failure

There are many causes for renal failure and none may be apparent when the patient presents himself for the first time. The general aim of the radionuclide investigations in patients with renal failure is to assess the extent of the individual kidney damage and to look for clues to help in the management of the patient, both on a short term as well as long term basis. In every case of unexplained deterioration of renal function, whether acute or chronic, the possibility of urinary tract obstruction should receive careful consideration, because this, when detected and promptly treated, will save the kidney from further damage or even reverse the damage that had already occurred. The assessment of relative function of each kidney is also important as it provides management strategies for intervention.

As mentioned earlier, a combination of ultrasound and radionuclide studies has almost replaced the use of contrast intravenous pyelography (IVP). Using ultrasound, renal size and the degree of dilatation of the collecting system can be estimated by a competent and experienced investigator. If the ultrasound confirms that both kidneys are small and there is no dilatation of the collecting system, there is little to be gained by further imaging investigations; but if there is evidence of dilatation of the collecting system, then neither the ultrasound nor the IVP can distinguish between a non-obstructed dilated collecting system and an obstructed dilated one; neither can they assess the contribution of each kidney to the total function. In such a situation $^{99}Tc^{m}$ DTPA gamma camera renography (or $^{99}Tc^{m}$ MAG3 renography if available) or ^{131}I OIH sequential imaging should be performed; though with ^{131}I OIH the radiation dose to the kidney will be higher than that with $^{99}Tc^{m}$ compounds. Even when there is no dilatation, the scintigraphy should be performed, if the kidney size is different on both sides, to assess the relative function. It is important to continue the study for as long as twenty four hours, if sufficient information is not obtained earlier.

Imaging is of limited value in the differential diagnosis of the actual disease process. Small contracted kidneys suggest chronic renal damage which is not of obstructive origin and is likely to be irreversible. Large kidneys may suggest a variety of causes like polycystic, medullary sponge or amyloid kidneys, but most importantly obstruction.

$^{99}Tc^{m}$ DTPA is the most useful radiopharmaceutical for scintigraphic studies in patients with renal failure. Sequential images should be obtained at appropriate intervals for as long as necessary. Time activity curves (renogram) of the initial thirty minutes of the study may or may not be helpful in determining the presence of obstruction, particularly when the background radioactivity is quite high, in the initial phase. Increasing retention of radioactivity in the collecting system as seen in the delayed images is an indication of obstruction. If non-obstructed, the accumulated activity is usually faint, is in the cortical region and is only slightly higher than the background activity.

All patients with renal failure do not require scintigraphic investigations. They are indicated only in those patients in whom the ultrasonography has shown dilatation of the collecting system or it has failed to clearly visualise the collecting system. Scintigraphic study is indicated also in patients in whom asymmetrical kidney disease is suspected.

4. Space-occupying lesions (SOL) in kidney

Common causes of SOL in kidney include cysts (simple or hydatid), multicystic disease, *polycystic disease, calculus, segmental hydronephrosis, segmental pyelonephritis, primary* malignant tumours and haematoma. The name SOL suggests that the lesion displaces normal renal tissue. Renal radiopharmaceuticals, when administered, fail to get concentrated in the region occupied by the lesion. At the same time, it gets concentrated in the surrounding normal tissue thus the negative uptake in the SOL, commonly described as "cold area" in the scintigram, is the hallmark of the presence of SOL. While scintigram may be quite sensitive in the detection of SOL, it does not help in the differential diagnosis of the SOL.

Radiopharmaceuticals which do not change their distribution rapidly within the kidney and which mainly display cortical accumulation are preferred for imaging of renal SOL. $^{99}Tc^{m}$ DMSA and $^{99}Tc^{m}$ Glucoheptonate (GH) belong to this class of renal agents. Tracers like $^{99}Tc^{m}$ DTPA, $^{99}Tc^{m}$ MAG3 and iodinated OIH are far less satisfactory agents because of their short cortical residence time and rapid accumulation in the collecting system. The latter may mask the presence of SOL. A gamma camera fitted with a high-resolution collimator is the instrument of choice for scintigraphic imaging of SOL's. Magnified images using pin-hole collimator are useful particularly in infants and young children. Addition of a nuclear medicine computer will allow dynamic scintigraphy of renal perfusion phase, which may be useful in characterising some SOL's. Until recently, IVP using radiological contrast media was the front-line investigation of SOL. With the advent of ultrasound and X-ray CT, the use of IVP is receding. Scintigraphic imaging is to be regarded as complimentary to US or CT imaging or supplementary when additional information such as the effect of SOL on the renal function and urine flow is desired.

Table III gives the usual scintigraphic findings of various SOLs in the kidney in the three different phases of the scintigraphic study.

5. Renal/renovascular hypertension

Early in the course of hypertension, detection of kidney abnormality has important practical significance. Radionuclide studies have been shown to have very high sensitivity, specificity and accuracy in detecting the presence of renal disease, especially when unilateral. When the radionuclide tests indicate renal disease in a patient with hypertension of recent onset, it is quite likely that the hypertension is renal. The nature of the disease can be determined by further investigations. On the other hand, if the kidneys are found to be normal after the radionuclide tests, renal causes can be excluded for the hypertension. Thus radionuclide tests have a well recognised role as a screening test for hypertension, particularly in children, young adults or in older people and when kidney disease is suspected.

TABLE III. USUAL SCINTIGRAPHIC FINDINGS IN SOL IN THE KIDNEY

S.No. I	Pathological entity II	Renal scintigraphic findings: Perfusion phase III	Early cortical and collecting system phase IV	Delayed cortical and collecting system V
1.	CYSTS (simple, hydatid)	Hypoperfusion, avascular appearance.	Focal photon deficient area. "Cold" lesion usually solitary and cortical location.	Same as the early phase.
2.	Multicystic disease	Same as above, involving multiple areas.	Same as above, but multiple. Usually unilateral.	Same as early phase.
3.	Polycystic kidney (adult)	Same as above. Usually Bilateral.	Large kidneys. Same as above. Usually bilateral and multiple.	Same as early phase.
4.	Calculus disease (with kidney function preserved)	Normal cortical perfusion.	Normal cortical uptake. Photon deficient area in the major collecting system corresponding to large stone.	Normal cortical uptake "Fixed" photon deficient area in the collecting system with evidence of statis or obstruction.
5.	Hydronephrosis	Generally shows diminished renal perfusion, the degree of decrease quite variable. Perfusion images show large central void due to distended collecting system (transient)	Cortical activity depends on overall renal function. Gradual appearance of radioactivity in the previously photon deficient central void.	Increasing activity in the collecting system.

TABLE III. (Cont.)

S.No. I	Pathological entity II	Renal scintigraphic findings: Perfusion phase III	Early cortical and collecting system phase IV	Delayed cortical and collecting system V
6.	Primary renal neoplasms (renal cell carcinoma)	Hyperperfusion. Hyper-vascular in appearance.	Focal decreased cortical uptake. Usually solitary	Same as early phase. Multiple lesions reduce the chance of primary carcinoma.
7.	Renal infarct	Focally decreased perfusion.	Cortical uptake defect.	Same as early phase, but better delineated.
8.	Renal trauma (hematoma, tear)	Focally decreased perfusion.	Uptake defect depending on the location of the lesion - cortical or central.	Same as early phase.
9.	Pyelonephritis (unifocal or multifocal)	Diminished perfusion.	Focal cortical uptake defect - single or multiple.	Same as early phase.
10.	Renal abscess	Diminished focal perfusion.	Focal cortical uptake defect.	Same as early phase.
11.	Renal pseudo-tumour (hypertrophic columns of Bertin).	Normal perfusion.	Normal cortical uptake.	Normal cortical uptake.
12.	Renal transplant (segmental ischaemia may be seen in a normally functioning or rejecting transplant).	Diminished focal perfusion	Focal cortical uptake defect.	Same as early phase.

Renovascular hypertension (R.H.) occurs in about 5% of the population with hypertension. Correct diagnosis of this condition is important because it is curable if detected in time. The use of the term renovascular hypertension implies that the narrowing of the renal arteries is causally related to the elevation of the arterial blood pressure and re-establishment of adequate blood supply results in the cure of hypertension. Atherosclerosis, fibrodysplasias and aortoarteritis involving renal arteries account for about 99% of cases of renovascular hypertension. One of the mechanisms which sustain hypertension in this disease is mediated through renin-angiotensin system, particularly in the early stages. The renin-angiotensin dependence of hypertension can be elucidated by the administration of an angiotensin-converting enzyme inhibitor called Captopril.

This forms the basis of using a Captopril renogram for the diagnosis of renovascular hypertension.

Various radionuclide techniques like probe renography, dynamic sequential imaging and clearance studies (GFR, RPF) have been used for the diagnosis of R.H. These investigations can be carried out in a nuclear medicine department which has a gamma camera, computer and appropriate software. The usual parameters of kidney function useful in patients of R.H. relate to renal blood flow (renal perfusion index), renal uptake function reflecting GFR or RPF as the case may be, and urine flow rate reflected by retention of radioactivity or abnormal transit time. However, the abnormalities in these parameters are not only seen in patients of R.H., but they are also seen in patients with other kidney diseases such as urinary obstruction, pyelonephritis and renal hypoplasia. Also, easy recognition of these abnormalities requires that the renovascular disease be confined to only one kidney and that the contra-lateral kidney be normal for reference and comparison. These shortcomings of the conventional radionuclide renal studies can be overcome by doing interventional studies, notably a Captopril renogram. Based on the mechanism of Captopril intervention, it is claimed that a Captopril renogram not only adds some degree of certainty to the diagnosis of R.H., but it also provides prognostic information regarding the beneficial outcome of therapeutic measures like balloon angioplasty and revascularisation surgery.

The sensitivity of rapid sequence IVP is also in the region of 70-80%. More sensitive and specific diagnostic tests like renal vein renin estimation and split renal function after ureteral catheterization are invasive and therefore cannot be considered for screening purposes. Digital substraction angiography has not been widely available as yet. Considering the simplicity, safety and accuracy, a Captopril renogram can be considered as a screening test for R.H. before sending the patient for selective renal arteriography.

6. Urinary system injury

Accidents and incidents causing morbidity and mortality to human life are on the increase. Road accidents, civil disorders, industrial accidents, building collapse, assault and natural calamities like earthquake and flood add to the overall incidence of accidents. Although head injury forms the major cause of death in such cases, abdominal trauma and injury to the urinary tract contribute significantly to the morbidity and mortality among the

victims of accidents. With the remarkable improvement in the practice of nephrology, early detection and proper management of such injuries can save many lives.

The successful management of patients with renal injury requires:

(a) recognition of the site of injury;

(b) assessment of individual renal function; and

(c) identification of any pre-existing renal disease.

Currently IVP and US scanning are considered front-line investigations in these patients. IVP is known to underestimate as well as overestimate the extent of injury. It cannot assess renal perfusion. In seriously-ill patients, it may be difficult to prepare the case for an IVP (bowel preparation) when the test needs to be carried out in an emergency. Ultrasonography, though very useful in detecting lacerations and haematomas, provides only limited information on renal perfusion and function. Functional evaluation of the individual kidney has a pivotal role in the management of patients with major renal injury but in stable clinical status.

Injury to renal vessels is one of the major factors which dramatically alter the therapeutic approach in patients with kidney injury. A normal $^{99}Tc^{m}$ DTPA dynamic study showing good and symmetrical renal perfusion rules out any major vascular injury. In patients with significant vessel injury, no perfusion or concentration of radioactivity is observed in the affected kidney. Transient vascular spasm leading to non-perfusion can be detected by appropriately timed serial studies. Assessment of relative function of the kidneys serves to form guidelines for further management. Clinically stable patients having moderate to good renal function may be treated conservatively; and unstable patients with moderate to poor function usually need surgical intervention. In short, there are good reasons to recommend radionuclide investigation to be part of frontline investigations in patients with known or suspected injury to the urinary system.

7. Congenital abnormalities

The various congenital abnormalities include:

(a) Congenital cystic disease (polycystic disease, medullary sponge, simple cysts)

(b) Horseshoe kidney

(c) Crossed renal ectopia

(d) Pelvic kidney

Usually, these abnormalities are detected as incidental findings while investigating the kidneys for other reasons. Congenital defects associated with malpositioning such as

horseshoe, ectopic and pelvic kidney render them prone to obstruction, infection and urinary stone formation. When ectopia is suspected and only one kidney is visualized, it is important and rewarding to specifically look for the other one by doing imaging at different projections (such as anterior view) and sites (pelvis). Radionuclide studies can play a significant role in the functional evaluation of the ectopic kidney; at times it can play a very useful role in locating the abnormally placed kidney that has eluded the search by ultrasound and radiological contrast studies.

8. Renal transplant dysfunction

Transplanted kidney is constantly under threat and has to struggle for survival. The very measures that protect it from the immune mechanisms of the host are potentially toxic and weaken the defences against infection. Accidents associated with transplant surgery such as ureteric ligation add further risk to its survival. Early detection and treatment of conditions which affect the transplant are crucial. Radionuclide techniques have been shown to be eminently suitable for renal transplant surveillance and evaluation. The common complications of renal transplant are:

(a) Ischemia
- Vascular obstruction
- Acute tubular necrosis

(b) Immunological
- Hyperacute, acute and chronic rejection

(c) Toxic
- Cyclosporin toxicity

(d) Urinary complications
- Leak, lymphocoele, obstruction

(e) Infection

All efforts should be made to evaluate critically any diagnostic procedure in terms of potential hazard to the transplant and the cost-effectiveness of the investigation. Because of the immune suppression, transplant patients are at a higher risk of infection. Catheterization, endoscopy, contrast angiography and allograft biopsy carry significant risk in these patients. IVP may not be very helpful in patients with diminished allograft function. Ultrasound examination is highly suitable in these patients to study structural abnormalities related to transplant dysfunction, it may be difficult to do US in the immediate post-operative period because of the bandages, wound, clips, sutures etc. It cannot also depict real time functional abnormalities which accompany the altered structure. Please note that in acute rejection and acute tubular necrosis, functional alterations are more prominent than structural alterations, if any. It is in the context of detecting real time functional abnormalities with virtually no

risk to the patient that radionuclide evaluation of renal transplant has gained wide acceptance in clinical practice.

Many centres in developing countries still use probe renography system and ^{131}I-OIH for performing renography. Probe renogram curves and blood clearance index of ^{131}I-OIH have been used for assessment of renal transplant function. A single study may not yield the required diagnostic information, but information regarding transplant dysfunction can be best obtained from serial studies in the same patient. It may also be not possible to derive at a differential diagnosis, hence differential management, of the various conditions which can affect the transplanted kidney. The main value of probe renogram study is in documenting the change of function serially during the life of a graft.

The gamma camera and a suitable data processing system (computer) is the instrument of choice. ^{99}Tc^{m} DTPA is currently the radiopharmaceutical of choice, since it can provide good quality images of all phases of renal transplant function such as perfusion, renal uptake and renal excretion. It also serves to study the function of the lower urinary tract and urinary bladder.

The renal transplant evaluation, using the gamma camera computer system, consists essentially of a dynamic first pass study followed by dynamic sequential scintigraphy. Static imaging of the entire region using a high resolution collimator at appropriate intervals helps in identifying morphological abnormalities (SOL's) that are suspected during the dynamic phase of the study. At times, it may be necessary to obtain delayed images even at 24 hours.

The first-pass scintigraphy helps in detecting significant obstruction to the blood flow to the transplant. Poor perfusion indicates renal artery or renal vein thrombosis. Total absence of perfusion can be due to renal infarction or hyperacute rejection which necessitates removal of the transplant. Quantitative parameters such as transplant perfusion index, not only serve to detect perfusion abnormalities, that are not well appreciated visually, as is the case sometimes with acute rejection, but also serve to document quantitatively changes in renal perfusion in follow-up studies. Sequential dynamic scintigraphy provides information on the uptake and excretory function of the transplant. It is possible to differentiate acute rejection and acute tubular necrosis at this stage. Normal perfusion, near normal uptake and delayed excretion are typical of early phases of acute tubular necrosis, whereas diminished perfusion, low uptake and delayed excretion characterise acute rejection. Bladder activity is better seen in cases of acute rejection than in cases of acute tubular necrosis. Transplant perfusion index is almost always abnormal in acute rejection and normal in early cases of acute tubular necrosis. Urinary outflow tract obstruction is easily diagnosed by continuous accumulation of activity in the transplant and poor activity in the urinary bladder. Delayed static images are very helpful in detecting and identifying space occupying lesions such as urinoma, lymphocoele, urinary extravasation and abscess. Transplant dysfunction, in the absence of such usual complications as acute rejection and acute tubular necrosis may be due to drug toxicity (Cyclosporin toxicity). Needless to say that the information obtained from radionuclide investigations has to be evaluated in the light of the overall clinical picture of the patient.

Radiopharmaceuticals such as $^{99}Tc^{m}$ sulphur colloid and ^{123}I fibrinogen have been used to differentiate acute rejection from acute tubular necrosis. A kidney being rejected accumulates these radiopharmaceuticals whereas the kidney with acute tubular necrosis does not.

9. Reflux nephropathy

Vesicoureteral reflux (VUR), i.e. retrograde flow of urine from the bladder into the ureters and upper urinary tract, is not an uncommon disorder. This phenomenon may be congenital or acquired. When VUR is congenital and not associated with other defects such as bladder neck obstruction and urethral valves, it may regress and spontaneously disappear with time. When the VUR reflux is associated with the above-mentioned defects of the lower urinary tract, it may progress relentlessly and result in functional and structural changes in the kidney and upper urinary tract. Structural abnormalities may include dilatation of calyces and ureters and scarring of the kidney following episodes of infection. These patients are at higher risks for urinary infection and repeated episodes of infection and consequent scarring may lead to renal failure termed reflux nephropathy. Acquired VUR can be seen in patients who have undergone ureter implantation surgery.

Adequate evaluation of patients with VUR remains a challenge. The diagnosis of VUR has been traditionally dependent on X-ray micturating cystourethrograms (MCU). Over the past two decades, several investigators have shown that radionuclide MCU using a gamma camera computer system and $^{99}Tc^{m}$ DTPA is more reliable and sensitive in detecting VUR than X-ray MCU.

Spot film X-ray MCU requires catheterization. It provides excellent anatomical details of the urinary system, but it gives more radiation to the gonads and involves added risk of infection in these patients in whom infection is to be avoided. It is also less sensitive in detecting VUR, especially when the reflux is intermittent. Radionuclide MCU overcomes many of these shortcomings of X-ray MCU and is ideal for repeat studies as it is non-invasive and involves less radiation to the gonads. For these reasons, it is particularly suitable in paediatric patients - the group which has the maximum incidence of VUR.

Radionuclide MCU can be performed in two ways, one as a follow-up of procedure of a $^{99}Tc^{m}$ DTPA renographic study. Following the renographic study, sufficient time is given for the radioactivity to accumulate in the bladder before performing the radionuclide MCU. The other method is to introduce radioactivity directly into the bladder either through a catheter or through a suprapubic puncture of the bladder. The first method is called indirect radionuclide MCU and the second method is called direct radionuclide MCU. In the author's experience, direct radionuclide MCU through the suprapubic puncture of the bladder using a disposable hypodermic needle has been simpler and risk-free. A direct radionuclide cystogram provides, in addition to the demonstration of VUR, quantitative indices like initial bladder volume, residual bladder volume, refluxing volume, maximum and minimum urine flow rate during the act of micturition. These are important parameters while evaluating a patient with VUR before or after the treatment.

VUR can be graded I-V, depending on the extent of retrograde urine flow and the structural alterations of the upper urinary tract induced by the reflux.

COMMON RADIONUCLIDE INVESTIGATIONS

1. Static renal imaging
2. Dynamic renal imaging
3. Quantitative estimation of GFR/RPE
4. Probe renogram (non-imaging)
5. Interventional studies
6. Cystourethrogram and urine flowmetry.
7. First pass renal angiography

1. Static renal imaging

Indications

(a) Assessment of mass lesions (cysts, tumours, pseudotumours)

(b) Congenital abnormalities (fusion, ectopia)

(c) Assessment of function (divided, regional)

(d) Detection of renal scarring (following infection)

$^{99}Tc^{m}$ DMSA is the radiopharmaceutical of choice for static renal imaging, since it is fixed to the kidney parenchyma for a long period, $^{99}Tc^{m}$ GH (glucoheptonate) is also used, but is not as good as DMSA.

Procedure for $^{99}Tc^{m}$ DMSA/GH static gamma camera imaging

(a) Prepare a dose of $^{99}Tc^{m}$ DMSA or $^{99}Tc^{m}$GHA. The adult dose is about 185-259 MBq (5-7 mCi). For children 74-185 MBq (2-5 mCi) of radioactivity should be used.

(b) Set up the gamma camera for $^{99}Tc^{m}$ with a low energy, high resolution, parallel hole collimator.

(c) The radioactivity is administered intravenously and renal imaging is done at 15 minutes, 30 minutes, 1 hour, 2 hours and at 4 hours. If necessary, a scan should also be done at 24 hours in patients with poorly functioning kidneys.

(d) Isotime renal imaging is done both in the anterior as well as in the posterior projection. Oblique views can be taken wherever necessary. (Isotime = Note the time taken to collect sufficient counts to get a good image in the anterior projection, and use the same time for all other projections.)

(e) Certain useful guidelines should be followed at the time of interpreting a static renal scan.

   (i) Observe the site, size and shape of the kidneys. Look for any asymmetry in size, abnormality in shape, irregular outline, etc.

   (ii) Observe qualitatively the degree of radionuclide concentration in each kidney.

   (iii) Observe the distribution of radioactivity in the renal parenchyma. Look for evidence of scarring, SOL, dilated pelvicalceal system and upper urinary tracts. Multiple, focal cortical uptake defects are suggestive of scarring.

(f) For the calculation of relative renal function, ideally the delayed images obtained at four hours are taken into consideration, and the calculations are done using a region of interest (RIO) selector and appropriate software.

(g) (i) First the posterior view is analyzed. ROIs are selected over the right kidney (RIO 1), left kidney (RIO 2), background below the right kidney (RIO 3) and background below the left kidney (RIO 4).

   (ii) The areas of RIO 1 and RIO 3 are first normalized followed by normalization of areas of RIO 2 and RIO 4. Using the normalized counts, the renal concentration of radioactivity is calculated for each kidney as follows:

Net cts.in rt kid. in post.view = cts.in rt kid. - Normalized bkg. cts.
Net cts.in lt kid. in post.view = cts.in lt kid. - Normalized bkg. cts.

(h) Similarly, the anterior views are also analyzed and net counts in both kidneys are calculated.

(i) The following data are now at our disposal:

Net counts in right kidney in the posterior view (RP)
Net counts in right kidney in the anterior view (RA)
Net counts in left kidney in the anterior view (LA)
Net counts in left kidney in the posterior view (LP)

(j) Percentage function of each kidney can be calculated by using the following formula:

$$\% \textit{ Function right kidney} = \frac{\sqrt{(RP \times RA)}}{\sqrt{(RP \times RA)} + \sqrt{(LP \times LA)}} \times 100$$

$$\% \textit{ Function left kidney} = \frac{\sqrt{(LP \times LA)}}{\sqrt{(LP \times LA)} + \sqrt{(RP \times RA)}} \times 100$$

2. Dynamic renal imaging

Indications

(a) Assessment of renal function (divided, regional)

(b) Assessment of dilated upper urinary tract (obstruction versus non-obstruction)

(c) Obstructive uropathy

(d) Vesicoureteral reflux

(e) Assessment of renal transplant function/complications

(f) Assessment of renal blood flow

Dynamic scintigraphy is done mostly using $^{99}Tc^{m}$ DTPA and gamma camera computer system. $^{99}Tc^{m}$ MAG 3 has been recently introduced, as replacement for iodinated Hippuran. MAG 3 (Mercapto acetyl triglycine) is a tubular agent.

Procedure for $^{99}Tc^{m}$ DTPA/MAG 3 gamma camera renography.

(a) Prepare a dose of $^{99}Tc^{m}$ DTPA or $^{99}Tc^{m}$ MAG3. The adult dose is normally about 185-259 MBq (5-7 mCi). For children, 74-185 MBq (2-5 mCi) of radioactivity should be taken.

(b) Set up the gamma camera for $^{99}Tc^{m}$ with the low-energy high-resolution parallel hole collimator or a low-energy all-purpose (LEAP) collimator.

(c) Position the patient for renal dynamic study. Normally, the study is performed

with the patient lying supine on the examination table with the detector placed below the patient. Ideally the renal area should be in the centre of the field of view of the detector.

(d) Set the computer ready to acquire data in frame mode.

(e) Administer the radioactivity intravenously into the patient as a bolus and simultaneously activate the computer. Acquire data in frame mode, in a total of 127 frames.

(f) Depending upon the information required, the acquisition of data can be made either uniformly (time per frame - 16 seconds for all 127 frames) or by applying the variable time acquisition facility (time per frame - 1 second for first 40 frames, followed by time per frame - 16 seconds for the rest of the frames).

(g) At the end of acquisition, examine the serial images frame by frame. Select regions of interest (RIO) over right kidney (RIO 1), left kidney (RIO 2), abdominal aorta (RIO 3) and background (RIO 4). Whenever necessary additional RIO's may also be selected over the ureters and the urinary bladder. Area normalized and background subtracted time activity histograms are obtained over the selected RIO's.

(h) Routinely, an X-ray printout of the serial images, selected RIO's, and time activity histograms along with the annotations are obtained for reporting and record.

NOTE: The study can be suitably modified to evaluate the single transplanted kidney.

3. Quantitative estimation of renal function (GFR/RPF)

Indications

Individual kidney function in unilateral or asymmetric bilateral renal disease:

(a) as a baseline parameter for future reference

(b) to decide about nephrectomy or salvation surgery

(c) for prognosis regarding beneficial effects of nephrectomy, in renal artery stenosis and hypertension

3A. GFR determination

Procedure for determination of GFR

(a) Prepare weighed dose of 74 MBq of a stock solution of $^{99}Tc^{m}$ DTPA and weighed standard of 74 MBq. Calculate the dose/standard ratio (correction factor - CF) from their net weights.

(b) Dilute the standard to exactly one litre (note the dilution factor - DF).

(c) Inject the dose intravenously. Note the time.

(d) Take 6 ml heparinized blood samples at 1.5, 2, 2.5, 3. 3.5 and 4 hours post-injection, again noting the actual collection time.

(e) Centrifuge the blood samples.

(f) Dispense 1 ml samples of plasma and diluted standard solution into labelled sample tubes.

(g) Measure the activity of each sample, using a scintillation well counter calibrated and set up for $^{99}Tc^{m}$.

(h) Using a semilog (log-linear) paper, plot the plasma activity as a function of time. Find the best straight line fit to these data and extrapolate the line to zero time. Note the extrapolated activity at zero time. Calculate the half time of clearance (T½).

(i) Calculate GFR by using the principle of single exponential analysis.

(j) First calculate the volume of dilution (V) as follows:

$$V = \frac{\text{Injected dose}}{\text{Plasma activity at 'O' time}}$$

$$= \frac{\text{Activity diluted standard x DF x CF}}{\text{Plasma activity at 'O' time}}$$

(k) Now calculate GFR as follows:

$$\text{GFR} = \text{Volume of dilution x clearance constant (Ml/min)}$$

$$= V \times \lambda \quad \text{(ml/min)}$$

$$= \frac{V \times 0.693}{T\frac{1}{2}} \quad \text{(ml/min)}$$

Note: Decay corrections for $^{99}Tc^{m}$ may be applied wherever necessary.

3B. Determination of renal plasma flow (RPF)

<u>Procedure for calculation of RPF</u>

(a) Prepare a weighed dose of 1.85 MBq of a stock solution of 131I Hippuran and weighed standard of 1.85 MBq. Calculate the dose/standard ratio (correction factor - CF).

(b) Dilute the standard to exactly one litre.

(c) Inject the dose intravenously.

(d) Take 4 ml heparinized blood samples at 10 minutes interval up to 60 minutes using a site other than that used for the injection of radioactivity and note the collection time of each sample.

(e) Centrifuge the blood samples.

(f) Dispense 2 ml samples of plasma and diluted standard solution in labelled sample tubes.

(g) Measure the activity of each sample using a scintillation well counter set up for ^{131}I.

(h) Using a semilog (log-linear) graph paper plot the plasma activity as a function of time. Calculate RPF by using the principle of biexponential analysis and the following formula:

$$\text{RPF} = \frac{\lambda 1\ \lambda 2 \times \text{dose injected}}{A\lambda 2 + B\lambda 1} \quad \text{ml/min)}$$

$$= \frac{\lambda 1\ \lambda 2}{A\lambda 2 + B\lambda 1} \times \text{Counts in diluted standard} \times \text{Dilution factor} \times \text{Correction factor CF (ml/min)}$$

Using curve splitting, separate the slow component from the fast component.

$\lambda 1$ - slope of slow component
$\lambda 2$ - slope of fast component
A - Zero time intercept of $\lambda 1$ at the activity axis
B - Zero time intercept of $\lambda 2$ at the activity axis

4. Probe renogram.

Indications

(a) Qualitative assessment of individual kidney function.

(b) Detection of obstructive uropathy, particularly acute obstruction.

(c) Assessment of dilated upper urinary tract (obstruction versus non-obstruction).

(d) Assessment of renal transplant function.

The radiopharmaceutical used is ^{131}I OIH and the instrument is a multiprobe (minimum two probes) scintillation detector and strip chart recorder for recording the time activity curves (complete renography system).

Procedure

Radio-Hippuran renogram

A radio-Hippuran probe renogram is the most common technique for recording the renogram, where a simple dual probe system is used. Two matched sodium iodide scintillation detectors through the standard system of electronics are connected to ratemeters, which in turn are connected to chart recorders. The chart recorders produce graphs of activity in the kidneys with respect to time following intravenous bolus injection of ^{131}I Hippuran. Additional information may be obtained from other detectors placed over the urinary bladder and the left infra-clavicular region.

Since the renogram records ^{131}I accumulation in the renal area, it is obvious that the position of the detectors has an important effect on the shape of the tracings obtained. Precise placement of the probes is a critical step in this study and wrong probe placement may lead to false positive results. In normal practice, before each study, the surface marking

of the patient's kidneys are drawn and the probes are placed in apposition to them. Some centres use a small pilot dose of ^{131}I Hippuran to locate the kidneys before injecting the major portion of activity for the renogram.

The patients generally do not require any preparation. It is a good practice to ensure that patients drink enough water and empty their bladder before starting the renogram. These two precautions will minimize false positive renograms mimicking outflow obstruction by dehydration and back pressure from a full bladder. It is better to avoid doing renograms soon after intravenous urography or angiography.

The test is usually performed with the patient sitting. Most patients do not have any difficulty in maintaining the posture for the duration of the test which is about twenty minutes. But this does not apply to sick patients and infants in whom the test is performed in prone position. A mild sedation may be necessary in young children and un-cooperative adults.

After the patient is positioned 2.2-3.7 MBq (60-100 microcurie) of ^{131}I Hippuran is injected intravenously into the antecubital vein as a tight bolus. Simultaneously the chart recorder is activated. Normally the study is conducted for a period of 20 minutes. The volume of the injected activity should be as small as possible in order to obtain a good bolus function and the injection must be accurate, since any extravasation will produce a flat curve due to slow and continued absorption of the tracer from the subcutaneous tissue.

The renogram curve.

The normal renogram performed in this way shows three classical phases **(Fig. 31.3)**. The first phase consists of a rapid rise in count rate during the first minute after injection. It corresponds mainly to the radioactivity in both the extra renal and renal vascular beds within the first few seconds following intravenous injection of ^{131}I Hippuran. After few seconds this gives way to a more gradual slope, the second phase, which corresponds chiefly to the renal handling of the Hippuran as it is extracted by the tubular cells and passed to the lumen of the tubules. In a normal well-hydrated subject, the second phase rises towards a maximum, the peak, which occurs 2.5 to 4.5 minutes after the injection. The shape and the duration of this part of the curve are dependent on several factors: renal blood flow, tubular extraction efficiency, intraluminal transit and forward drainage. The rising curve is due to the fact that more and more Hippuran is arriving at the kidney through recirculation while none has left the renal pelvis. The period of declining amplitude after the peak is the third phase of the renogram. In this phase, more radioactivity leaves the renal pelvis than what is arriving in it. The beginning of the third phase of the renogram corresponds to the time at which activity first appears in the bladder. The third phase of the renogram curve reflects predominantly the drainage function of the kidney. In normal subjects the slope of the third phase usually begins to plateau near the base line about 20 minutes after intravenous injection.

Each phase of the renogram is determined by a number of simultaneously occurring intra- and extra- renal activities. The overlapping of these multiple physiologic processes determine the shape of the renogram curve but precludes the separation of the individual contributing factors. Because of the difficulty in separating these factors, and the changes in their relative contribution to the three phases of the renogram, it is not possible to quantitate the physiological parameters of renal function from the analysis of the curve. In spite of all these limitations, the renogram still provides a truly functional assessment of the kidney and upper urinary tract. It provides useful information in various renal disorders, such as obstructive uropathy **(Fig. 31.4)**, nephropathies of various etiologies **(Fig. 31.5)** and renovascular hypertension **(Fig. 31.6)**.

Renogram curve interpretation

Certain useful guidelines should be followed at the time of interpreting a renogram curve. They are as follows:

(a) First, observe the deviation from the normal pattern of the renogram curve.

(b) Translate these deviations to possible alterations in the physiology of the kidney in terms of blood flow, kidney uptake function, urine flow rate and drainage function.

(c) Such derived information as to the changes in kidney physiology is useful in providing differential diagnosis.

(d) The most probable diagnosis is selected out of the differential diagnosis on the basis of the clinical setting of the patients's disorder.

(f) It may be pointed out that a renogram curve should not be used primarily to pinpoint a diagnosis, but should be used mainly to identify the altered kidney physiology as a result of the disease in the patient.

5. Interventional techniques

(a) Diuresis renography

Increased urine flow caused by diuresis overcomes simple stasis and helps in clearing the radioactivity from the upper urinary tract. On the other hand, if the stasis is due to true mechanical obstruction, increased urine flow cannot overcome the hold-up and the radioactivity tends to accumulate in the urinary tract proximal to the obstruction. This is the principle underlying the diuretic intervention. The urinary tract may or may not be dilated depending on whether the impediment to urine flow is chronic or acute. It has been shown that diuresis renography can distinguish between dilated obstructed upper urinary tracts from dilated non-obstructed ones - an issue that cannot be resolved by other investigations like ultrasound, IVP and X-ray CT. It is important to realise that the predictive value regarding

the presence of obstruction is closely related to the function of the kidney. Good function increases the predictive value and poor function decreases it.

There is no consensus regarding the time of diuretic intervention as to whether it should be before the start, at the beginning or in the middle or at the end of the renograpy procedure. The most commonly used diuretic is Frusemide administered intravenously in the dose range of 0.3-0.5 mg/kg body weight. It is important to standardize the technique, validate it, and establish values for different groups of patients, so that the interpretation of the test has a meaningful basis.

Following diuresis, a significant decline in the third phase of the renogram curve rules out any organic obstruction and the need for any urgent therapeutic measures. Absence of a significant fall in the third phase indicates obstruction and the need to watch that kidney closely and to undertake measures to remove the obstruction and save the kidney from further damage. Attempts to quantitate the decrease or the change in radioactivity following diuresis have given rise to various indices such as diuretic index, and diuretic excretion index, etc., which help in interpreting the results and in monitoring the changes with time and with treatment more objectively.

In situations of compromised renal function, (such as sub-total/partial obstruction, equivocal or low-grade pelviureteric functional stenosis, grossly dilated pelvicalyceal system, immediate post-operative period, and severe vesicoureteric reflux), the test may give results which may be difficult to interpret unequivocally.

(b) Captopril renography.

In renovascular hypertension, the elevated systemic arterial blood pressure is sustained in the early stages mainly through the renin-angiotensin mechanism. Angiotensin I is converted to angiotensin II, which is a powerful vaso-constrictor, by the angiotensin-converting enzyme (ACE) present in lungs.

Captopril is used as an antihypertensive drug. It acts by inhibiting the ACE and thereby blocking the production of angiotensin II. In the kidney with a stenotic renal artery, filtration at the glomeruli is maintained by vaso-constriction of the efferent arterioles sustained by angiotensin II. Captopril relieves this vaso-constriction, resulting in a fall of glomerular filtration. This is the pathophysiological mechanism underlying Captopril intervention and the principle of Captopril renography.

Demonstration of a decrease in the renal uptake of $^{99}Tc^{m}$ DTPA by the kidney with a stenotic artery following Captopril administration indicates that the renal artery stenosis is functionally significant in triggering the renin-angiotensin mechanism responsible for hypertension. When iodinated OIH or $^{99}Tc^{m}$ MAG3 is used, there is progressive accumulation of radioactivity in the kidney with the stenotic artery due to prolongation of transit time. This is also considered to be the result of shutting off glomerular filtration by Captopril administration.

Captopril is administered orally, in the dose of 25-50 mg for the adult patient, one hour before the renographic procedure. Haemodynamic parameters like pulse and blood pressure of the patient are regularly monitored until they become stable. Hypotension and transient renal failure (pre-renal) are occasional complications. Adequate volume replacement by intravenous fluids takes care of these complications.

It is believed that a Captopril renogram is more sensitive in the diagnosis of renovascular hypertension than the conventional one. It is also considered to indicate the outcome of revascularization procedures to establish adequate blood supply to the kidney. A positive Captopril test suggests successful results after revascularization. As the test is new, these observations are based on few studies, and there is a need to substantiate these by larger experience with Captopril renography.

6. Cystourethrogram and urine flowmetry

Evaluation of bladder function and urethral patency had not received much attention from nuclear medicine specialists so far. This had largely been due to lack of demand from the urologists who were not made aware of the potentials of nuclear medicine techniques in this field. This situation is slowly changing. Nuclear medicine techniques for the evaluation of urinary bladder functions are becoming popular especially with the paediatric urologists, mainly because of the sensitivity, accuracy and unmatched safety and simplicity.

A cystourethrogram essentially demonstrates the distribution and kinetics of radioactivity in the bladder during different phases of its function, i.e. filling and micturating phases. This helps in detecting vesico-ureteral reflux (VUR) and in measuring urine flow rates at given bladder volumes. This has been found to be very useful in the management of patients with VUR, neurogenic bladder, bladder neck obstruction, posterior urethral valves and urethral stenosis.

The radioactivity in the bladder can be from the renographic study using $^{99}Tc^{m}$ DTPA, if the cystourethogram is planned soon after (indirect radionuclide cystourethogram - IRCG). Radioactivity can be also directly introduced into the bladder either through a urethral catheter or through a suprapubic puncture of the urinary bladder by a needle (direct radionuclide cystourethrography - DRCG). Both have certain advantages and disadvantages; DRCG is described in some detail here as it is the procedure favoured by the author.

DRCG by suprapubic puncture is more physiological than a conventional X-ray micturating cystourethrogram (MCU) and catheter-aided DRCG. It avoids complete emptying and refilling of the urinary bladder.

Technique

In performing direct radionuclide cystogram (DRCG) and urine-flowmetry (UFMT), the patient is permitted normal activity prior to the examination. Primary requirement is a

reasonably full urinary bladder. The patient is encouraged to drink a lot of water and if he/she is able to hold urine, the patient is instructed not to pass urine for at least 1-2 hours prior to the procedure.

The patient lies supine on the examination table. The lower abdomen and suprapubic area is thoroughly cleaned with antiseptics. Suprapubic puncture of the urinary bladder is done using a 22 gauge needle about 2 cm above the pubic symphysis in the mid-line. A small amount of urine is drawn into the syringe to make sure that the needle has entered the bladder cavity. 37-74 MBq (1-2 mCi) of $^{99}Tc^{m}$ DTPA is injected in a volume of about 1 cc into the bladder and the needle is withdrawn. In children, a normal size (38 mm length) disposable needle is good enough to enter the urinary bladder. However, in adults, a large needle, preferably an intracath with a 45 mm long needle, is required. In a properly prepared patient with a reasonably full urinary bladder, it is possible to enter the urinary bladder without much problem. Care should always be taken not to inject any radioactivity into the bladder wall. After this the patient is placed before the gamma camera, and serial images of the urinary bladder in anterior projection are obtained at rest, every ten seconds for a period of 5-6 minutes. Following this, the patient stands with his back facing the gamma camera detector and passes urine without interruption into a container. While the patient is micturating, images of the urinary bladder and lower abdomen are obtained in frame mode (total frames: 120, time per frame: 1 second) using appropriate computer software. The total amount of urine passed is accurately measured.

Serial images of the urinary bladder are visually inspected frame by frame to see evidence of VUR. Sometimes contrast enhancement of the images may be essential for the detection of minor degrees of reflux. Following this, an appropriate frame or a combination of a few frames is selected to mark the regions of interest (RIO) by a computer light pen (or any other suitable device). RIO's are selected over the urinary bladder, areas corresponding to the upper urinary tracts on both sides and background. Time activity curves are generated over these regions. This particular technique is based on certain assumptions, which are:

(a) concentration of radioactivity in the bladder is uniform at the time of study; and

(b) there is a linear correlation between external counting and radioactivity inside the bladder, while its contents are progressively decreasing. Based on these assumptions, the counts recorded during the study will be proportional to the volume(s).

From the relationship derived from the volume and radioactivity of the voided urine sample, it is possible to quantitate the bladder volumes - initial and residual, i.e. before and after the act of micturition, respectively. It is also possible to quantitate the urinary flow rate by processing the bladder activity clearance curve and calculating the maximum urine flow rate for a given bladder volume. There is of course the need to standardize the test, validate the results and define normal ranges for these parameters in the given population before the test results can be interpreted for clinical management of patients.

7. First-pass renal angiography

Radionuclide first-pass scintigraphy has become an established procedure after the advent of the gamma camera computer system and the most favoured radionuclide for this is $^{99}Tc^{m}$. Both qualitative and quantitative evaluation of organ perfusion are done with this procedure. In relation to the kidney, this technique has been applied in the diagnosis of patients with renal artery stenosis and renovascular hypertension, in the differential diagnosis of mass lesions of kidney, and in the evaluation of patients with renal trauma. But this technique has proved its usefulness most illustratively in the evaluation of renal transplant dysfunction, particularly to distinguish acute rejection from other causes of transplant dysfunction.

Renal transplant perfusion index (RTPI)

RTPI is one of the quantitative indices derived from the first-pass renal scintigraphy study. Essentially, this parameter defines the ratio of blood flow to the kidney transplant and the artery that supplies blood to it. This index is expressed in many ways. The following is the technique used by the author to determine the RTPI.

The test is performed with the patient lying in supine position with the transplanted kidney under the field of view of the camera. The technique involves the intravenous bolus injection of 185-370 MBq (5-10 mCi) of $^{99}Tc^{m}$ DTPA. Immediately following the intravenous injection, rapid sequence dynamic images of the transplant are obtained in frame mode (in a total number of 127 frames) by using a histo-acquisition programme with variable time facility. For the first forty frames, the acquisition of data is done at a faster rate (time per frame: 1 second). After that the acquisition is done at a slower rate (time per frame: 16 seconds). This is done in order to have a good separation between the first-pass perfusion curve and the subsequent uptake function curve of the kidney (Fig. 31.7).

The sequential images are first examined frame by frame to assess qualitatively renal perfusion, renal cortical and collecting system morphology, and to select appropriate frame for marking the regions of interest (RIO). RIO's are selected over the transplant, external iliac artery, urinary bladder and background. As and when necessary, an RIO can also be selected over the ureter. All the RIO's are normalized prior to background correction. Corrected time activity histograms are generated from each RIO.

The transplant perfusion index is determined by taking into consideration the area under the normalized arterial and renal curves up to the peak of the arterial curve caused by the first passage of the radioactive bolus and is calculated by the following formula:

$$\text{Renal transplant perfusion index (RTPI)} = \frac{\text{Area under the arterial curve up to the peak}}{\text{Area under the renal curve up to the time of peak arrival of arterial curve}}$$

When the two areas are identical, the TPI has a value of unity. As relative blood flow through the kidney is reduced, the area under the perfusion phase of the renal curve becomes smaller, and the value of TPI increases. **(Fig. 31.8).**

Fig. 31.8 shows diagrammatically the determination of RTPI. The RTPI has been shown to have a very discriminating value in the differential diagnosis between acute rejection and acute tubular necrosis, two common conditions affecting a renal transplant. **Fig. 31.9** illustrates this discriminating value of RTPI in 70 scintigraphic studies done in renal transplant recipients.

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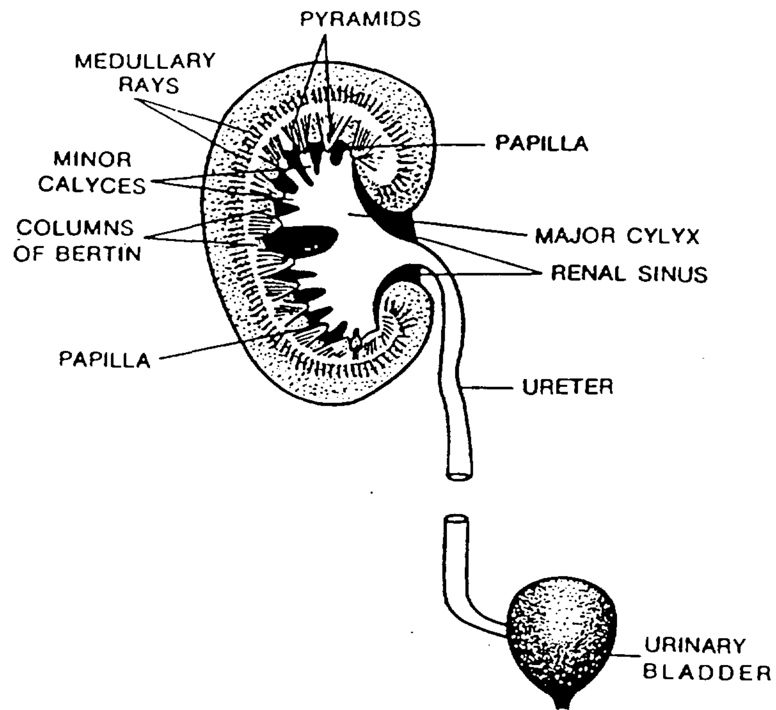

Fig. 31.1 Gross anatomy of kidney and upper urinary tract.

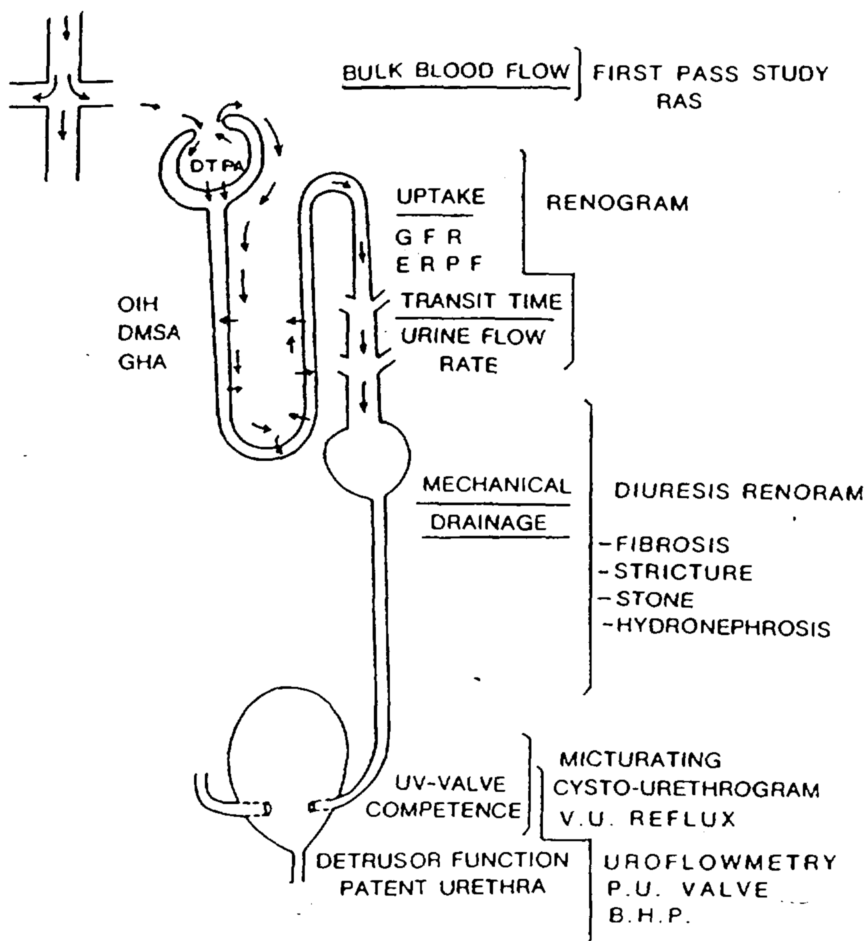

Fig. 31.2 Functional anatomy of nephron. The radiopharmaceutical arrives at the nephron via renal artery (top left). It is handled by filtration and/or tubular secretion and passed into the lumen of collecting tubule. The physiological parameters and the related functional segment of nephron are indicated. The radionuclide investigations and their major indications are also mentioned.

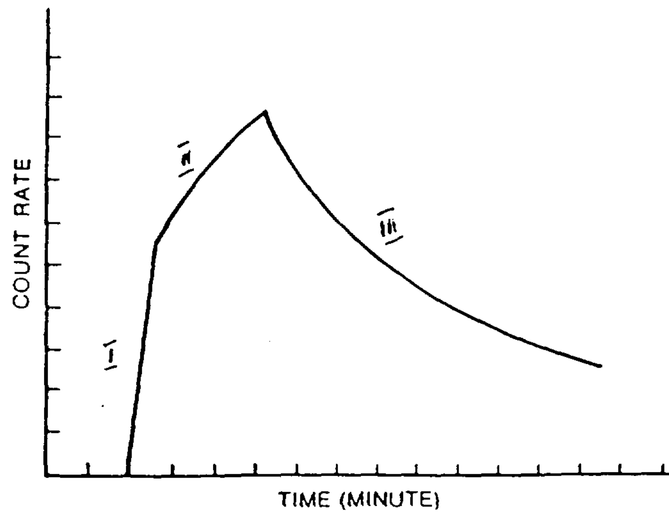

Fig. 31.3 The normal renogram curve showing the classical three phases.

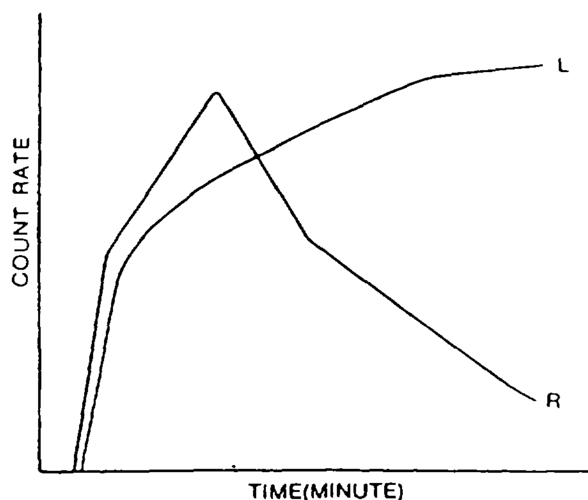

Fig. 31.4 The renogram pattern in a patient with unilateral obstructive uropathy (LT side) showing an absent third phase.

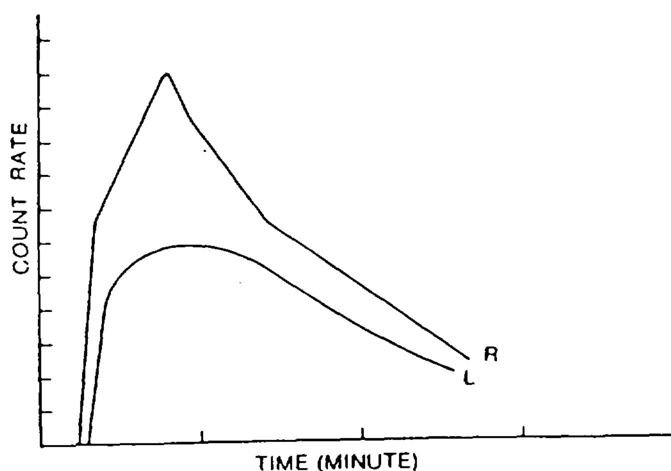

Fig. 31.5 The renogram pattern in patient with left-sided parenchymal disease showing a flattened phase II and delayed peak due to compromised renal function, but no evidence of any obstruction to urine outflow.

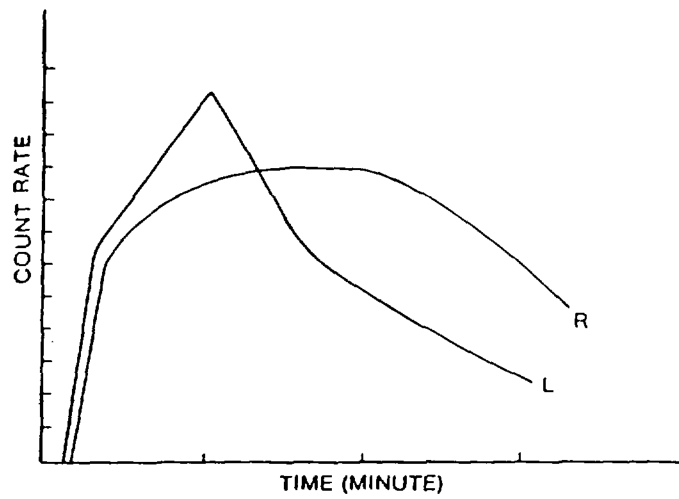

Fig. 31.6 The renogram pattern in patient with unilateral (right side) renal artery stenosis showing flattened phase II, delayed peak and prolonged phase III.

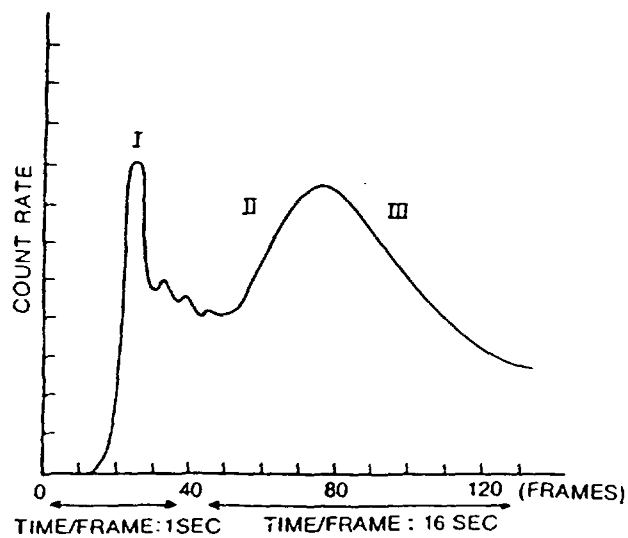

Fig. 31.7 $^{99}Tc^{m}$ DTPA study using a gamma camera-computer system with variable time acquisition. Normal study. Note the separation between the first-pass perfusion curve (I) and the function curve (II and III)

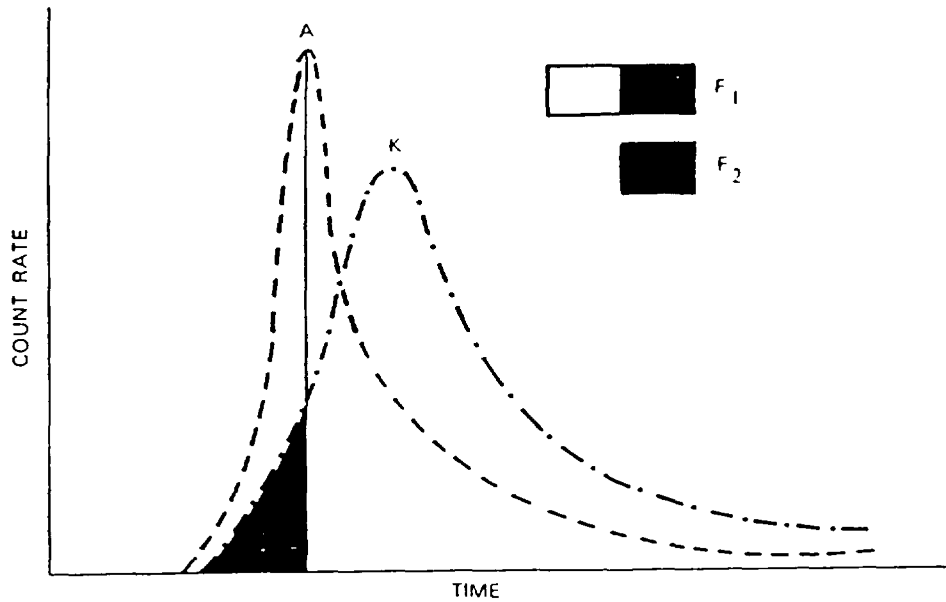

Fig. 31.8 Determination of perfusion index from the first-pass time activity curves over the external iliac artery and the transplant RTPI = F_1/F_2.

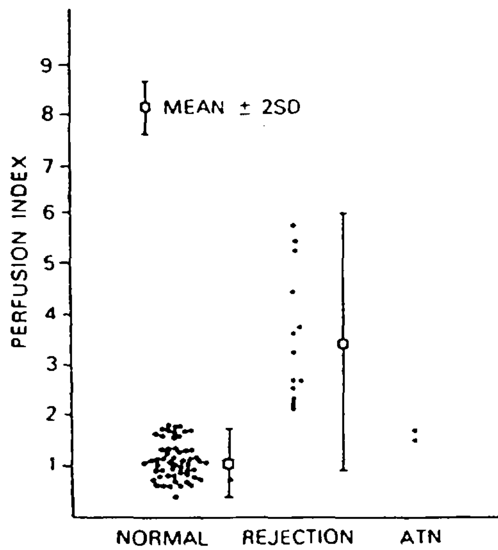

Fig. 31.9 Perfusion index in normal, acute rejection, and acute tubular necrosis

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Chapter 32

TRAINING IN NUCLEAR MEDICINE

Based on the recommendations of IAEA/WHO Seminar
8-12 August 1988, Vienna, Austria

Introduction

Nuclear Medicine is defined as a clinical specialty that utilizes the radionuclides for diagnosis, therapy and medical research. The radionuclides are used as unsealed sources of radioactivity. The diagnostic applications include both in vivo and in vitro uses of radioisotopes. There is hardly any medical research which does not use radioactive compounds. Only clinical research is considered within the purview of nuclear medicine. The above seminar reviewed the needs of training in nuclear medicine mainly for the physicians with special emphasis on the needs of the developing countries.

A somewhat similar Seminar was held under the auspices of the WHO and the IAEA at Vienna, Austria, on 18-29 November 1974. At that time nuclear medicine was one of the emerging clinical disciplines of medicine. The recommendations of the Seminar described in details the preparation and training of those likely to be involved in its application and use. Since that time, nuclear medicine has developed into an independent medical specialty and has already established reputation as a reliable diagnostic and effective therapeutic tool in clinical medicine, as well as establishing itself as indispensable in many academic and investigative undertakings. Although several new diagnostic technologies have emerged in recent years and have influenced the pattern of the utilization of nuclear medicine; in the main, nuclear medicine procedures continue to provide unique, useful and accurate clinical information in a cost-effective manner. Consequently, training programs in nuclear medicine should respond to the demands of this ever-growing and dynamic field.

The need for guidelines for training of nuclear medicine physicians such as those set in 1974 have become essential as more medical workers have taken up nuclear medicine as their field of specialization and as technology and instrumentation have become more complex and elaborate.

"Components of Professional Competence of Nuclear Medicine Physician" published by the American Board of Nuclear Medicine [Journal of Nuclear Medicine 27, 1986, 863-865] is a comprehensive guide to a syllabus for nuclear medicine. A complete syllabus like this may seem an unrealistic for a developing country that has yet to obtain its first gamma camera. It is, however, essential that those who are going to be the leaders in nuclear medicine over the next 20-30 years should be aware of current and possible future developments in the field.

The approach to the training of nuclear medicine specialists in the developing countries implies that a substantial proportion of students would acquire knowledge and experience in more advanced countries, in spite of the attendant danger of frustration upon their return home and possible "brain drain".

The establishment of centres of excellence with advanced facilities and training schemes, located on a regional basis in developing countries, appears to be an ideal solution but there would be problems in selecting suitable sites in most areas.

The duration of existing courses in nuclear medicine varies widely between countries; from the one year M.Sc. course of the University of London to four or five years courses in some of the advanced countries. It should be recognized that the duration of the training would vary from one country to another to suit the local conditions and to conform to the pattern and format of other postgraduate training programs in the University. However, it was felt that a training period of less than two years in an advanced laboratory would be inadequate.

Statements.

1. Nuclear medicine should be identified as a recognized medical specialty and departments of nuclear medicine should be established in all post-graduate teaching hospitals.

2. Nuclear medicine should be included in the undergraduate medical curriculum for the purposes of informing medical students of its clinical utility and to identify nuclear medicine as a career option.

3. Candidates for nuclear medicine postgraduate training must be qualified and licensed medical graduates.

4. Nuclear medicine is a clinical specialty and its graduates must be competent clinicians.

5. The duration of nuclear medicine training program shall be left to the discretion of the certifying board of its country but should not be less than two years.

6. In spite of the development of non-radionuclide imaging modalities, nuclear medicine continues to thrive especially in functional studies. Moreover, newer applications continue to develop with their own unique advantages.

7. The capital and operating costs of a nuclear medicine facility are small compared to CT and NMR.

8. Standards for training programs should not differ between developed and developing countries.

9. Formal certification in nuclear medicine should provide a career structure for specialists. There should be enough job opportunities in the country to attract new trainees.

10. Introductory courses and short special courses serve an useful purpose and can meet specific needs but should not be equated with a formal specialty training program.

11. Core curriculum should be uniformly defined, and should be inviolable. Additions may be made as required by the respective national training programs.

12. The teaching of the theoretical aspects of nuclear medicine should be adequate to explain the basis of pre-clinical and clinical work and to enable the future specialists to adapt to the evolving modes of practice throughout his career.

13. Training in-vitro assays should be an integral part of the curriculum.

14. Training in nuclear medicine can be advantageously accomplished in accredited Regional Training Centers.

15. When allocating fellowships and grants, regional possibilities should be explored before consideration of placements abroad.

16. Fellowship and other grants for training abroad should have a specified purpose and plan of application in the home country.

17. Institutions desiring to start a training program in nuclear medicine should be using a representative array of current generation equipment.

18. Operation of nuclear medicine unit is a multi-disciplinary effort with the participation of all relevant professional groups in the instruction of trainees.

19. Agencies such as the IAEA and the WHO should closely integrate their activities in support of nuclear medicine, particularly in developing countries.

Entrance qualifications, types of training and examinations:

Within any country, the authority for establishing standards of training and the certification of nuclear medicine specialists should lie with a body concerned with the entire field of nuclear medicine.

The certification should be after an integrated program of training in the entire field of nuclear medicine.

The clinical training and demonstrations, and "on the job" practical training in nuclear medicine, should take place in centralized departments of nuclear medicine or in an integrated program involving a group of associated departments. The training program should be under the direction of a full-time physician qualified and experienced in nuclear medicine.

NM is a clinical specialty and sound clinical training and experience are essential for the practice of NM. Physicians entering the field of nuclear medicine should therefore have to obtain, during training, a sound background of broad clinical experience, in accordance with national program requirements.

For nuclear medicine specialists, there should be at least two years of full-time training. The duration may vary from country to country so as to be commensurate with the university requirements for other medical qualifications but should not be less than two years.

A comprehensive examination should be held on completion of training under the direction of the certifying body, including the basic aspects and methods of nuclear medicine, and its clinical applications. In view of the latter requirement, the examination should include testing on actual clinical situations.

Institutional requirements for training.

General considerations.

Specialization training should be given in centralized nuclear medicine departments of such a nature that the full-time specialist in the whole field of nuclear medicine could receive his full training in them, or in an integrated rotation through affiliated establishments or specialized institutions. In the latter case, it would be essential that the training program be coordinated, with guidance from one of the establishments, and that the total content of the program would be equivalent to that based on a single centralized nuclear medicine department.

The facilities provided for the training of a full-fledged nuclear medicine specialist should be of such an extent and composition as to provide ample clinical experience. The program should allow adequate opportunity for trainees personally to study patients undergoing nuclear medical procedures, in vitro and in vivo laboratory studies, and nuclear medicine therapy. It should be of sufficient scale and diversity to provide a broad experience of the various types of clinical applications of radionuclides in diagnosis, treatment and follow-up. There should be adequate case notes and records of the results of investigations and treatment, so that the role of nuclear medicine in the care of the patient and in the diagnosis and control of disease could be fully demonstrated and incorporated in the training. It is desirable for training, as well as for practice, that departments of nuclear medicine should have beds available to them for the admission of patients for certain types of investigations and/or therapy.

Adequate lecture and seminar rooms, audiovisual aids, library, laboratory equipped to conform to radiation safety codes and other related facilities should be readily available in the training environment. The library should include adequate nuclear medicine literature including basic textbooks, monographs on special nuclear medicine topics and nuclear medicine periodicals.

Staff

The training staff should, whenever appropriate, include physicians certified in nuclear medicine and an array of scientists which may typically, but not exclusively, include medical physicists, radiopharmacists, radiochemists, specialists in medical informatics, electronic engineers and nuclear medicine technicians. The above list should not be considered to be exclusive and other medical specialists could, with advantage, contribute to the training program.

Curriculum

Specialized training in nuclear medicine is discussed in this report under fields of application into which the practice may be conveniently, albeit arbitrarily, divided.

In-vivo studies

These include all studies in which radioactive substances are administered to patients. Static or dynamic imaging and non imaging dynamic studies are examples.

Imaging methods are of great diagnostic importance in a large number of clinical fields, and ordinarily represent the most widely used group of in vivo methods. While the basic forms of instrumentation, by gamma camera or by rectilinear scanner, are common to most such applications as well as to organ function studies, the particular techniques applicable to different problems vary widely, and the subject requires detailed attention in training.

Organ function studies

While the functional activity of some organs or parts of organs can be best studied by quantitative examination of imaging data, the simple in vivo determination of the rate of uptake, turnover or discharge of a radionuclide or radiopharmaceutical with simpler instrumentation is also of great clinical value in a number of instances. The techniques involved differ for different organs and need emphasis in training.

In-vitro studies

In a rapidly increasing number of important determinations, the radionuclide is not administered to the patient but is added to a sample in vitro to determine in blood very low concentrations of substances of biological interest such as hormones by techniques like radioimmunoassay.

These purely in vitro assays apply to a wide variety of determinations of clinical importance, and probably represent the types of radionuclide procedures that are most commonly being performed, particularly in developing countries.

Therapy

Although nuclear medicine is essentially diagnostic there are a limited number of therapeutic applications. They are possible because of the local concentration or retention of radioactive materials administered (in unsealed form) by various routes, and effective as a result of selective irradiation, destruction or inhibition of pathological tissues.

Stages of training

The various levels of training in nuclear medicine are:

(a) Education in nuclear medicine at undergraduate level to all medical students to acquaint them with the scope and applications of nuclear medicine procedures, the general basis of its methods and radiation protection aspects. The student should also be made aware of other sources of exposure to ionizing radiations including that from natural sources.

(b) Postgraduate training in other medical specialties should include information on the application of nuclear medicine in the specialty concerned.

(c) Postgraduate training in all aspects of nuclear medicine was considered to be the preferred training for all those engaged in the practice of nuclear medicine. The training for this group should have a core of basic scientific training which will include training in relevant aspects of nuclear physics, instrumentation, mathematics, radiation biology, radiation protection, radiopharmaceuticals and data processing.

(d) **Continuing education** is essential since the physiological understanding and the technological basis underlying nuclear medicine studies continue to evolve rapidly. The requirement can be met in part through periodic participation in continuing education courses. Such courses may be of value when given for specialists in nuclear medicine, specialists in other branches of medicine dependent upon the application of the results of radionuclide procedures in their work, and other non-medical professionals engaged in work with the medical uses of radionuclides.

Outline of subjects required at the various stages of training

TRAINING IN NUCLEAR MEDICINE

Undergraduate education.

The following outline deals with those subjects relevant to nuclear medicine in which instruction should be received as part of the general training of a doctor up to the stage at which he attains his medical degree or diploma qualifying him to practice medicine. In countries where the degree becomes registrable only after a year of clinical work in hospital, the "undergraduate" period is taken as that preceding this year of hospital duty.

Pre-clinical education.

It was not thought that nuclear medicine should be dealt with as a separate subject in this already overcrowded part of the students' instruction, but it would certainly be valuable that the methods used in nuclear medicine and the results obtained by it should be illustrated during teaching of the traditional subjects involved in this stage in training.

Anatomy.

The imaging procedures of nuclear medicine regularly give evidence of the outline of anatomical structures in the living subject and illustrate the normal variations in size, position and shape of such organs as the liver, spleen and thyroid. In view of the development of tomographic techniques it is essential that sectional anatomy be taught and its significance emphasized.

Physiology and medical biophysics.

The seminar considered it to be most important that teachers of physiology should be aware of, and should utilize, the findings of nuclear medicine in the teaching of these subjects to undergraduate students. It should be emphasized that a wealth of physiological information can be obtained from nuclear medicine investigations with examples such as neurological studies etc.

Nuclear medicine should also have a prominent role in the teaching of endocrinology, particularly in regard to the thyroid of which the functional behavior has been elucidated extensively by radionuclide techniques.

Biochemistry.

Teachers of biochemistry will ordinarily be familiar with, and likely to describe or demonstrate, the use of tracer methods in an analysis of biochemical systems. Various applications of radioisotope techniques to areas such as molecular biology, intermediate metabolism, neurology etc, should be taught at this stage.

Pharmacology.

The use of a variety of labelled chemical agents as radiopharmaceuticals in nuclear medicine involves the need for appropriate instruction at the pregraduate stage in the metabolic pathways, kinetics, and routes of excretion of these substances and of their radioactive labels.

The student should thus have some knowledge of the metabolism of the chemical elements and substances most commonly used in nuclear medicine.

Mathematics.

It was recognized that this subject would not usually be taught as a separate part of a pre-clinical course. It is recommended, that the student at his pre-clinical stage should master mathematics as far as elementary calculus.

Some general knowledge of statistics is clearly required in any pre-clinical training and the particular requirements of nuclear medicine add very little to the general training needed at this stage, although it is important that some knowledge of the Poisson distribution should be acquired.

With regard to computers, he should know the scope and limitations of computer procedures and should be able to formulate a problem in a form that is computer compatible. He should be taught how to use computers and when they should be used.

Clinical work.

During the clinical undergraduate stage, the methods of nuclear medicine should be illustrated continuously when patients are being investigated by these procedures. It is an important aspect of clinical training that students should accompany their patients referred for nuclear medicine investigation to the nuclear medicine department so that they can observe and evaluate the procedures involved and the results. In addition it is recommended as a desirable part of clinical training that the student should be attached for a suitable period to a centralized nuclear medicine department.

Radiation exposure and protection.

It was strongly recommended that at some, preferably early, stage in his course the student should receive general instruction in the amount of radiation exposure received from natural sources and the added amounts from artificial sources, including the medical procedures. He could thus attain a proper perspective as to the potential dangers of ionizing radiations, the benefit derived from the medical uses and risks of radiation exposures. If he becomes aware of radiation as a normal component of the environment and that typical nuclear medicine diagnostic studies add little to this exposure, he will be better able to

reassure patients requiring such investigations or to assess the justification for such investigations.

This instruction would entail some knowledge of effective dose equivalent, quality factor, exposure and the related elements of radiation physics together with definitions of relevant units.

During the teaching of micro-anatomy or cytology, in the description of sub-cellular constituents including chromosomes, the attention of the student should be drawn to the effects of radiation on these structures and to the importance of radiobiological studies as a whole.

THE SYLLABUS FOR COMPREHENSIVE TRAINING IN NUCLEAR MEDICINE.

The nuclear medicine physician requires broad knowledge and experience in medicine. The nuclear medicine physician must be prepared intellectually to extend the scope of nuclear medicine beyond current methodology as the specialty advances.

Upon referral of a patient for consultation, a nuclear medicine physician must be prepared to:

(a) Obtain pertinent information from the patient and other relevant sources,

(b) Perform a physical examination related to the consultation,

(c) Select and carry out appropriate procedures in a manner that is safe to both patient and staff.

(d) Interpret the results, arrive at a reasonable diagnosis after correlating all clinical and laboratory information available, and issue a timely report.

(e) Recommend further investigations or treatment when appropriate.

(f) Assume full responsibility for patient management, if nuclear medicine therapy is indicated.

(g) Institute quality control measures in the practice of nuclear medicine,

In order that a nuclear medicine physician can effectively and efficiently shoulder these responsibilities a special knowledge in the following areas is essential.

1. **Physical sciences.**

   (a) Elementary aspects of the structure of matter.

   (b) Modes of radioactive decay with special emphasis on radionuclides commonly used in nuclear medicine.

   (c) Emissions accompanying radioactive decay, and their biological implications.

   (d) Interactions of radiation with matter.

   (e) Basic physics of other imaging procedures including X-ray computed tomography, nuclear magnetic resonance, and ultrasonography.

2. **Instrumentation.**

   (a) Principles of radiation detection and detectors.

   (b) Nuclear medicine instrumentation including gamma scintillation cameras, scanners, dose calibrators, tomographic imaging devices, positron imaging instruments, whole body counters, gamma well counters, liquid scintillation counters, monitoring devices and bone densitometers.

   (c) Quality control of nuclear medicine instruments.

   (d) Collimation of radiation detectors, the characteristics of parallel hole and other types of collimators, their response to point, line, and plane sources.

   (e) Electronic instruments, such as pulse amplifiers, pulse height analyzers, scalers, count rate meters and computer interfaces including gating systems.

   (f) Image production and display technology including photographic principles, with special emphasis on sensitivity, resolution, contrast, latitude, and film processing.

   (g) Principles and application of other imaging modalities as a correlate to nuclear medicine procedures.

3. **Mathematics, statistics, and computer sciences.**

   (a) Fundamental concepts of mathematics including algebra, geometry, and calculus.

   (b) Probability distributions and parametric and non-parametric statistics.

   (c) The principles of medical decision making and comparative effectiveness of tests and therapeutic procedures.

   (d) Basic aspects of computer structure, function, and programming.

   (e) Computer applications with emphasis on digital image acquisition, analysis, processing and enhancement, tomographic reconstruction, display, and recordings of findings.

   (f) Compartmental analysis and mathematical models of physiologic systems.

4. **Radiation biology and protection.**

   (a) The biological effects of radiation exposure, with emphasis on the effects of low level exposure.

   (b) Administrative and technical means of reducing unnecessary radiation exposure to patients, personnel and environment.

   (c) Immunology, molecular biology and genetics.

   (d) Calculation of the radiation dose from internally administered radionuclides.

   (e) the diagnosis, evaluation, and treatment of radiation overexposure in any form.

   (f) International recommendations and governmental regulations regarding limits of radiation exposure, handling of radioactive patients, transport of radioactive material and disposal of radioactive wastes.

   (g) Management of radiation accidents, including monitoring, decontamination, and subsequent control.

5. Radiopharmaceuticals.

(a) The safe handling of radioactive material.

(b) General principles of production of radionuclides by reactors, cyclotrons, other particle accelerators and the use of radionuclide generators preparation and labelled compounds.

(c) Formulation of radiopharmaceuticals considering chemical properties and quality control with special emphasis on Tc-99m chemistry.

(d) labelled monoclonals for immunoscintigraphy

(e) Biochemistry, physiology, and pharmacokinetics of radiopharmaceuticals.

(f) An understanding of the role of regulatory bodies applicable to the practice of and research in nuclear medicine

(g) Economic aspects of radiopharmaceutical production and distribution.

6. In vivo diagnostic studies.

(a) General: Clinical indications for and limitations in their appropriate usage; normal and altered anatomy, physiology, biochemistry, and metabolism of the various organs or processes to be examined; technical performance of the procedure, including proper patient preparation and patient management before, during, and after the procedure.

(b) In vivo imaging and/or function studies, including the brain, cerebrospinal fluid, thyroid, salivary glands, lung, heart and vessels, oesophagus, stomach, biliary system, liver, spleen, kidney, adrenal, bladder, bones, joints, bone marrow, tumors and abscesses, etc.

(c) The use of imaging devices, external detectors and computers for body organ imaging, and for time-dependent and differential function studies.

(d) The use of physiologic gating techniques for functional studies.

(e) Patient monitoring during interventional tests such as exercise and pharmacological administrations with special emphasis on electrocardiographic interpretation and cardiopulmonary resuscitation.

(f) Cellular kinetics, absorption and excretion analyses, nuclear hematology and balance studies using radiotracers.

(g) Body composition tests, including compartmental analysis.

(h) Whole-body counting and total-body scanning.

(i) The use of stable isotopes.

(j) Relationship between nuclear medicine procedures and other pertinent imaging modalities such as diagnostic radiology, ultrasound, X-ray computed tomography, nuclear magnetic resonance and digital radiography in a given situation.

7. In vitro studies.

(a) Principles of radioisotopic microanalytical techniques, such as RIA, IRMA and related procedures, pertinent background of immunology, molecular biology and bio-technology with special reference to the production of monoclonal antibodies,

(b) Methods of quality control and data analysis.

(c) Setting up radioassays with bulk reagents or with kits,

(d) Binding capacity studies such as receptor assays etc.

(e) In vitro nuclear techniques in the diagnosis of communicable diseases,

(f) Comparative evaluation of nuclear and non-nuclear assays,

(g) DNA probes and related procedures in human diagnosis,

(h) Principles of activation analysis

(i) Principles of autoradiography.

8. Therapeutic uses of radionuclides.

(a) Patient selection, including the diagnostic procedures necessary to establish the need for radionuclide therapy, indications and contraindications for the use of radionuclide therapeutic procedures and their efficacy in relation to other therapeutic approaches.

(b) Dose administration in patient management; estimating dose to the target area, to the surroundings tissues and/or other organ systems, and total-body exposure; the range of doses in each specific application; the special problems of patient care during the radionuclide therapeutic procedure, potential early and late adverse reactions, evaluation of the clinical response, and the follow-up care.

(c) Specific applications: radioiodine in hyperthyroidism and thyroid carcinoma; radiophosphorus (soluble) in polycythemia vera and other myeloproliferative disorders, treatment of metastatic bone disease with P-32 labelled organic phosphates, colloidal radiophosphorus and other radiocolloids for intracavitary therapy of malignant effusions and treatment of chronic osteoarthritis of joints; radiolabelled antibodies.

9. Organizational considerations.

(a) Design of laboratories

(b) staff structure

(c) care and maintenance of instruments

(d) organization of QC of nuclear medicine procedures

(e) waste disposal in a nuclear medicine laboratory

(b) Cost-effectiveness of nuclear medicine procedures

(c) Public relations

(d) Role of International Organizations.

Practical work.

The detailed list given in the 1974 report of the IAEA/WHO Seminar on Training in Nuclear Medicine, 18-29 March 1974 can be used as a guide to the range of experiments and demonstrations that are of value to those studying nuclear medicine. The choice must depend upon local facilities and needs.

Experiments and demonstrations should be supplemented where possible by audio-visual aids. The IAEA and the WHO can provide help with this type of teaching material.

Continuing education.

The content or purpose of further education courses would include:

(a) New developments in the field.

(b) Availability of new facilities.

(c) Improvements in existing technology and knowledge.

(d) Demands for new technology and knowledge.

(e) Improving economic efficiency of a nuclear medicine installation.

(f) Changes in generally used regulations or units.

It is of special importance that participants selected for special courses should:

(a) Have a homogeneous background of knowledge and experience in the topics of the course.

(b) Have a good knowledge of the language in which the course is to be given.

(c) Be likely to apply what they have learned on their return to their parent institution.

Special courses should be arranged only by organizations that are highly competent in the subject fields. It is desirable that some system of follow-up should be available, to evaluate the benefit given by the course to the participant and to his parent institution, so that future courses may, where necessary, be better adapted to the needs of participants.

No special course of the types described can, however, be regarded as replacing the need for full training in the practice of nuclear medicine or for some limited number of applications.

CHAPTER 32

CONCLUSIONS AND RECOMMENDATIONS.

The nuclear techniques applied to medicine and comprised under the term nuclear medicine are of great benefit to humanity.
The full potential of the contributions of nuclear medicine to health care still remains to be realized and appreciated in many countries.

In developing countries, the IAEA and the WHO could provide a service of great value by establishing or developing local or regional training programs, and by facilitating and supporting the attendance of students from developing countries for periods of training at such advanced centers. Such assistance by IAEA and WHO in establishing and maintaining regional training centers for nuclear medicine, particularly in regions of the world where adequate training facilities in nuclear medicine do not at present exist, will be of immense value.

A statement about certification of the competence of a nuclear medicine physician was published in The Journal of Nuclear Medicine, Vol. 12, December 1971; a revised version was published in Vol. 22, December 1981. The following revision was published in Vol. 27, June 1986.

NUCLEAR MEDICINE PHYSICIANS

The nuclear medicine physician requires broad knowledge and experience in medicine. The nuclear medicine physician must be prepared intellectually to extend the scope of nuclear medicine beyond current methodology as the specialty advances. Upon referral of a patient for consultation, a nuclear medicine physician must be prepared to:

(a) Obtain pertinent information from the patient and other sources.

(b) Perform a physical examination related to the consultation.

(c) Select and carry out appropriate procedures in a manner that is safe both to the patient and the staff.

(d) Interpret the results, arrive at a reasonable diagnosis on the basis of correlation of all clinical and laboratory information available, and issue a timely report.

(e) Recommend further study or treatment when appropriate.

(f) If requested, assume full responsibility for patient management if nuclear medicine therapy is indicated.

(g) Demonstrate oral and written communications skills adequate for full exchange of information with patients and referring physicians.

(h) Perform quality assurance programs, quality control measures and their applications to nuclear medicine practice.

The practice of nuclear medicine requires a special knowledge in the following areas:

I. Physical Science

(a) Elementary aspects of the structure of the matter.

(b) Modes of radioactive decay.

(c) Emissions accompanying radioactive decay, and their biological implications.

(d) Interactions of radiation with matter.

II. Instrumentation

(a) Principles of radiation detection and detectors.

(b) Nuclear medicine instrumentation, with special emphasis on gamma scintillation cameras, but also including scanners, tomographic imaging devices, "positron imaging instruments," whole body counters, gamma well counters, liquid scintillation counters, monitoring devices, and dose calibrators.

(c) Collimation of radiation detectors with special emphasis on the characteristics of not only parallel hole, diverging and converging, and slant-bole collimators, but also pinhole and coded-aperture collimators and their response to point, line, and plane sources.

(d) Electronic instruments, such as pulse amplifiers, pulse height analyzers, scalers, and count rate meters.

(e) Image production and display technology including photographic principles with special emphasis on sensitivity, resolution, contrast, latitude, and film processing,

(f) Principles and application of other imaging modalities as a correlate to nuclear medicine procedures.

III. Mathematics, statistics, and computer sciences

(a) Fundamental concepts of mathematics including algebra, geometry, and calculus.

(b) Probability distributions and parametric and nonparametric statistics.

(c) The principles of medical decision making and comparative effectiveness of tests and therapeutic procedures.

(d) Basic aspects of computer structure, function, and programming.

(e) Computer applications with emphasis on digital image acquisition, analysis, processing and enhancement, tomographic reconstruction, display, and recordings of findings.

IV. Radiation biology and protection

(a) The biological effects of radiation exposure. with emphasis on the effects of low level exposure.

(b) Administrative and technical means of reducing unnecessary radiation exposure to patients, personnel, and environment.

(c) Immunology, molecular biology, and genetics.

(d) Calculation of the radiation dose from internally administered radionuclides.

(e) The diagnosis, evaluation, and treatment of radiation overexposure in any form.

(f) Governmental regulations regarding limits of radiation exposure, handling of radioactive patients, and disposal of radioactive wastes.

(g) Management of radiation accidents, including monitoring, decontamination, and subsequent control.

V. **Radiopharmaceuticals**

(a) Production of radlonuclldes by reactors, cyclotrons, other particle accelerators and the use of radionuclide generators.

(b) Formulation of radiopharmaceuticals considering chemical properties and quality control.

(c) Biochemistry, physiology. and pharmacokinetics of radiopharmaceuticals.

(d) An understanding of the role of regulatory bodies applicable to the practice of and research in nuclear medicine.

VI. **Diagnostic use of radionuclides**

(a) General: Clinical indications and limitations for the appropriate usage; normal altered anatomy, physiology, biochemistry, and metabolism of the various organs or processes to be examined; technical performance of the procedure, including proper patient preparation and patient management before, during, and after the procedure.

(b) In vivo imaging and/or function studies, including the brain, cerebrospinal fluid, thyroid, salivary glands, lungs, heart and vessels, esophagus, stomach, biliary, liver, spleen, kidney, pancreas, tumors and abscesses, bladder, bones, joints, and the bone marrow.

(c) the use of imaging devices and external detectors for body organ imaging, and for time-dependent and differential function studies.

(d) the use of physiologic gating techniques for functional studies.

(e) Patient monitoring during interventional tests such as exercise and pharmacological administrations with special emphasis on electrocardiographic interpretation and cardiopulmonary resuscitation.

(f) Cellular kinetics, absorption and excretion analyses, and balance studies using radiotracers.

(g) Body composition tests, including compartmental analysis.

(h) Whole-body counting and total-body scanning.

(i) Relationship between nuclear medicine procedures and other pertinent imaging modalities such as diagnostic radiographic techniques, ultrasound, x-ray computed tomography, nuclear magnetic resonance, and digital radiography.

VII. **In vitro studies**

(a) Methodology and quality control of radioligand assay,

(b) Binding capacity studies.

(c) Principles of activation analysis and autoradiography.

VIII. **Therapeutic uses of radionuclides.**

(a) Patient selection; including the diagnostic procedures necessary to establish the need for radionuclide therapy, indications and contraindications for the use of radionuclide therapeutic procedures and their efficacy in relation to other therapeutic approaches.

(b) Dose administration in patient management: including dose to the target area, to the surrounding tissues and/or other organ systems, and total-body exposure; the range of doses in each specific application; the special problems of patient care caused by radionuclide therapeutic procedure, the potential early and late adverse reactions, the timing and parameters of anticipated clinical response and the follow-up care and evaluation as needed.

(c) Specific applications: radioiodine in hyperthyroidism and thyroid carcinoma; radiophosphorus (soluble) in polycythemia vera and other myeloproliferative disorders, and metastatic bone disease: colloidal radiophosphorus and other radiocolloids for intracavitary therapy of malignant effusions; radiolabelled antibody therapy.

SUGGESTED READING.

[1] Royal College of physicians and surgeons of Canada, Journal of Nuclear Medicine

[2] Report of an IAEA/WHO Seminar on Training in Nuclear Medicine, 18-29 March 1974

[3] Components of Professional Competence of Nuclear Medicine Physicians, Journal of Nuclear Medicine 27 (1986), 863-865.

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Chapter 33

GUIDELINES FOR STARTING A NUCLEAR MEDICINE LABORATORY

[2]Excerpts from a booklet published by
Bhabha Atomic Research Centre, India.

Introduction

Nuclear medicine is concerned with diagnostic and therapeutic uses of artificially produced radioisotopes. Nuclear medicine procedures are of two types:

1. In-vivo procedures in which radioisotopes are administered to patients, and

2. In-vitro procedures where radioactivity is added to the samples collected from the patient. Again, in-vivo tests are classified into:

   (a) imaging procedures, and

   (b) non-imaging procedures.

   An imaging procedure, which is more popularly known as scintigraphy, provides an image of the distribution of administered radioactivity in the organ or tissue of interest at any given time. Non-imaging procedures are aimed at measurements of gross radioactivity in the organ of interest at any particular time. Serial measurements provide a time-activity curve.

With the availability of many short lived radionuclides, nuclear medicine has now become an important medical specialty with wide applications in various branches of medical science. The following Table lists some of the major applications of nuclear medicine.

Major applications of nuclear medicine.	
(i)	imaging of various organs such as thyroid, liver, brain, bone, kidneys etc.,
(ii)	thyroid function studies, and therapy of thyroid disorders,
(iii)	investigations of central nervous system,
(iv)	absorption studies in gastroenterology,
(v)	nuclear haematology, e.g., blood volume studies, iron kinetics, etc.,
(vi)	renal function studies,
(vii)	nuclear cardiology,
(viii)	in-vitro studies like radioimmunoassay (RIA) of various hormones.

[2]Portions which had information of strictly local nature have been omitted (for example, what is available where in India).

With the help of an on-line computer, coupled to a gamma camera, a variety of dynamic function studies can also be performed using appropriate radiopharmaceuticals. Therapeutic uses of radionuclides are mainly limited to treatment of thyrotoxicosis and thyroid cancer with ^{131}I, polycythemia vera with ^{32}P and intracavitary instillation of radiocolloids for malignant ascites and effusions.

A nuclear medicine department caters to the need of all clinical departments, and, therefore, should be located at a central place. At the same time, because of radiation hazard associated with the use of radionuclides, planning of the departments should be done in such a way that there is no radiation exposure to non-radiation workers and the general public, and also that radiation workers handling radioisotopes receive minimum exposure.

When a decision to set up a nuclear medicine department is taken, the authorities are faced with a number of questions regarding the location, planning for the premises, equipment needed, availability of trained medical and paramedical personnel and the procedure for obtaining clearance from various authorities.

2. Categories of Nuclear Medicine Laboratories

On the basis of the nature of work carried out and the facilities required for different types of radioisotopic diagnostic procedures, nuclear medicine laboratories can be classified into 4 categories, two pertaining to in-vitro procedures only and two to in-vivo as well as in-vitro procedures.

2.1. Category 1: Laboratories performing in-vitro radioassays with ready to use kits.

2.2. Category 2: Laboratories carrying out radiolabelling of ligands with preparation of kits and using them for in-vitro radioassays.

2.3. Category 3: Laboratories performing in-vivo non-imaging procedures and in-vitro assays.

2.4. Category 4: As category 3, but also performing in-vivo static/dynamic imaging procedures.

2.5. Radionuclide Therapy: Categories 3 and 4 nuclear medicine laboratories can also undertake treatment of thyrotoxicosis with ^{131}I and polycythemia vera with ^{32}P, with a few added facilities. The treatment of thyroid cancer with ^{131}I, however requires elaborate arrangements for hospitalization and barrier nursing of patients, high activity radioactive waste collection and disposal, etc., and is not dealt with in this chapter.

3. Basic Requirements for various categories of Nuclear Medicine Laboratories

The basic requirements for a nuclear medicine laboratory are considered under three main headings:

(1) space;

(2) equipment, and

(3) staff.

Exact requirements depend very much on the number and types of studies to be carried out in the laboratory. However, a broad outline of these requirements for different categories of nuclear medicine laboratories are given below.

3.1. Category 1 Nuclear Medicine Laboratory

3.1.1. Space: A simple in-vitro laboratory requires only two rooms, one for storage and handling of radionuclides kits and the other for counting. The total area of the laboratory may be about 30 m^2. A typical layout for a Category 1 laboratory is given in **Fig. 33.1.**

3.1.2. Equipment: Major items of equipment normally required for an in-vitro laboratory are:- A well type scintillation counting system for ^{125}I measurements (an automatic counting system will be needed if the workload is large), a refrigerator, a clinical centrifuge, a shaking water bath, a magnetic stirrer, a vortex mixer and an interval timer. Enough quantities of pipettes, dispensers, disposable syringes, needles, assay tubes, etc., are also required. A refrigerated centrifuge capable of taking 100 or more assay tubes may be needed for larger workloads and for some type of radioimmunoassays.

3.1.3. Staff: Staff requirements for a category 1 in-vitro laboratory depends primarily on its workload. For a modest laboratory analyzing about 100-200 samples per week, one or two trained persons may be adequate.

3.2. Category 2 Nuclear Medicine Laboratory

3.2.1. Space: In addition to rooms for sample collection and counting, an RIA laboratory carrying out radiolabelling of ligands requires a separate room for radiolabelling. Iodination leads to considerable amount of radioactive waste, which calls for an additional room for radioactive waste handling and decontamination. A Category 2 in-vitro nuclear medicine laboratory with a moderate workload, therefore requires an of about 50 m^2 having 4 rooms. A typical layout is given in **Fig. 33.2.**

3.2.2. Equipment: For routine assay work the equipment mentioned in Category 1 are adequate. But, for iodination work, a ventilated fume-hood, automatic fraction collectors, iodination tubes, various sized columns for chromatography, a portable contamination monitor for monitoring and enough number of radioactive waste receptacles - foot operated waste bins for solid waste collection and polythene carboys for liquid waste collection - will also be required.

3.2.3. Staff: For a Category 2 laboratory, in addition to staff recommended for category 1, one or more technologists trained in iodination of compounds and preparation of kits, etc., are required. One of the staff members who has undergone training in RIA or in handling radionuclides, must be designated as Radiation Safety Officer (R.S.O.) for ensuring safe handling of radionuclides.

3.3. Category 3 Nuclear medicine laboratory

3.3.1. Space: The space needed for a nuclear medicine laboratory carrying out in-vitro and in-vivo non-imaging procedures would depend on the number and types of investigations. Generally, a laboratory carrying out thyroid function studies, renography, haematology etc. would require an area of about 100-120 m^2. Separate rooms should be provided for radionuclide storage and handling, dose administration, counting, decontamination and waste handling. A suggested plan for Category 3 laboratory is shown in **Fig. 33.3**.

3.3.2. Equipment: Equipment needed for non-imaging procedures would be dictated by particular procedures to be carried out. A collimated scintillation probe and a counting system is required for carrying out thyroid function measurements, whereas for renography a double probe system with ratemeter-recorder attachment is required. An isotope calibrator for dose measurement and a portable contamination monitor for routine monitoring of work surfaces, hands, etc., are essential. For in-vitro procedures all the equipment mentioned above are needed.

3.3.3. Staff: The staff requirement again depends on the workload. For a medium sized nuclear medicine laboratory, the staff requirement is - one nuclear medicine specialist and one or two technologists. The nuclear medicine specialist is a clinician who has undergone training in nuclear medicine for a period of at least one year, leading to a degree or diploma in nuclear medicine. The technologists should be science graduates, having undergone training in medical radioisotope technology. One member of the staff should be designated as R.S.O., with responsibility to ensure safe handling of radionuclides.

3.4. Category 4 Nuclear medicine laboratory

3.4.1. Space: Space requirement for a nuclear medicine laboratory depends on the number of imaging devices available and the workload. If radionuclide imaging is to be carried out with ready to use radiopharmaceuticals obtained from the supplier, then, in addition to the space provisions indicated for Category 3 laboratory, an additional space of about 25 m^2 for

locating the gamma camera / rectilinear scanner should be adequate (a total area of about 125 m^2). It should be borne in mind that rectilinear scanners are almost obsolete and it is advisable to procure a gamma camera which is more versatile and also permits dynamic studies with the help of a computer. In order to utilize the capabilities of the gamma camera to the maximum, radiopharmaceuticals with short lived radionuclides, such as $^{99}Tc^m$, are essential. Preparation of $^{99}Tc^m$ radiopharmaceuticals from cold kits requires an extra space for an in-house radiopharmacy and hence additional area is required. A Category 4 laboratory having a gamma camera with computer system and an in-house radiopharmacy, therefore, requires an area of 200 m^2. A typical layout of Category 4 laboratory is given in **Fig. 33.4.**

3.4.2. Equipment: The main equipment needed for a Category 4 laboratory, in addition to those for category 3, is a gamma camera system or rectilinear scanner. A trustworthy isotope calibrator for dose measurement and one or two portable contamination monitors are indispensable.

3.4.3. Staff: The staff requirement again depends on the workload. At least one nuclear medicine specialist and two or more technologists are essential. One of the staff members should be designated as R.S.O. with the responsibility to ensure safe handling of radioactive materials and safe disposal of radioactive waste.

4. Special Aspects of Radiation Protection

4.1. Radiation protection is an important aspect to be taken care of in all types of nuclear medicine laboratories. Salient features of radiation protection requirements are outlined below.

4.2. Adequate number of radiation monitoring instruments, as mentioned above, should be procured for routine monitoring and survey. A variety of radiation monitoring instruments are available from many suppliers. A portable contamination monitor with aural alarm is an essential instrument for each type of nuclear medicine laboratory. In addition, beta gamma survey meter with measurement ranges from a few mR/hr to a few R/hr is also required where large activities are handled.

4.3. Persons handling radionuclides should wear personnel monitoring badges while working in the nuclear medicine laboratory. However, the staff members of Category 1 nuclear medicine laboratory need not have personnel monitoring service as the activities handled in these laboratories are very small.

4.4. For effective implementation of radiation safety in the nuclear medicine laboratory, following points are to be ensured:

(a) All walls and doors of the radioisotope laboratory are to be painted with a good quality washable paint.

(b) Minimum furniture should be used.

(c) Top surfaces of work tables should have a smooth laminated finish.

(d) Adequate number of lead containers and interlocking lead bricks should be procured for providing adequate shielding in storage and handling rooms.

(e) Remote handling devices for different operations in the radioisotope laboratory should be procured.

(f) Ventilated fume hoods for handling large doses of ^{131}I and for carrying out extraction of $^{99}Tc^{m}$ should be installed.

(g) The drainage ducts of the radioisotope laboratory, i.e. of sinks, wash basins, toilets, etc., should be connected directly to the sanitary sewage system.

4.5. Ordinary wall thickness is sufficient, in general, for the diagnostic nuclear medicine laboratory. However, extra wall thickness may be required for rooms where patients administered with ^{131}I therapy doses are to be hospitalized.

4.6. A qualified and trained Hospital Physicist can be employed in big hospitals handling large activities of radionuclides for diagnosis, therapy and substantial R & D work.

5. Radioactive Waste Collection and Disposal

The radionuclides used in nuclear medicine are mostly short lived, the half life being in the range from a few hours to a few days. Normally, $^{99}Tc^{m}$ and ^{131}I are the two major radionuclides that are used for nuclear medicine procedures in large quantities. Other radionuclides used at relatively low levels of activities are ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{32}P and ^{125}I and a few others. Because of the short half lives, the disposal of radioactive waste is done by the simple method of decay and disposal.

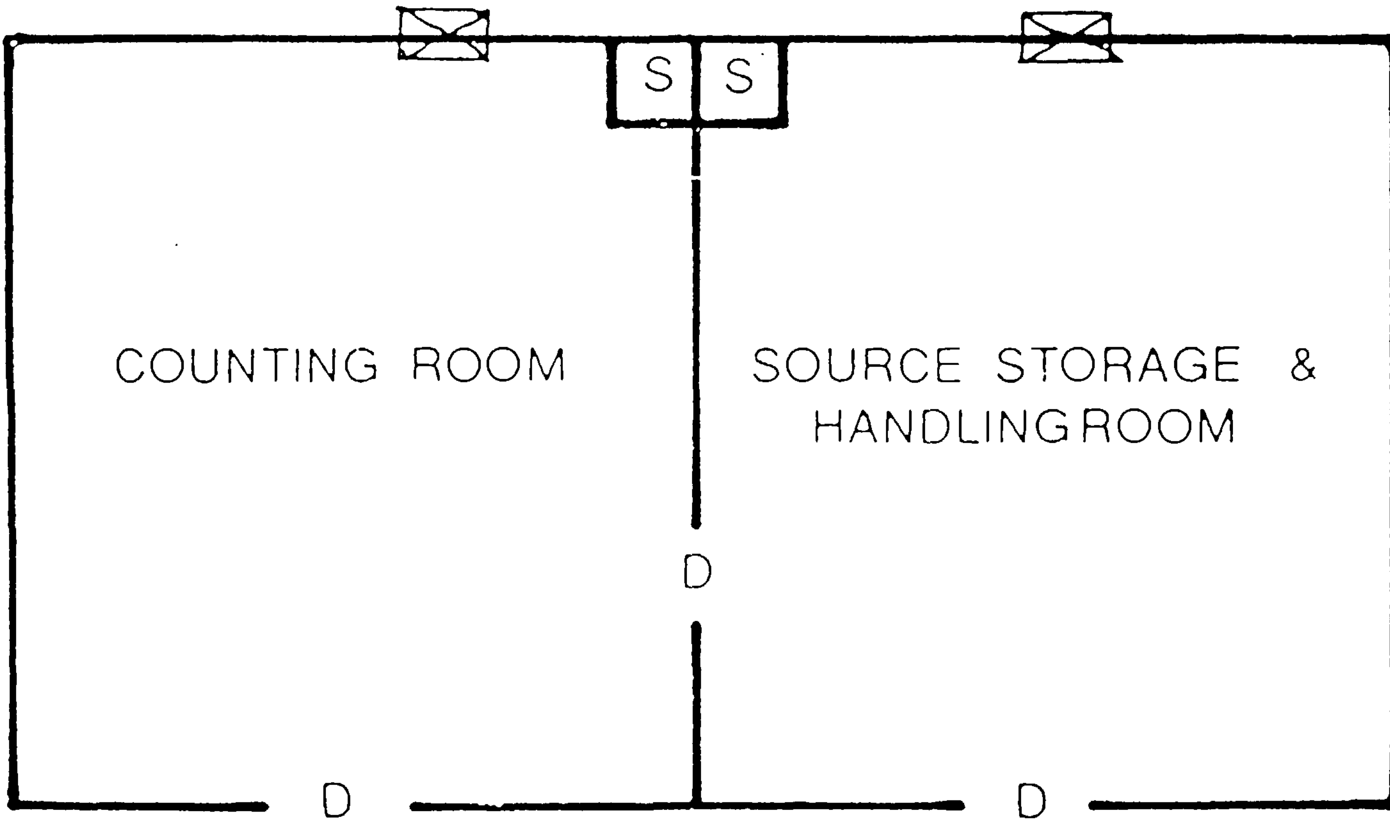

Fig. 33.1 A typical plan for a RIA laboratory (only kits). Total area: ~ 30 m^2

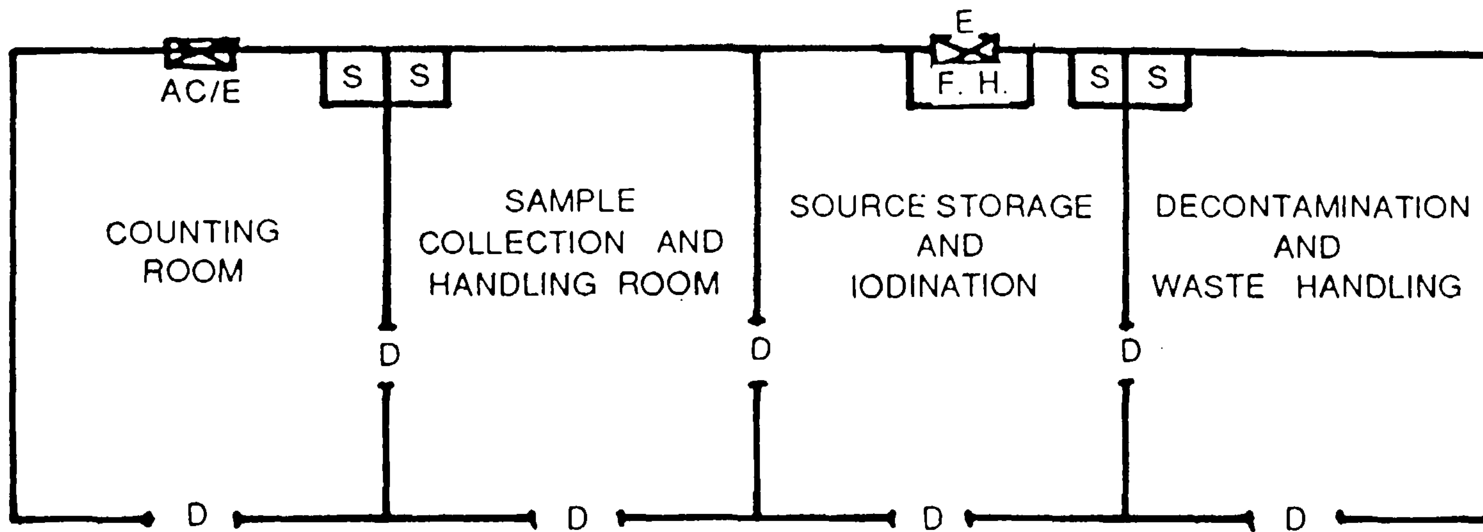

Fig. 33.2 A typical plan for a RIA laboratory including facility for iodination. Total area: ~ 50 m^2

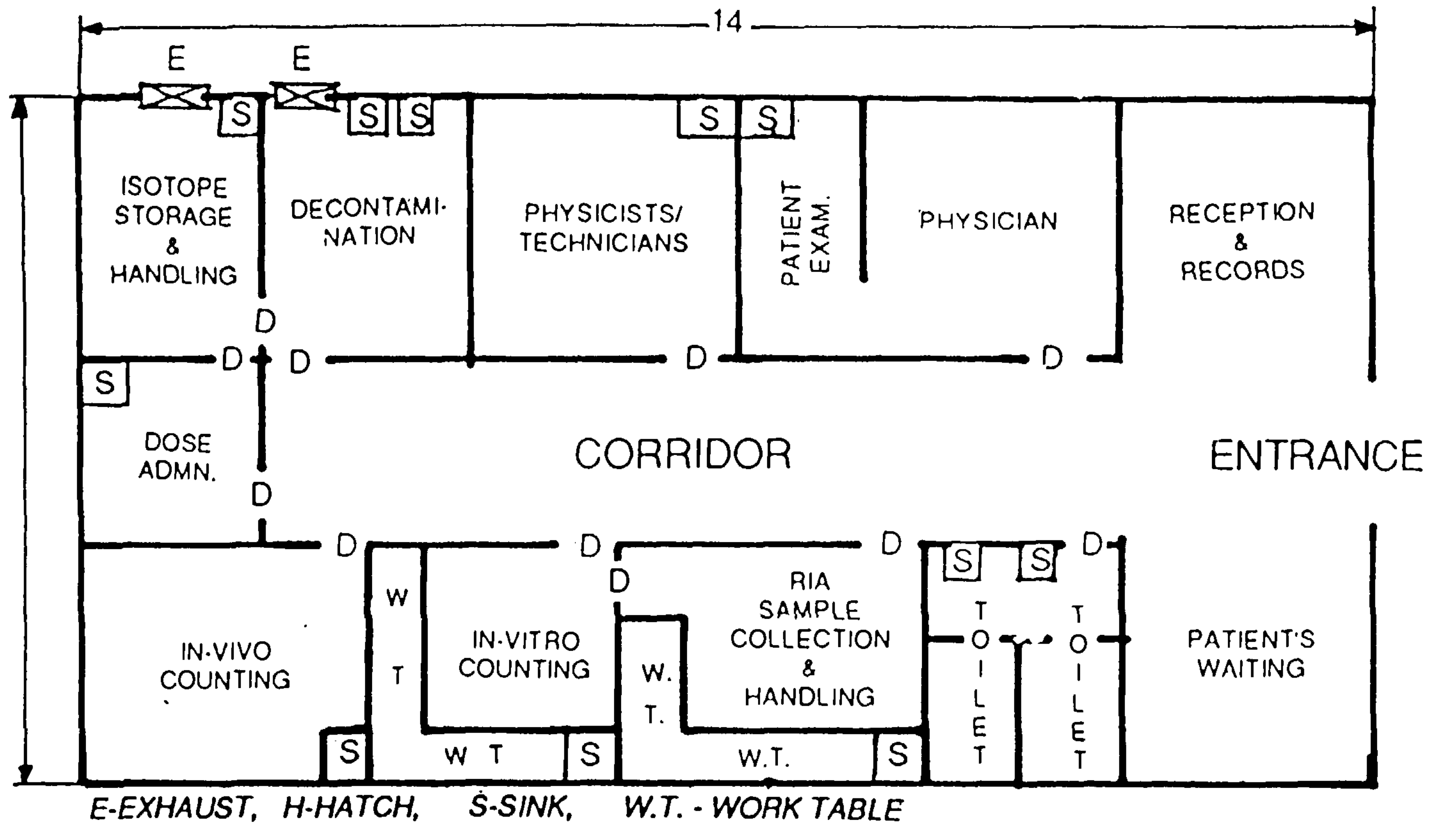

Fig. 33.3 A typical layout for a nuclear medicine laboratory (in-vivo non-imaging studies and RIA work only). Total area: ~ 100 m^2

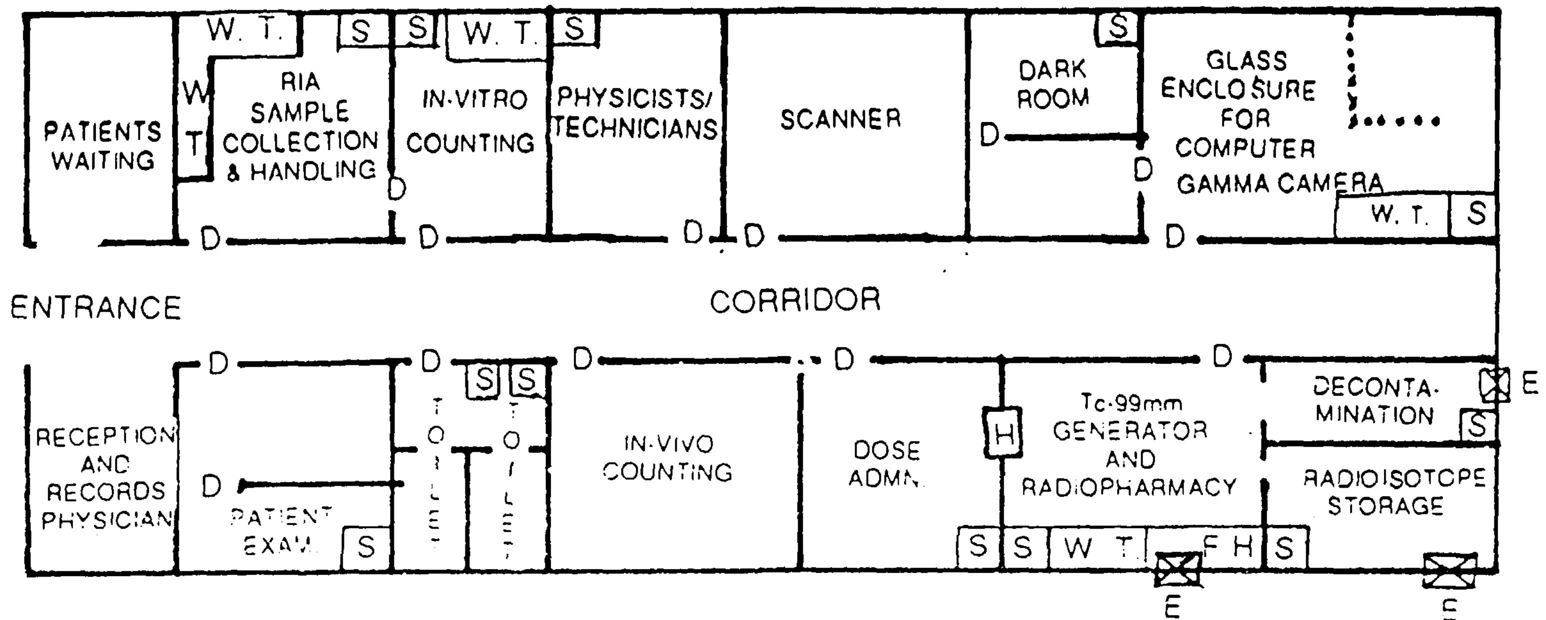

Fig. 33.4 A typical layout for a diagnostic nuclear medicine centre (in-vitro laboratory, in-vivo non-imaging and imaging diagnostic procedures). Total area: ~ 200 m^2

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Chapter 34

SPECIAL PROBLEMS OF SETTING UP NUCLEAR MEDICINE IN A DEVELOPING COUNTRY

R.D. Ganatra

There are some special problems in setting up nuclear medicine in a developing country. They can be briefly described in the form of the following general rules.

1. Impossible triangle. For the practice of nuclear medicine, three things are needed: Instrument, Radiopharmaceutical and a Patient. In a developing country, these three become three sides of an impossible triangle. When the radiopharmaceutical is available, the instrument may not be working; when the instrument is functioning, the radiopharmaceutical may not have been obtained from the foreign supplier; and when both are there, the patient might no longer be in the hospital. Three sides of this triangle never join to become a congruent whole.

2. Reverse square law. Further away one is from the source of supply of instruments and radiopharmaceuticals, the problems multiply by the square of this distance.

3. Future of nuclear medicine is tied to the electrical supply available in a developing country. These problems related to power supply are described in the Chapter on maintenance of instruments.

Location of a nuclear medicine department in the hospital

It is particularly essential to keep all nuclear medicine work of a hospital in a developing country in one centralized place for the following reasons:

1. Effective and economical utilization of costly equipment, easier to create ideal working environment for these instruments, simpler to organize their maintenance and repairs.

2. Indenting and dispensing of all radioactive material from one place in the hospital, safe storage and custody at one place, promotes safe handling of the radioisotopes under guidance and supervision, easier to monitor and contain radioactive contamination.

3. Availability at one central location of trained technologists for operating the instruments and for handling of radioactivity.

Not only it is advisable to have a centralized nuclear medicine department in a hospital, it is equally desirable to have a consortium of interested clinicians to associate with the unit because of the diverse range of medical disciplines that nuclear medicine serves. No single unit can serve a wide variety of clinical interests with equal emphasis. It is first of all, therefore, necessary to decide what are going to be the major interests and activities of the department. It is easier to set up facilities on the basis of such a decision. Orders can then

be placed for the instruments which would be most useful for the intended work. The total available space of the unit can be planned in an effective way. If financial resources are limited, as is always the case in a developing country, it may be better to start with a small unit with limited range of procedures and then gradually enlarge into a larger unit, reaching out to newer and more sophisticated applications.

Staff

All scientific units prosper when woven around a trained competent person. It is wrong to create a unit first by an administrator, who knows next to nothing of nuclear medicine, and then go hunting for a person, who can be sent for training and pray that he would return some day to take charge of the unit, which by then would be in a ramshackle state.

The training of a physician in nuclear medicine has been described at great length in another chapter. Nuclear medicine is a clinical discipline and therefore an appropriate person to be in charge of such an unit will be a medical doctor. Usually such a person is either an internist, radiologist or a pathologist, because it is in these disciplines that the major applications of nuclear medicine lie.

A medical physicist is included as a staff member of a large nuclear medicine department but this post is not considered essential in case of small units. His (or her) duties would normally include the following:

1. Supervising radiation protection.

2. Carrying out quality control procedures for the instruments, which can be done by a trained technician also.

3. Supervising the general running of the whole unit as such, but he has normally no training in in vitro procedures or radiopharmacy, which are two important components of modern nuclear medicine practice.

4. Supervise waste disposal but which is less burdensome with the present use of short half life radiopharmaceuticals. A full-time trained nuclear medicine physician can do all the above mentioned functions in a small unit. There is a need to define the training and functions of a medical physicist so that his posting as a staff member can be property justified to a budget chopping hospital director. Ideal solution may be to share this posting with Radiology and Radiotherapy departments.

Technicians form the main bulwark of the department. How many of them should be there in the department will depend upon the range and the volume of work. There should be several for the imaging work because that is the heaviest workload of a nuclear medicine department. The technicians operating the instruments should preferably have academic background in physics. The technician working in radiopharmacy or radioimmunoassays should have a background of chemistry. A person with qualification in pharmacy is

desirable, if kits for radiopharmaceuticals are made locally. Similarly a qualified biochemist is needed if radioimmunoassays are set up with bulk reagents, and not with kits.

A person with a diploma in or experience of the electrical work is an useful person to have around. A full fledged electronics engineer solely attached to a nuclear medicine unit is not considered necessary. Further views about the maintenance of the equipment are discussed at length in another chapter.

As far as possible technicians should receive their training in their own countries or regions. After several years experience, they can be deputed abroad for further training in a specialized field, but such a person should have good theoretical background and should have the calibre of training others, after his return. A foreign expert working with the technicians in the local milieu is a better way of training the technicians.

If a nuclear medicine department engages in the radioiodine therapy of the thyroid disorders, it is absolutely mandatory to have a full-time full fledged radiation protection officer or a medical physicist or a health physicist (whatever be the name) on the staff.

Layout of the nuclear medicine laboratory

The size of the laboratory will depend on the nature and the extent of the work that is to be carried out in such a laboratory. One should first decide what he wants to do, how many techniques he wants to set up in the initial phase, and, last but not the least, the amount of funds available for the initial capital cost and the recurring annual operational cost. If all these priorities and needs are known first, it is easier to plan the space requirements of the Unit or rather the clothes can be cut to the size required.

The first major decision in this respect concerns the therapy. Is the unit planning to undertake therapeutic applications of radioisotopes? If therapy is proposed, special planning is necessary from the viewpoint of the radiation protection. Therapy of a thyroid cancer patient requires administration of 100 to 200 mCi of radioiodine. After the dose, the patient has to be isolated in a room with an attached toilet. Urine of the patient requires to be collected in a separate container. The collected urine should be stored for decay or disposed off in a manner prescribed by the radiation protection authorities. For the nursing care of such a patient, it is necessary to train the nursing staff in radiation protection measures.

Therapy of the patient with thyrotoxicosis is carried out by administration of the doses less than 30 mCi. At this level, it is not mandatory to keep the patient in the hospital. However, the patient who has been given such a dose excretes significant amounts of radioactivity in the urine for first few days. In a developing country, satisfactory sanitary conditions and toilet facilities may not be available at the patient's home. It is often advisable to keep this kind of patients in the hospital for few days after the therapy dose.

The following general guidelines should be followed in planning the layout of a nuclear medicine department:

1. Nuclear medicine facilities in a hospital should be as far away from other sources of radiation as possible (e.g. Radiology, radiotherapy) so that the counting equipment does not have to work in a high background area.

2. Some of the nuclear medicine equipment is quite heavy and it is convenient to locate the unit on a lower floor, so that the weight bearing capacity of the floor is adequate.

3. The layout of the unit should be such that the open unsealed sources of radiation are kept away from the counting instruments. The patients who have been administered radioactivity come into this category of unsealed and leaky sources of radiation.

4. Present practice of using short half life generator produced radionuclides requires a radiopharmacy area which has a fume hood and a sterile working area for making the final parenteral preparation. If kits for the radiopharmaceuticals are made locally, the radiopharmacy set up should be quite elaborate.

5. It is a common practice nowadays to inject the patient while he is under a gamma camera detector or in front of a detector probe for dynamic functional studies. A small corner needs to be set up in the camera room itself for handling of radiopharmaceuticals for injection.

6. If the unit makes kits for the radiopharmaceuticals, a small animal house may be needed to carry out periodically biological toxicity and the biological specificity testing. The responsibility for the quality control of the kits prepared in the hospital radiopharmacy lies with the Head of the nuclear medicine unit. Scrupulous care should be taken in setting up this laboratory.

Chapter 33 gives diagrammatic sketches of different layouts of various nuclear medicine laboratories. There can be many variations on the basic theme and the general principles outlined in these drawings. There are three kinds of flows in a nuclear medicine department: the patient flow; the staff flow and the flow of the radioactivity. All these flows should be independent of each other and should intermingle at predestined points for a specified purpose.

Instruments

Nuclear medicine is very much instrument - oriented and its practice depends upon diagnosis of sick patients by healthy instruments. Health and care of the electronic instruments is in the hands of the elusive electronics engineers. This is a sort of triangular

relationship, where a doctor diagnoses ill patients by the electronic instruments, but the diagnosis and care of the malfunctioning instruments depend on the electronic experts.

We follow the same custom in establishing a department in a hospital as in setting up marriages. A bride is found for the bridegroom by the family elders and then it is expected that they would find love for each other after the marriage. In setting up nuclear medicine in a hospital, it is usually a well meaning administrator who provides money to a person who is usually ignorant and unwary. They seek advice of a equally well meaning elder who recommends some instruments. Everyone then hopes that the instruments and the user are compatible with each other and would make a successful combination in life and be useful to the hospital. Quite often the things do not go as planned and the doctor does not know what to do with his instruments and they remain idle or are misused.

Instead of the above customary way, there is another way of starting a nuclear medicine Department, i.e. first to find a right person, get him trained in nuclear medicine, let him decide what are his interests and needs and then buy the most appropriate instruments for this. By this approach a doctor is able to meet most of his requirements by fewer instruments.

The following Table I shows different types of instruments in nuclear medicine and their functions:

TABLE I. INSTRUMENT		PURPOSE
Contamination monitors, survey meters, etc.	-	Radiation protection
Well-type scintillation probe with spectrometer (manual)	-	in vitro counting
Well-type scintillation probe with spectrometry (automatic) attached to a PC.	-	RIA Counting
Scintillation probe (single)	-	thyroid uptake
Scintillation probes (multiple)	-	Renograms
Scintillation gamma camera	-	for static imaging
On line gamma camera and a computer with appropriate software	-	for static and dynamic functional studies
Liquid scintillation counter	-	in vitro counting of ^{14}C and ^{3}H labelled compounds; mostly for research applications
Radioisotope dose calibrator	-	for Measurement of radioactivity

Selection of instruments

Buy what you need. A small nuclear medicine department with a restricted range of procedures will need less equipment while a department intending to provide a comprehensive nuclear medicine service will need at least all the equipment listed above.

Need based shopping list should be as much a good doctor's creed as a good housewife's. If one is interested in thyroid uptake studies only, imaging devices are superfluous for him. For many of the haematological applications, a simple well counter may be sufficient. To buy a gamma camera to do only thyroid scans is like buying a cannon to kill a cat. Worse than that is to buy a gamma camera and not to invest in short half life generator system. That would be like driving an elegant automobile with a horse because there is a shortage of gasoline!

Servicing an instrument.

Further away one is from the source of supply, the problems increase by a square of the distance. A good repair engineer is a scarce commodity. On the whole, it is easier to repair a modern microprocessor based equipment than the old instruments. The engineer has to diagnose a fault and substitute a new part for the old one.

Like medicine, in electronics also, prevention is better than the cure. The following prescription is for the general well-being of the instruments:

1. Provide a comfortable air cooled home for the equipment. The Head of the Department can sit in this home because "inactive" material is allowed in the Counting room.

2. Let the equipment be installed by the trained Engineer of the firm. Install always before the warranty period expires.

3. Read the instrument manual, howsoever boring it is. Why do all the instruction manuals have to be so dull and obfuscating?

4. Keep the instruments ON constantly, irrespective of whether you plan to work or not.

5. Calibrate the equipment daily and maintain a log book for each instrument. This activity is often called as quality control of the instruments.

6. Leave enough room behind the instrument for even a fat service engineer to go there.

7. Little malfunctions are the early warning signals. Do not disregard them as your spouse's nagging.

8. Do not contaminate your counting equipment ever. It will make your laboratory inactive for days.

9. If your instrument gives bizarre results, ask your technician to read the instruction manual (which you never read in the first instance).

One needs a spark to start the engine of the car. That spark comes out of friction, friction between what you want to do and what you can do.

CHAPTER 34

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Chapter 35

BASIC REQUIREMENTS OF NUCLEAR MEDICINE SERVICES

Based on an IAEA document by E.H. Belcher

Introduction

Technological progress in nuclear medicine continues, not always to the immediate advantage of the developing world. The capital expense, operational demands and maintenance requirements of ever more complex equipment, the consequent need for highly trained staff, the necessity to assure regular supplies of costly radioactive materials, all present problems to which compromise or alternative solutions must often be sought.

This Chapter constitutes an attempt to define the basic requirements for the practice of nuclear medicine with respect to staff, equipment, accommodation, supplies and supporting services with particular reference to the needs of institutions in developing countries.

Nuclear medicine has been defined as embracing all applications of unsealed radioactive materials in diagnosis or treatment of disease or in medical research [1]. This definition includes not only procedures for clinical diagnosis and in vivo investigation and radiation therapy, but also radioimmunoassay and related procedures - radioassay procedures - for clinical diagnosis and in vitro investigation. It also includes a multitude of procedures and applications in medical research which, while of great potentiality to developing countries, fall outside the scope of this Chapter.

There are strong reasons for the close co-ordination, if not the integration, of all such applications, whether in vivo or in vitro, within a medical institution. Such co-ordination is prerequisite not only to ensure that proper facilities and equipment are available for the procedures to be carried out and at the same time to avoid wasteful duplication, but also to provide necessary infrastructure for the reception, preparation, quality control and dispensing of radioactive materials, including radiopharmaceuticals, the safe handling and safe disposal of radioactive wastes and the maintenance and quality control of equipment. The reasons are stronger with regard to institutions in developing countries, where the scale of work may be limited. It should be emphasized in this connection that nuclear medicine is a collaborative discipline involving not only the skills of the clinician but also those of the medical engineer, medical physicist and radiopharmacist.

It has, however, to be recognized that radioassay procedures in vitro have more in common with clinical laboratory procedures in general, especially those of clinical chemistry, than with other nuclear medicine procedures. More radioassays are performed in conjunction with clinical chemistry procedures; others have replaced such procedures or have counterpart assays in clinical chemistry - for example enzyme assays and fluorescence assays - which differ from them only in so far as a non-radioactive label is used in place of the radioactive one and which may be preferred to them on grounds of convenience. It is important for these reasons that a radioassay laboratory, whatever its administrative status, have close connections

not only with any nuclear medicine service in the institution concerned but also with other clinical laboratories there, which may provide further necessary infrastructure, for example in relation to the collection or reception of blood and other specimens. A radioassay laboratory should in any case be set apart from areas where nuclear medicine procedures are conducted in vivo, because of the need to avoid the radioactive contamination which could result from the higher levels of radioactivity used therein.

FACILITIES FOR NUCLEAR MEDICINE PROCEDURES IN VIVO

General remarks

As regards procedures for clinical diagnosis and investigation in vivo, a distinction may be made between non-imaging and imaging procedures. Non-imaging procedures still constitute a significant, though declining, part of the work of nuclear medicine services - for example in relation to haematology. At the present time, however, the emphasis in such services, at least in advanced countries, is on static or dynamic radionuclide imaging by scintillation cameras for the most part with $^{99}Tc^{m}$ labelled radiopharmaceuticals.

The widespread introduction of scintillation cameras in developing countries is still hindered by the capital cost, operational demands and maintenance requirements of these instruments, as well as by restrictions in the use of $^{99}Tc^{m}$ labelled radiopharmaceuticals due to the costs of these generators and their short useful life and the further costs of radiopharmaceutical preparation kits.

The rectilinear scanner, as a moderately-priced and relatively robust imaging instrument, had offered a partial solution to these problems and was at least useful for brain, liver and thyroid imaging. Unlike the scintillation camera, it is well adapted to work with Indium labelled radiopharmaceuticals, the use of which is attractive in some localities because of the long useful life of ^{113}In generators. Unfortunately, the manufacture of the scanner has now been mostly discontinued.

The purchase of new rectilinear scanners and of spare parts for existing ones is thus increasingly difficult. Because of this and in view of the wider possibilities for static and dynamic imaging with the scintillation camera, the rectilinear scanner seems no longer a generally appropriate instrument on which to base imaging procedures in a new nuclear medicine service, although many such instruments remain in efficient use, mostly in the developing countries.

For reasons already indicated, an integrated service is essential to the efficient conduct of nuclear medicine procedures in vivo. None the less, the interrelations of radionuclide imaging and other imaging modalities, among them angiography, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) should be appreciated and the competing claims of the latter given due recognition. CT and MRI remain outside the

reach of most institutions in developing countries. US, on the other hand, is in widespread use and, moreover, may now be preferred for imaging certain organs - for example, liver and thyroid.

For these reasons, it may be convenient to locate facilities for radionuclide imaging adjacent to other imaging facilities in the institution concerned, so that the results obtained by different modalities may be more readily compared and also so that some of the necessary infrastructure - for example, in relation to the reception of patients and darkroom facilities - may be shared.

Radiation therapy

Radiation therapy with unsealed radioactive materials is likely to be limited to the use of ^{131}I in the treatment of thyrotoxicosis and thyroid cancer and the use of ^{32}P in the treatment of polycythemia vera. Use of ^{131}I-MIBG for neuroblastoma is gaining acceptance now. Specific monoclonal antibodies labelled with an appropriate radionuclide is looming on the horizon as a therapeutic possibility.

Any nuclear medicine service undertaking clinical diagnosis and investigation in vivo may also practice these applications. However, the relatively large activities which they employ necessitate some special measures for the protection of the staff and the general public against radiation hazards. These measures should include the segregation of the patients undergoing therapy, the instruction of nursing staff in their management,the containment of radioactive contamination and the storage of radioactive wastes to allow appropriate radioactive decay before disposal.

CATEGORIES OF NUCLEAR MEDICINE SERVICE

Three categories of nuclear medicine service for which different facilities are needed may be considered, viz.:

Category 1: Performing non-imaging procedures for clinical diagnosis and investigation in vivo.

Category 2: As Category 1, but also performing static imaging procedures with preparation of relevant radiopharmaceuticals.

Category 3: As Category 2, but also performing dynamic imaging procedures with preparation of relevant radiopharmaceuticals.

An additional Category may be considered to designate those laboratories which undertake radioisotope therapy. The radioiodine therapy of thyroid cancer needs special additional features.

Category 1 functions may be adequate if the service relates particularly to another specialty. In general, however, services should be established at least in Category 2. The eventual upgrading of a proportion of Category 2 services to Category 3 may be foreseen. Such upgrading implies the interfacing of a computer for digital data processing to an existing scintillation camera. However, interfacing a new computer with an old gamma camera may be difficult if such an upgrading is undertaken after a lapse of many years. If sufficient capital funds are available there are distinct advantages in starting a nuclear medicine service as a Category 3 service.

In a locality with several nuclear medicine services, the establishment of a centralized radiopharmacy for the preparation and distribution of radiopharmaceuticals is clearly advantageous, above all in relation to the use of $^{99}Tc^{m}$. This may be done within an existing service or independently - for example, in the laboratories of a national atomic energy authority.

Basic requirements of Category 1, 2 and 3 services as regards staff, accommodation, equipment, supplies and supporting services will now be presented. The details given in these respects are representative only and should be modified to accord with the particular circumstances of any one service.

REQUIREMENTS FOR A CATEGORY 1 SERVICE

Staff

Staff requirements for a Category 1 service depend on both its functions and its workload. A modest service may be operated by 1-2 professionals plus 1-2 technicians, all employed full-time, with secretarial and other support as appropriate.

The professionals should be physicians and may be specialists in nuclear medicine or in some other specialty to which the service particularly relates. In the former case, they should have received comprehensive training in nuclear medicine extending over at least one or two years. In the latter, they should have received training in nuclear medicine as related to their own specialty extending over at least six months. More specialized training should be provided according to needs as the work of the service expands.

The technicians should already have clinical or medical laboratory experience - for example, in diagnostic radiology or clinical chemistry - in which case their specialized training can be given in the service itself.

One member of the staff should be designated as Radiological Health and Safety Officer [2] to the service, with the responsibility to ensure safe handling of radioactive materials and safe disposal of radioactive wastes.

Accommodation

Accommodation requirements for a Category 1 service depend on its functions and workload. There should be a simple radiopharmacy for the dispensing of radiopharmaceuticals, with corresponding storage facilities. There should be a laboratory for radioactivity measurements in vitro. There should be a clinic for the administration of radiopharmaceuticals and for radioactivity measurements in vivo. There should be waiting rooms for patients.

There should also be office accommodation for staff and for the keeping of records. Separation of the various functions is imperative.

In general, a radiopharmacy area of 10 m^2, a laboratory area of 10 m^2, a clinic area of 25 m^2, and an office area of 10 m^2.

The radiopharmacy should meet the requirements of Type B working places for handling radioactive materials [2] and those of a simple clean Room for pharmaceutical operations [3]. Special facilities for the disposal of liquid radioactive wastes are not normally required, provided that the effluent is discharged to a main sewer, but attention should be paid to the national regulations in this regard, which differ greatly from country to country.

A well-ventilated fume hood to provide protection against airborne radioactive contamination during dispensing operations is a desirable facility, and an essential one if radiation therapy with unsealed radioactive materials is to be practised. The latter would also require special storage facilities for radioactive wastes.

The laboratory and clinic should meet the requirements of Type C working places for handling radio-active materials [2]. Again, special facilities for the disposal of liquid radioactive wastes are not normally required, provided that the effluent is discharged to a main sewer, but attention should be paid to the national regulations in this regard.

The radiopharmacy and laboratory should have ample workbenches, strong enough to carry lead bricks for radiation shielding, as well as centrifuges, electronic equipment and similar items. There should be adequate space for free-standing items such as refrigerators and appropriate facilities for the storage of radioactive materials, radioactive wastes, reagents and supplies. Electricity and water services should be liberally available. Air conditioning is an essential requirement in tropical climates.

In the clinic, electricity and water services should also be liberally available. Air conditioning is again an essential requirement in tropical climates. Chairs, couches, tables and other furniture should be provided as appropriate, likewise in the waiting rooms and offices.

A separate power line fed directly from the final step-down transformer in the institution and a separate earth line are desirable for electronic equipment. Outlets of these should be

available in the radiopharmacy, laboratory and clinic as appropriate, so that an independent conditioned power supply may be provided for each major equipment.

Equipment and supplies

Equipment requirements for a Category 1 service are dictated by the particular procedures to be carried out. Major items of electronic equipment normally needed are a probe scintillation counting system for radioactivity measurements in vivo, a manual well-type scintillation counting system for measurements in vitro, a radionuclide "dose" calibrator and two or more portable radioactivity monitors. There should be the means to provide conditioned power supplies for this equipment. Appropriate radiation sources should be available for equipment quality control.

Thyroid ^{131}I uptake measurements would require a single-probe system for radioactivity measurements in vivo, with scaler output; this could also be used for studies of red cell destruction processes with ^{51}Cr or ferrokinetic studies with ^{59}Fe. Renography with ^{131}I would require at least a double probe system, with ratemeter-recorder output. If on the other hand, no procedures based on radioactivity measurements in vivo are foreseen, the probe system can be omitted. A heavy workload involving the manual well-type system for measurements in vitro would justify the eventual provision of an automatic system for such measurements. The manual system could then be retained as a standby. A trustworthy radionuclide "dose" calibrator is indispensable; so also are portable radioactivity monitors.

There should be a refrigerator, clinical centrifuge with accessories, mini-autoclave and drying oven for glassware; there should be shielded devices for the handling, dispensing and storage of radioactive materials and initial supplies of laboratory glass and plastic ware, disposable gloves, disposable syringes and needles etc.

The total cost of these items may be estimated at US $ 40,000, depending on the type of probe scintillation counting system required.

Running costs in respect of radiopharmaceuticals in a Category 1 service vary widely according to the particular procedures carried out. It is important in planning any new service to obtain assurances that such costs will be covered.

Supporting services.

An efficient and fast mechanism for the reception of radioactive materials and other supplies is indispensable to the smooth operation of any nuclear medicine service. Delays in importation should above all be avoided. If such a mechanism does not already exist when a service is being established, one should be put into effect. Facilities for the maintenance and repair of electronic equipment should also be assured.

Measures for the protection of staff against radiation hazards must be enforced [4]. Film badges or other devices should be provided to monitor external radiation exposure. Regular

checks for possible internal radioactive contamination - for example, thyroid ^{131}I uptake - should be conducted.

While the staff requirements given for a modest Category 1 service do not include medical physicists or radiopharmacists, support in medical physics and radiopharmacy is desirable, at least on an occasional basis. This may be possible to arrange from neighbouring services.

REQUIREMENTS FOR A CATEGORY 2 SERVICE.

It is assumed that the minimum equipment requirement for static imaging in a new Category 2 service should now be defined as a single scintillation camera and that such a Service may eventually be upgraded to Category 3 by interfacing a computer for digital data processing to this camera.

Staff

Staff requirements for a Category 2 service again depend on both its functions and its workload. Minimum requirements may be 2-3 professionals plus 3-4 technicians, with secretarial and other support as appropriate.

One of the professionals should be a physician with specialization in nuclear medicine, who has received comprehensive training in nuclear medicine extending over at least two years and is employed full-time in the service. Also available should be a medical physicist and a radiopharmacist. The former should have received post-graduate training in medical physics extending over at least one year; the latter should have received similar training in radiopharmacy. Their initial employment on a part-time basis may be adequate.

The technicians should already have medical laboratory experience- for example in diagnostic radiology or clinical chemistry- in which case their specialized training can be given in the service itself.

One member of the staff should again be designated as Radiological Health and Safety Officer [2] to the service, with the responsibility to ensure safe handling of radioactive materials and safe disposal of radioactive wastes.

Accommodation

Accommodation requirements for a Category 2 service also depend on its functions and workload. Additionally to that for a Category 1 service, accommodation is required to house the scintillation camera and to allow the preparation and/or dispensing of $^{99}Tc^{m}$-labelled and other radiopharmaceuticals according to needs. There should thus be a more extensive radiopharmacy for the preparation and dispensing of radiopharmaceuticals, with corresponding storage facilities. There should be a laboratory for radioactivity measurements

in vitro. There should be a clinic with separate rooms for clinical examination, for the administration of radiopharmaceuticals, radioactivity measurements in vivo and imaging procedures. There should be waiting rooms for patients. There should also be office accommodation for staff and the keeping of records. Separation of the various functions is again imperative.

In general, a radiopharmacy area 30 m^2, a laboratory area of 20 m^2 , a clinic area of 60 m^2, and a waiting room area of 20 m^2 and an office area of 20 m^2, totalling 150 m^2, should meet initial requirements. It is good policy when installing a scintillation camera for static imaging to allow space for the eventual addition of a computer and other facilities for dynamic imaging, since otherwise such upgrading may require relocation of the camera.

The different sections should meet the various requirements previously specified for a Category 1 service. A laminar-air-flow (LAF) work station in which radionuclide generators can be installed is an appropriate, if not essential, facility in the radiopharmacy.

Equipment and supplies

Equipment requirements for a Category 2 service are again dictated by the particular procedures to be carried out.The major items needed additional to those of a Category 1 service are the scintillation camera itself and accessories for its quality control and use, among them a formatter for multiple-view registration on X-ray film. The preparation and quality control of $^{99}Tc^{m}$ -labelled radiopharmaceuticals require some additional facilities in the radiopharmacy - a water bath, a microbalance, equipment for membrane filtration and sterility testing etc.

A trustworthy radionuclide "dose" calibrator is again indispensable; so also are portable radioactivity monitors. The total additional cost of these items may be estimated at US $ 175 000, depending on the model of scintillation camera selected.

Running costs in respect of radiopharmaceuticals in a Category 2 service vary widely according to the particular procedures carried out. The costs of Tc generators must be taken into account. An important issue is whether or not commercial radiopharmaceutical preparation kits are used. A considerable saving may result if locally prepared reagents or kits are used instead, but careful product quality control is then more than ever essential. It is important in planning any new service to obtain assurances that such costs will be covered.

Supporting services

Supporting services in respect of the reception of radioactive materials and other supplies, the maintenance and repair of electronic equipment and the protection of staff against radiation hazards are needed in a Category 2 service as in Category 1.

ADDITIONAL REQUIREMENTS FOR A CATEGORY 3 SERVICE

It is assumed that a Category 2 service may be upgraded to Category 3 by coupling a computer for digital data processing to an existing scintillation camera. The acquisition of an integrated scintillation camera/computer system in the first place would, of course, provide Category 3 facilities directly.

Staff

Additional staff may not be necessary if a Category 2 service is upgraded to Category 3; this depends on its existing staff and workload. However, at least one of the physicians with specialization in nuclear medicine should have undergone or should undergo training in the clinical applications of dynamic imaging procedures with scintillation camera/counter systems and a medical physicist or other professional with training in computer applications should be available.

Accommodation

Additional accommodation may not be necessary if a Category 2 service is upgraded to Category 3, provided that such upgrading was foreseen. Ample space should be available, however, for the computer itself and for necessary accessories.

Equipment and supplies

Additional equipment requirements if a Category 2 service is upgraded to Category 3 are the computer itself, its interface with the scintillation camera, accessories for its quality control and use, and various other accessories that may be necessary in dynamic imaging procedures. If the main applications are in cardiac function studies, the latter include a cardiac stress system and an electrocardiograph synchronizer. The computer should be supplied with the necessary software for a wide range of clinical applications. The total additional cost of these items may be estimated at US $ 100,000, depending on the model of computer selected.

Supporting services

Supporting services are needed in a Category 3 service as in Category 2.

In addition, a maintenance contract with the computer manufacturer's agents is desirable to assure adequate facilities for its maintenance and repair.

CHAPTER 35

FACILITIES FOR RADIOASSAY PROCEDURES IN VITRO

General remarks

Radioassay procedures are now the methods of choice for the measurement of most hormones, many vitamins and drugs and various indicators of specific diseases in blood and other specimens in vitro. Since they may be performed in a central laboratory on samples sent from external institutions, they find particular uses in developing countries. For many analytes, commercial assay kits are available and provide a means for laboratories with limited facilities and workloads to perform assays. They are, however, costly and their use may entail various inconveniences, for which reason assays based on acquired or locally prepared individual reagents are often to be preferred.

An important consideration is that the majority of radioassays are now based on the use of reagents labelled with ^{125}I. Requirements for radioactivity measurements are thereby greatly simplified.

Radioassays in general require a separation step for the separation of a liquid and a solid phase before radioactivity measurements. This separation is commonly effected by centrifugation; for certain assays the centrifuge should be refrigerated. A current development which seems likely to have important consequences, however, is the introduction of magnetic particulate material as the solid phase, whereby the separation is achieved simply by allowing the particles to sediment towards a permanent magnet placed below the tubes containing the reaction mixtures and then decanting the supernatant. If this becomes a standard practice, the need for a centrifuge in the separation step will vanish, with significant savings in equipment costs and labour.

CATEGORIES OF RADIOASSAY LABORATORY.

Two categories of radioassay laboratory for which different facilities are needed may be considered [5], viz:

Category 1: Performing assays with assay kits or acquired reagents.

Category 2: As Category 1, but also preparing ^{125}I-labelled assay reagents for internal use.

Category 1 laboratories should be the first to be established. Since assays may be performed in a central laboratory on samples sent from external institutions, it may be desirable that individual laboratories assume responsibility for different groups of assays - for example those for thyroid-related hormones - within a defined area. This may avoid much wasteful duplication. The eventual upgrading of a proportion of Category 1 laboratories to Category 2 may be foreseen.

In a locality with several radioassay laboratories, the establishment of higher category laboratories undertaking the preparation and distribution of assay reagents or kits and also performing external assay quality control is clearly advantageous.

Basic requirements of Category 1 and 2 services are given below. The details given are again representative only and should be modified to accord with the particular circumstances of any one laboratory.

REQUIREMENTS FOR A CATEGORY 1 LABORATORY.

Staff.

Staff requirements for a Category 1 laboratory depend primarily on its workload, increasing with the variety and number of the assays performed. Radioassay may be performed to a limited extent by a single person, either a professional or a technician, though a graduate in medicine should be responsible for the interpretation of the clinical results. Initial requirements for a modest laboratory providing a clinical service and assaying 100-200 samples per week are 1-2 Professionals plus 1-2 technicians, all employed full time, with secretarial and other support as appropriate.

The professionals may be graduates in medicine or science. If not already trained in radioassay, they should undergo preliminary instruction, preferably through a formal training course of a few weeks' duration followed by in-service training for 3-6 months in an established radioassay laboratory. If such training can be given locally, this should be done. More specialized training should be provided according to needs as the work of the laboratory extends.

The technicians should already have medical laboratory experience - for example in clinical chemistry - in which case their specialized training can be given in the laboratory itself.

Accommodation.

Accommodation requirements for a Category 1 laboratory also depend primarily on its workload. If facilities for the collection or reception of blood and other specimens do not already exist elsewhere - for example in association with the clinical chemistry laboratory in the institution concerned - there should be a clinic for this purpose. There should be an assay section for the performance of assays. There should also be office accommodation for staff and for the keeping of records. While all these can in fact be provided in a single room of appropriate size, separation of the clinic and office from the laboratory functions is desirable. In general, a clinic area of 15 m^2 , an assay section area of 25 m^2 and an office area of 10 m^2, totalling 50 m^2, should meet Initial requirements for the laboratory designated. A more limited workload can be handled in a correspondingly smaller place. An expansion of 25 % during the first year and 15% per year thereafter should be foreseen.

For reasons given previously, the chosen location should be such that the laboratory can have close connections, not only with any nuclear medicine service in the institution concerned but also with other clinical laboratories there. The laboratory should in any case be set apart from areas where nuclear medicine procedures are conducted in vivo because of the need to avoid the radioactive contamination and increased radiation background which could result from the higher levels of radioactivity used in such procedures.

Depending on the clinical situation, it may be desirable to provide separate rooms for in-patients and out-patients from whom specimens are to be collected. In any case, careful design of @he clinic is necessary to minimize risks of infection. Electricity and water services should be available in this area. Chairs, couches, tables and other furniture should be provided here and in the offices as appropriate.

The assay section should meet the requirements of Type C working places for handling radioactive materials [2]. Special facilities for the disposal of liquid radioactive wastes are not normally required, provided that the effluent is discharged to a main sewer, but attention should be paid to the national regulations in this regard, which differ greatly from country to country.

The assay section should have ample workbenches, strong enough to carry centrifuges, water baths and similar items. There should be adequate space for free-standing items such as freezers and refrigerators, and appropriate facilities for the storage of reagents and supplies. It may be desirable to provide a separate room for equipment for radioactivity measurements and another for storage purposes. Electricity and water services should be liberally available. A standby power supply for the freezer is desirable. A separate power line fed directly from the final step-down transformer in the institution and a separate earth line are desirable for electronic equipment, so that a conditioned power supply may be provided. Air conditioning is an essential requirement in tropical climates.

Equipment and supplies.

Major items of equipment normally needed in a Category 1 laboratory are an automatic well-type scintillation counting system for ^{125}I measurements, a manual well-type counting system as a standby to the latter, one or more portable radioactivity monitors for ^{125}I measurements and a refrigerated centrifuge capable of accepting 100 or more assay tubes at a single loading. There should be the means to provide a conditioned power supply for the electronic equipment. A simulated ^{125}I radiation source should be available for calibration of the counting system. The automatic well-type counting system should incorporate facilities for data processing on-line. There should be additional facilities in the form of a simple personal computer for data processing.

There should be a refrigerator, freezer, clinical centrifuge, shaking water bath, magnetic stirrer, vortex mixer and interval timer; there should be single-delivery and repeating micropipettes with disposable tips, repeating dispensers and an initial supply of laboratory

glass and plastic ware, disposable syringes and needles, disposable assay tubes etc. The total cost of these items may be estimated at US $ 50,000.

Needs may be modified, however, in the case of a laboratory with limited workload. If only a few assay batches are to be handled per week, a second manual well-type counting system may be substituted for the automatic system. If appropriate, a non-refrigerated centrifuge may be substituted for the refrigerated one. Finally, if all assays to be performed utilize the magnetic separation technique or some other technique not involving centrifugation, this centrifuge may be omitted altogether. Such changes can reduce the total cost by more than half.

The laboratory should have access to an analytical balance and a pH meter. Ample supplies of deionized or distilled water and ice should be available.

Whereas assays for most analytes of importance in clinical diagnosis and investigation can be performed with the items listed, some call for additional facilities. Assays with ^{3}H-labelled reagents require a liquid scintillation counting system for radioactivity measurements. An initial supply of counting vials and liquid scintillator is then needed. Access to a tissue homogenizer and an ultracentrifuge is necessary for sample preparation in radioreceptor assays. It is not recommended, however, that such assays form part of the initial work of a Category 1 laboratory.

Running costs in respect of assay reagents in a Category 1 laboratory also depend on its workload but very much too on whether or not commercial kits are used. The prices of kits vary widely. Costs of assays with bulk reagents are likely to be several times less, however, but careful assay quality control is then more than ever essential. It is important in planning any Category 1 laboratory to obtain assurances that such costs will be covered.

<u>Supporting services.</u>

An efficient and fast mechanism for the reception of reagents and other supplies is indispensable to the smooth operation of a Category 1 laboratory. Delays in importation should above all be avoided. If such a mechanism does not already exist when a laboratory is being established, one should be put into effect. Facilities for the maintenance and repair of electronic equipment should also be assured.

ADDITIONAL REQUIREMENTS FOR A CATEGORY 2 LABORATORY

Staff

Additional staff may not be necessary if a Category 1 laboratory is upgraded to Category 2; this depends on its existing staff and workload. However, at least one member of the staff should have undergone or should undergo specialized training in the preparation of ^{125}I-labelled reagents.

One member of the staff should be designated as Radiological Health and Safety Officer [2] to the laboratory, with the responsibility to ensure safe handling of radioactive materials and safe disposal of radioactive wastes.

Accommodation

In addition to the accommodation of a Category 1 laboratory, a Category 2 laboratory should have a separate reagent preparation section comprising at least one room of 15 m^2 or more in which ^{125}I-labelled reagents can be prepared without risk of general radioactive contamination.

This section should meet the requirements of Type B working places for handling radioactive materials [2]. Special facilities for the disposal of liquid radioactive wastes are not normally required, provided that the effluent is discharged to a main sewer, but attention should again be paid to the national regulations in this regard, which differ greatly from country to country. A well-ventilated fume hood to provide protection against airborne radioactive contamination during labelling operations is an essential facility.

Like the assay section, the reagent preparation section should have ample workbenches strong enough to carry moderately heavy equipment. Electricity and water services should be liberally available. Outlets of a conditioned power supply should be provided for electronic equipment. Air conditioning is an essential requirement in tropical climates.

Equipment and supplies

Additional equipment requirements if a Category 1 laboratory is upgraded to Category 2 are a further manual well-type scintillation counter for ^{125}I measurements, a further portable radioactivity monitor for ^{125}I measurements, a further, small refrigerator and a fraction collector. There should also be an additional supply of laboratory glass and plastic ware, including items required in labelling with ^{125}I. These items are for use in the reagent preparation section and should he segregated from other equipment in the laboratory to avoid the spread of possible radioactive contamination. The total additional cost of these items may be estimated at US $ 15 000.

Running costs in respect of reagent preparation depend on workload. It is again important in planning any Category 2 laboratory to obtain assurances that such costs will be covered.

Supporting services

Supporting services in respect of the reception of reagents and other supplies and the maintenance and repair of electronic equipment are needed for a Category 2 as for a Category 1 laboratory.

Measures for the protection of staff against radiation hazards, particularly in the

preparation of ^{125}I-labelled reagents must be enforced [2]. Film badges or other devices should be provided to monitor external radiation exposure. Regular checks for possible internal radioactive contamination - for example, thyroid ^{125}I uptake - should be conducted. If nuclear medicine procedures in vivo are performed in the institution concerned, such services should in any case be available.

ASSISTANCE THROUGH IAEA TECHNICAL CO-OPERATION PROGRAMS

Assistance in projects aimed at the establishment of nuclear medicine services or radioimmunoassay laboratories in the categories considered may be available through IAEA technical co-operation programs. Such assistance may take the form of fellowships for the necessary specialized training of project staff, appropriate equipment for the initiation and development of the project and/or expert advice or assistance in its planning and execution. Relevant requests should be directed to national atomic energy authorities.

This Chapter is based on an IAEA document entitled " Basic requirements of nuclear medicine services" , prepared by E.H. Belcher, and presented at the International Symposium on "Nuclear Medicine in the developing countries" held at Vienna in 1985. Some of the financial estimates from the original text are revised to reflect the current prices. Few differences may be noted between this Chapter and other Chapters dealing with the same theme in this Handbook. These differences serve to emphasize that planning of a laboratory should be based on national guidelines and local facilities.

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Chapter 36

RADIATION PROTECTION IN NUCLEAR MEDICINE

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Introduction

The goal of this Chapter is to give a general outline of the essential principles and procedures for radiation protection in a nuclear medicine department where radionuclides are used for diagnosis and therapy.

More detailed recommendations regarding radiation protection in nuclear medicine are given in the publications of the International Commission on Radiation Protection (ICRP, publications 25, 57, 60) and in the ILO/IAEA/WHO Manual on Radiation Protection in Hospitals and General Practice (Volume 2: Unsealed Sources, WHO, Geneva, 1975), on which this Chapter is based. This chapter is not intended to replace the above-mentioned international recommendations on radiation protection, as well as existing national regulations on this subject, but intended only to provide guidance for implementing these recommendations in clinical practice.

One of the basic principles of radiation protection in nuclear medicine is that the radiation exposure of the patient, the staff, and the members of the public should be as low as reasonably achievable, (ALARA principle).

In general, the scope of radiation protection in nuclear medicine includes the following:

1. planning, construction and organization of nuclear medicine departments;
2. organization and administration of radiation protection services within the nuclear medicine department;
3. guidance for the transportation and safe handling of unsealed radioactive sources prior to their use;
4. monitoring of working areas;
5. waste management and disposal;
6. preparation for dealing with accidents.

It should be emphasized that all radiation protection measures in an individual nuclear medicine department should be developed and followed in the light of the above-mentioned international recommendations and national radiation protection regulations.

CHAPTER 36

Planning of a Nuclear Medicine Department

According to the recommendations of the ILO/IAEA/WHO "Manual on radiation protection in hospitals and general practice", nuclear medicine diagnostic departments serve almost all the clinical departments of a hospital, and should, therefore, be located in a central area. From the organizational point of view, the grouping of all diagnostic radiological services in an imaging department has proved most successful in large and medium sized hospitals.

An adequate distance between the diagnostic and therapy departments will avoid the problem of shielding the counting rooms, from therapeutic radionuclide sources and also from teletherapy units, such as linear accelerators, cobalt-60 units, and even conventional x-ray therapy units. This will provide a considerable saving in the cost of the shielding of the equipment.

In planning the premises where the unsealed radioactive services are used for diagnostic and therapeutic purposes, two basic principles of radiation protection should be observed. Firstly, that all unnecessary exposures of the staff and members of the public should be avoided, and, secondly, that exposures should not exceed the dose limits established by the competent local regulatory authority or, in their absence, the dose limits recommended by the International Commission on Radiation Protection (ICRP) and other international organizations.

The radiation measuring equipment used for imaging as well as for in-vitro nuclear medicine studies is highly sensitive and may require additional shielding. The background radiation in these premises should not exceed that in the general premises of the hospital.

Additional shielding may be required in the walls and doors of rooms used for patients who have received high doses of radionuclides such as ^{131}I for therapeutic or even diagnostic purposes at times.

For structural shielding design considerations, an "optimization" process as recommended by the ICRP in its publication No. 60 should be used. For these purposes, movable lead shields are commonly used. In rooms, the walls and doors leading to the corridor should be adequately shielded.

At the planning and design stage, special attention should be given to the provision of a reliable power supply, which is imperative for the normal functioning of modern nuclear medicine instruments.

The ventilation system of the department should be adequate for the requirements and functions of each section of the department (radiochemical laboratory, rooms for storage, preparation and dispensing of radioactive materials, counting rooms, wards for patients, etc.) and should not be connected with the central ventilation system of the building.

When radioactive gases are used, care should be taken to prevent inflow of the air exhaled by patients into the working area. A special exhaust system should be used for this purpose.

Organization, staffing and responsibilities

The International Commission on Radiation Protection recommends that the authority in charge is ultimately responsible for all radiation protection matters associated with the handling, administration, storage, etc. of radionuclides within the establishment or working area and its environment.

In a large nuclear medicine department, the person in charge should appoint a competent specialist to act as radiation safety officer, who will work in close collaboration with the hospital's medical physics and radiation protection departments, wherever they are available.

The main task of such an officer is to provide advice on all matters related to radiation protection in the department and to ensure compliance of appropriate rules or recommendations on radiation protection.

In a large nuclear medicine department, a responsible person should also be appointed to take care of the delivery, storage, and transportation as well as dispensing and waste disposal of radioactive materials. The physician is responsible for radiation protection of the patient and must balance the benefits of the diagnostic and therapeutic procedures against the harmful effects of radiation, in view of the dose delivered to the patient as a result of a particular procedure.

An important requirement for efficient radiation protection measures in a nuclear medicine department is training of the staff. Staff should be instructed in the principles of radiation protection, related to the type of work, at the beginning of employment and given periodic refresher courses during their employment. These instructions should include information on biological effects of ionizing radiation, types of radioactive sources used in the department, counting equipment, principles and practical instructions on radiation protection in the department. Special attention should be given to action of the staff during accidents with radioactive sources and in emergency situations, e.g. during a fire, etc. Clearly written instructions, regarding each type of routine operations with radioactive materials, as well as accidental and emergency situations, should be readily available to the staff.

Depending on the nature of the radionuclides and the types of operation with radioactive materials, the authority in charge may need to establish an area to which access is limited and controlled for radiation protection purposes. Such areas should be clearly marked by using warning signs of radiation danger.

CHAPTER 36

Safe handling of unsealed radioactive sources

Most of the radioactive materials, used for diagnostic and therapeutic purposes in a Nuclear Medicine department, are in liquid form as true solutions, colloidal solutions, suspension, or gases dissolved in liquids. In general, the safe handling procedures depend on the physical form of radioactive materials, their radiotoxicity, the type of operation to be carried out and amount of activity to be handled.

A radioactive source, from the storage to the handling room within the department, should be moved in a special lead container. Operations with radioactive sources involving opening of containers and withdrawal and delivery of their contents are described in the ILO/IAEA/WHO Manual of radiation protection in hospitals and general practice (Geneva, 1982). They are based on the following basic rules:

(a) The operations should be carried out in a laboratory specially reserved for work with radioactive materials.

(b) The glassware and instruments used should be reserved for the laboratory in which the sources are handled and should bear distinctive markings.

(c) Every handling operation involving radioactive material should be conducted with care, precision, and neatness.

(d) Generally speaking, every handling operation requires a certain minimum of preparation. The working surface must be prepared and certain items, including all the instruments that will be needed during the work, must be ready and nearby. A bag of impermeable material into which contaminated waste can be dropped should be placed in an immediately accessible position.

(e) Handling operations that are not routine should be planned in advance and rehearsed with non-radioactive materials.

(f) Operators should wear special apparel - e.g., laboratory overalls and gloves.

(g) Pipetting should never be carried out orally because of the danger of radioactive material entering the mouth.

(h) As far as possible, all receptacles containing radioactive liquids should remain sealed when not in use.

(i) Sources should be returned to storage as soon as possible, when no longer required.

(j) After the handling operation, the technician should clean the working area and monitor all surfaces, equipment, and tools, as well as his own clothing and hands, for contamination.

All necessary precautions should be taken to avoid opening or holding radioactive sources by hand. Tongs and other remote handling devices should be used for these operations. The gamma emitting radioisotopes should be handled from behind a protective sheet of lead glass or a wall made of lead bricks. A plentiful supply of lead bricks should always be available in a nuclear medicine department.

An operation with radioactive gases or aerosols should be carried out in a fume hood or a laminar air-flow cabinet with exhaust ventilation. The ventilation system must be regularly controlled regarding air-flow velocity and directions and effectiveness of the filter. More detailed information on this matter can be found in the book by K. Kristensen "Preparation and control of radiopharmaceuticals in hospitals" (IAEA's Technical Report Series No. 194, Vienna 1979).

All operations with radioactive sources should be carried out carefully but in as short a time as possible.

During the handling of radioactive materials, before their administration to patients, all necessary precautions must be taken to prevent contamination of working surfaces as a result of spillage of liquid, formation of aerosols, etc.

Special attention should be paid to preserve sterility of radioactive liquid sources to be administered parenterally to the patient.

The elution of the short-lived radionuclides from generators, e.g. $^{99}Tc^{m}$ and $^{113}I^{m}$ should be carried out in accordance with the instructions attached to the generators and in complete safety and sterile conditions.

To avoid pipetting by mouth, "remote" pipettes with rubber bulbs, or rubber tubes encased in protective sheaths, should be used. Surgeon's gloves, preferably disposable, should also be used by personnel to avoid contamination of the hands. The washing and decontaminations of small items of equipment after use is always a problem. Therefore, the widest possible use of small disposable items of equipment such as unbreakable sterilized tubing, pipettes, and syringes, which are treated after use as radioactive wastes, should be encouraged. However, such disposable items may not be available in many developing countries and even if available, they may not be economically acceptable.

The working surfaces in rooms, where the handling of radioactive materials is carried out, are usually of stainless steel or covered by plastic specially designed for this purpose. The working surfaces have slightly raised edges to retain any spilled liquid. It is essential that, before the handling of radioactive sources, the working surface is covered by one or more layers of absorbent paper, which can be disposed off easily after use. Cotton wool and paper towels must be available near to the operator. The floor in the dispensing room also should be covered by special leak-proof plastic and walls are painted by a special paint which allows easy cleaning.

It is a general rule that the operations with radioactive sources should never be carried out in every day clothing. It is necessary to have enough special protective clothing (overshoes, caps, jackets, trousers, etc.) in the nuclear medicine laboratory.

Good house cleaning practice (wet-mopping), avoiding raising any dust in controlled and supervised areas of the nuclear medicine department, is obligatory. The access of hospital staff and public to the department should be restricted and personnel dosimeters should be available for medical and technical personnel working in the department. The access of personnel to storage and dispensing rooms, as well as to any other parts of the controlled area, should be strictly limited to staff working in these premises.

As required by some national regulations, and as is always considered to be a good safety practice the following rules should be observed in laboratories and nuclear medicine departments [U.K., NRPB, 1983]:

(a) No food or drink should be brought into the area, e.g. for storage in a refrigerator. Smoking or even carrying of open packets of cigarettes should also be similarly banned.

(b) There should be a ban on the use of handkerchiefs; an adequate supply of paper tissues should be provided to replace them.

(c) Any cut or break in the skin should be covered before a person enters the controlled area. Dressing should incorporate a waterproof, adhesive strapping.

(d) The walls, floor and ceiling and all apparatus in the area should be cleaned often to ensure that contamination is kept as low as reasonably achievable.

(e) It is essential that radioactive solutions to be administered are clearly labelled, indicating the radionuclide, chemical form, and activity at a given date and time. The terms "millicurie" and "microcurie" (where still used) should be written out in full to avoid mistakes.

Special equipment for the safe handling of radioactive materials in hospitals, according to the recommendations of ILO/IAEA/WHO Manual on Radiation Protection in Hospitals and General Practice is needed for the following operations:

(a) Storage: lead bricks, lead shielded containers, remote handling tools.

(b) Transportation within the hospital premises: lead shielded cart, lead shielded containers.

(c) Safe handling: remote pipettes, disposable gloves, remote handling tools, lead shielding.

(d) Preparation and calibration of deliveries: safe handling tools (see above), activity calibration meter (Dose calibrator).

(e) Administration and measurement of radioactive substances: disposable gloves, shielded syringe.

Radionuclides for diagnostic and therapeutic applications are generally stored together. It is desirable that storage and dispensing rooms are separate, but in a small nuclear medicine laboratory, this may not be possible.

Each shipment of a radionuclide should be kept separately in individual lead containers, clearly labelled, and having the required wall thickness. It is recommended that the exposure rate should never exceed 0.2 m Gy/h at a distance of 5 cm from the surface of the containers. If necessary, additional interlocking lead bricks should be used to provide adequate shielding. It is not advisable to keep diluted radionuclide solutions in the storage room, e.g. a small volume of ^{131}I in solution prepared for an individual test dose. Special precautions should always be taken to prevent spillage. The storage room should have an efficient ventilation system.

Remote handling equipment such as tongs, forceps, tweezers and more sophisticated devices, with a ball and socket joint, should be available in storage and dispensing rooms. Other safe handling devices and equipment for transportation have been already mentioned in relation to handling of radioactive sources.

The equipment for preparation and calibration of radiopharmaceuticals and other radioactive sources in dispensing laboratories should include activity meters with the range from 37 MBq to 37 CBq. It should be noted that gamma activity can be measured accurately by the well-type ionization chamber, only if a small volume of the solution (about 0.1/5.0 cm^3) is in the centre of the chamber and a spherical geometry can be assured.

Radioactive sources containing carbon-14 and tritium can be measured by comparison with standard sources in a liquid scintillation counter.

Written instructions for the handling and dispensing of radiopharmaceuticals for diagnostic and therapeutic applications should always be available and periodically reviewed. Good records should be kept in relations to all operations with radioactive materials from delivery to dispensing or disposal. Concerning records, the following information is considered noteworthy:

Regarding the radiopharmaceutical;

Name of radiopharmaceutical,
Manufacturer, and batch number,
Delivery date,
Expiry date,
Activity, and date of measurement,
Volume.

Regarding administration to the patient;

Date and time of patient examination,
Type of study and route of administration,
Volume and activity administered,
Volume and activity remaining in the container.

Protection of patients

With respect to the protection of patients, in relation to diagnostic and therapeutic application of radionuclides, it should be emphasized that a nuclear medicine test or treatment with radionuclides should be carried out by a nuclear medicine physician, only if there is a sufficient clinical justification for these procedures. As a general rule, the radiation dose resulting from the administration of radionuclides should be kept at the lowest possible level but without sacrificing the quality of the necessary information or the desired therapeutic effect.

The measuring equipment including single- and multi-probe counting systems, rectilinear scanners and gamma cameras should be regularly checked, in accordance with recommended quality control procedures [Quality Assurance in Nuclear Medicine, WHO, Geneva, (1982) and Quality control of nuclear medicine equipment, IAEA-TECDOC-317] to ensure that each instrument produces optimum performance with respect to its capability and thus contributes to the reduction of patient dose. The practice of using instruments with poor sensitivity which leads to an increase of the activity of the radionuclides administered to patients, should be avoided.

The radiation dose to some organs (e.g. thyroid) can be reduced by using suitable drugs or other compounds which either block the uptake of radionuclides in organs where its presence is not required for diagnosis or treatment or will accelerate the clearance of radionuclides from the body after the examination has been performed. In this connection,

special care must be taken to reduce the organ doses to children undergoing a nuclear medicine examination.

Special attention should be given by the nuclear medicine physician, if a woman undergoing an examination is pregnant. Due to high radiosensitivity of the embryo and fetus, it is advisable that the examination should be postponed until the end of pregnancy or until the second half of it unless such examinations are unavoidable for clinical reasons and the dose to the fetus is minimal. The possibility of pregnancy should always be taken into account in women of reproductive age and, therefore, the radionuclide examination should be carried out during the ten days from start of menstruation. However, the validity of the ten day rule is a matter of debate at present. The breast feeding should be avoided till the level of radioactivity is low enough to avoid any potential harmful effects to the newborn.

In conclusion, it should be emphasized once again that before an examination or treatment with radionuclides, careful consideration should be given to the effectiveness and risk of such diagnostic or therapeutic procedures, in comparison with effectiveness and risk of other methods which do not expose a patient to radiation. For further information and examples, the report of the WHO Scientific Group on Clinical Diagnostic Imaging, published as "Effective choices for diagnostic imaging in clinical practice", TRS-795, WHO, Geneva 1990, should be consulted.

Handling and disposal of radioactive waste

For practical reasons, all radioactive wastes can be classified as liquid, solid or gaseous wastes. All actions related to disposal of radioactive wastes to the environment must be carried out in full compliance with national regulations on radiation protection.

Liquid wastes include the remains of unused radioactive suspensions and solutions, rinsing water that had been used for cleaning contaminated equipment, water from contaminated laundry, and excreta from patients.

The excreta from patients who have received relatively low amount of activities for diagnostic purposes can be released to the sewage system. When the patient has received the therapeutic amounts of radionuclides, e.g. ^{131}I for treatment of thyroid cancer, the excreta should be released into a specialized sewage system, which could be available in large radio-oncological hospitals or kept in nuclear medicine department in bags or specially constructed containers, until the level of activities allows safe disposal into ordinary sewage system.

The disposal of liquid radioactive wastes into the normal sewerage system should strictly follow the recommendations of national regulations. It should be mentioned that general principles and detailed recommendations on waste disposal are given in an internationally accepted code of practice [Safe handling of radionuclides, IAEA Safety Series No. 1, Vienna (1973)] which is, at present, under revision.

The solid radioactive wastes include contaminated equipment (e.g. vials, syringes, drinking glasses, etc.), laundry, dressings, disposable paper, cotton, etc.

All used linen should be checked for contamination and sent to a specialized laundry or washed in a room specially designed and designated for this purpose. Other solid radioactive waste should be kept in a properly ventilated room until it can be disposed as low-level radioactive waste or the radioactivity decays sufficiently, so that it can be disposed as non-radioactive waste.

The radioactive wastes containing long-lived carbon-14 and tritium should be kept in special containers and sent to industrial waste depositories. However, these two radionuclides are seldom used in clinical nuclear medicine practice.

The handling of cadavers (preparation for burial or cremation) containing substantial radioactivity must be carried out under supervision of the radiation safety officer. Autopsy in such cases is not advisable and must be restricted to minimum handling, if postmortem examination is necessary. All necessary radiation protection measures for the staff and the decontamination of instruments and the working place should be taken, in consultation with the radiation safety officer.

Monitoring and surveillance procedures

The radiation safety rules and procedures in nuclear medicine departments should be established in collaboration with the institution's radiation protection officer, or the local radiation protection authority, if appropriate, and must follow the requirements of the existing national regulations. It is recommended that written instructions be available for all techniques and procedures involving use and handling of radioactive sources and radioactive wastes.

An important radiation safety procedure in nuclear medicine departments is monitoring. According to the statement of the International Commission on Radiation Protection (publication No. 25) radiation monitoring implies the performance of relevant measurements on individuals in the work area and of the environment, and the interpretation of these data for the purposes of controlling personnel exposure and environmental contamination. Monitoring is very important also for the protection of sensitive and expensive measuring equipment, which can become inoperational due to radioactive contamination.

In monitoring, attention must always be given to both external irradiation and radioactive contamination. The choice of equipment for monitoring depends on the media and objects to be monitored and the type of the radiation which is to be measured.

There are many types of instruments which are suitable for the monitoring of gamma and beta irradiation. According to the recommendation of the ILO/IAEA/WHO Manual on Radiation Protection in Hospitals and General Practice, the most practical device is the acoustic dose-rate meter, which gives an audible warning when the wearer is subjected to a high rate of irradiation. As the dose rate increases, the pitch of the sound rises. In addition to this portable warning dose-rate meter, it is advisable to have in the nuclear medicine laboratory a hand and foot monitor, which should be placed at the door of the active room,

containing the radioactive materials. According to the ILO/IAEA/WHO recommendations, in the small departments, an end-window counting tube with long cable permits the monitoring of hands and feet and may also be used for monitoring contamination.

The routine monitoring should be carried out on all persons or things which may have had contact with unsealed radioactive sources, and in areas, where the work with these sources has been performed. This would include protective clothing of the staff, bed linen and patient's clothing, protective gloves, working surfaces, and radioactive wastes. The room should also be monitored after work with radioactive gases and substances which may form radioactive aerosols.

Special attention should be given to the monitoring of staff working with radioactive materials. Additionally, it is recommended that staff be provided with appropriate personnel monitoring devices, such as personal pocket monitors or film badge (or TLD badges).

The IAEA publication [Recommendations for the Safe Use and Regulation of Radiation Sources in Industry, Medicine, Research and Teaching, Safety Series No. 102, IAEA, Vienna, 1990] recommends schemes for monitoring of the work-place and personal dosimetry:

Monitoring for internal contamination is a more difficult task. Usually an indirect assessment of this contamination is made based on an estimate of the quantities of radionuclides entering the body in the working area through inhalation and ingestion; and, in case of accidents, through wounds. The monitoring of internal contamination can be also carried out by radioactive measurement or radiochemical analysis of the biological excreta. This approach is based on the assumption that the biological elimination of a radionuclide from the body depends on its nature and may be expressed by mathematical formulae.

In large nuclear medicine departments with radiochemical laboratories, where ^{35}S, ^{14}C and ^{3}H are used as labelled compounds, appropriate monitoring instruments should be available to check possible contamination in the working areas. Since these radionuclides are pure beta emitters, in case of internal contamination, external measurement is not possible. Therefore, periodic monitoring of the laboratory staff should be carried out by analysis of the excreta (urine) in a well-type scintillation counter.

Appropriate records should be maintained regarding the procurement and the day-to-day utilization of radionuclides, monitoring of working areas, accidents and remedial actions taken, radioactive waste disposal, monitoring of personnel and information on calibration of monitoring instruments.

Measures for dealing with radiation emergencies and accidents.

The most common types of accidents which may occur in a nuclear medicine department involve fire, explosions, spillage of a significant amount of radioactive solution, misplacing or losing radioactive sources, and the inadvertent administration of large activities of radionuclides.

Despite the fact that these events are usually unpredictable, it is advisable that adequate plans and written instructions are prepared in advance to deal with accidents, which may occur in the laboratory. The staff must be trained to implement the plan and strictly follow the instructions in the event of an accident.

When an accident occurs, the hospital administrative authorities, the nuclear medicine department head and the radiation protection officer must be informed immediately and necessary remedial measures must be taken.

In case of a serious accident, involving bodily injury, the first priority is to give first aid to the persons, affected by the accident. Then, assisted by suitable monitoring instruments, radioactive substances should be removed from the skin, hair and other parts of the body by using physical methods. Contaminated, small wounds can be washed, preferably with a product that makes the radionuclide insoluble, e.g. magnesium sulphate for the alkaline earth elements or chelating agents which form complexes with high valence elements.

In case of contamination through the gastro-intestinal or respiratory tract, the decontamination measures should be carried out under the supervision of a trained physician.

Within the area affected by the accident, action must be taken to prevent further spread of contamination or the intake of radioactive materials.

The borders of the contaminated area must be clearly marked and the decontamination of the working surfaces should be carried out by using physical methods (absorbent materials, washing with detergent and water) and then using recommended chemical compounds. A recommended list of chemicals for use in such circumstances should be available in the nuclear medicine department.

Protection of personnel and members of the public.

The radiation protection principles and rules for clinical staff of the nuclear medicine department and, in particular, for personnel involved in the preparation and administration of radionuclides are based on the concept of optimization in radiation protection and dose limits established by the national regulations, in accordance with international recommendations. These limits should not be exceeded during the work with unsealed radioactive sources in the department. Every effort should be made to keep these limits as low as reasonably achievable.

The International Commission on Radiological Protection [ICRP Publication 60, 1990] recommends a limit on effective dose of 20 mSv per year, averaged over five years (100 mSv in five years), with a further provision that the effective dose should not exceed 50 mSv in any single year. The five-year period would have to be defined by the regulatory agency, e.g. as discrete five-year calendar periods. It is implicit in these recommended dose limits that the dose constraint for optimization should not exceed 20 mSv in a year. It is assumed

that the dose constraints may be established on basis of "conclusion about the level of individual doses likely to be incurred in well-managed operations".

Usually the amount of radioactivity administered to the patients for diagnostic purposes is small and does not create any hazards for medical and auxiliary personnel, because of external irradiation from the patient. In this case, radiation protection efforts are concentrated in ensuring that the necessary precautions are observed during the process of preparation and administration of radiopharmaceuticals.

The main danger for personnel from external irradiation arises from the patients and their excreta, when therapeutic amounts of radionuclides are administered. The contacts of the staff with these patients, particularly during the first few days after the administration of radionuclides should be limited to the most essential visits, examinations, and procedures. The installation and use of television systems for remote surveillance is highly desirable if practicable. Special precautions should be taken during the collection, storage and disposal of excreta. After optimizing the working procedures, the rotation of personnel could be used to ensure that dose limits established for the staff is not exceeded.

The employer shall ensure that each female worker engaged in work with ionizing radiations is informed of the possible resulting danger to the foetus and the importance of informing the employer as soon as she knows that she is pregnant.

When a female worker declares that she is pregnant, the employer shall take all reasonably practicable steps to ensure that working conditions, including the likelihood of accidents, are such that during the remainder of her term of pregnancy the dose to the surface of her abdomen is less that 2 mSv and the dose to the woman from radionuclides intake is less than 1 mSv.

Regarding protection of members of the public, who may have personal contact with patients, who have received diagnostic amounts of radionuclides, it should be pointed out that this does not create any hazard because of external irradiation. It is only necessary that the members of the patient's family are instructed by medical personnel regarding the basic rules on how to collect excreta, if such collection is necessary.

The possible contact of members of the public with patients, to whom therapeutic amounts of radionuclides are administered, is a more serious problem. Measures should be taken to avoid exposures, in excess of the dose limits established for members of the public. Visits to patients, who are hospitalized in special wards after administration of therapeutic amounts of radionuclides, should be limited and a reasonable distance should be kept between visitors and the patient's bed.

The possible visits to a patient by pregnant women and children should be restricted to the most indispensable circumstances.

After treatment is completed, the patient may be allowed to leave the hospital, when the activity in his body does not exceed the level recommended by national regulations.

During the travel to his home, it is advisable that the patient be accompanied by a member of the medical staff to prevent contamination of the environment and other people, if the patient needs to vomit or excrete. (This may not be practical in cases where public transport has to be used.) Members of the family should be instructed regarding precautions against possible contamination and how to minimize their exposure to external irradiation from the patient's body.

SUGGESTED READING.

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Chapter 37

ROLE OF INTERNATIONAL ORGANIZATIONS IN PROMOTING NUCLEAR MEDICINE IN THE DEVELOPING COUNTRIES

M. Nofal[3]

Introduction

When the IAEA was established in 1957 as an autonomous member of the United Nations family, one of its prominent assignments was to "seek to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

The inclusion of health reflects an important fact - that medical uses of radiation and radionuclides were among the first and most widespread applications of "atomic energy". Indeed in one developed country, it has been estimated that these applications now contribute, in some measure, to the diagnosis or treatment of one out of every three hospitalized patients. Of even greater significance, is their role in research, helping to clarify the very nature of health and disease.

Today, because of the diversity of its applications - radiation and radionuclides for medical and biological purposes are used in more countries and in more laboratories than any other application of atomic energy. International organizations, mainly the IAEA and the WHO, have played a significant role in the spread of this nuclear technology in developing countries.

There are altogether 112 member states of the Agency, about 71 of them can be classified as developing countries. Out of them, nearly 56 have some kind of nuclear medicine. By that I mean there is some medical use of radioisotopes, be it imaging, radioimmunoassay or the old thyroid uptake! In most of these countries, the personnel working in nuclear medicine has been trained abroad. Training can be as short as few weeks abroad in the form of attendance at one of the four or six week training courses offered by an international organization. Occasionally it is through a fellowship offered by the same organizations. In terms of technology and training, Nuclear Medicine, in its present form, can thus be considered a high technology imported medicine in many of these countries.

The first question that arises is whether nuclear medicine is necessary in all these countries particularly when it is usually at a minuscule level with hardly any impact on the medical practice of that hospital. Is nuclear medicine a luxury or a status symbol? RIA? Some of the radioimmunoassays are obviously useful but if you do very few assays with kits, the whole exercise is not cost effective. Space occupying lesions by imaging? If you do not have neurosurgery, what use is the finding of a space occupying lesion in the brain? Abdominal organs can be scanned effectively with an ultrasound. Nuclear cardiology needs gamma camera-computer complex which is beyond the means of many of the hospitals in the

[3]. Based on the address given at the World Congress of nuclear medicine held at Montreal in Aug. 1990.

developing countries. As far as the radiopharmaceuticals are concerned, the situation is still dismal. Most radiopharmaceuticals are imported from developed countries. Technetium generator needs at least a fortnightly supply. Indium generator is out of fashion and even difficult to obtain.

The Agency conducts many programs to strengthen the indigenous capabilities of developing countries in using radionuclides in the medical field. These programs account for some 10% of the Agency's promotional efforts. Where large amounts of funds are required for establishment of nuclear medicine facilities in a country, it is usually through the Technical Cooperation Program. To do this function effectively in a cost effective way with minimum of failure rate, lots of safeguards are necessary. The first question which faces an Agency is whether a particular hospital or a country qualifies for a large scale assistance for instituting nuclear medicine.

People who say how great is nuclear medicine, how pressing is the need for setting up nuclear medicine in various hospitals, how a tiniest hospital will benefit by its wonders and so on and so forth, are usually nuclear medicine specialists. Nuclear medicine in a developing country sometimes start in a hospital as a status symbol. There is an impossible triangle of: patients, radiopharmaceuticals and the instruments. If you have the radiopharmaceuticals, the instruments do not work; if the instruments are in order, the right kind of radiopharmaceuticals are not available; when both of them are there, the patient is not there. It is usually said for the modern instruments that when they are out of order they require minimum time for repairs because it only involves replacement of a board or an integrated circuit. This is not as easy as it sounds. In a developing country, it may be easy to obtain an instrument costing thousands of dollars through a technical assistance program of an international organization but sometimes extremely difficult to obtain a small sum for spare parts through local foreign exchange resources.

We must recognize that nuclear medicine, being a high technology medicine, demands certain infrastructure for its successful institution in a developing country. It should be in a hospital where specialties like radiology, and clinical pathology are well established, where at least some of the clinical specialties are flourishing. These specialties should have other ancillary investigational facilities, where it is possible to get well defined clinical material referred to the nuclear medicine unit so that you are trying to answer specific rational questions and not trying to get unconfirmable results which do not lead any where as far as the management of the patient is concerned.

To justify nuclear medicine in a developing country we have to see it in a new role. It is not for putting the diagnostic labels, not for differential diagnosis as we have been conditioned to think so far. In a developing country, it should be for differential management. How does it alter the management decision in respect to a particular patient? If management outcomes are restricted, there is no need for an investigation which does not help the management of the patient. If there is no bypass surgery, what use is the thallium perfusion? Although primarily a diagnostic discipline, for its justification and survival in a developing country it should contribute to a sensible differential management.

ROLE OF INTERNATIONAL ORGANIZATIONS IN PROMOTING NUCLEAR MEDICINE IN THE DEVELOPING COUNTRIES

There are always few apical hospitals in every country. Not many, but one or two are certainly there. Before instituting nuclear medicine, one needs certain basic conditions to be fulfilled. The hospital should have a good stable uninterrupted power supply and reliable air conditioning. This is a condition difficult to satisfy in many of the developing countries. If the culture of preventive maintenance is not there in a hospital, it would be difficult for nuclear medicine to survive. The hospital also should be in a city on the main trunk route of an international airline. Without this, import of radiopharmaceuticals would be impossible. Apart from these, it should have necessary provision for foreign exchange for import of these radiopharmaceuticals and spare parts for the instruments.

It is possible to get technical assistance from the IAEA if you satisfy these conditions. The request for the assistance comes from national atomic energy authority or some such administrative agencies. The Agency's primary function is not the transfer of the equipment but the transfer of technology.

The most crucial part is who is the counterpart at the other end? Who is going to set up nuclear medicine in the recipient country? We need a person who is well versed in one of the branches of medicine, who is a seeker, is keen on diagnostic aspects of medicine and does not want to parade as a healer, is capable of learning a new discipline and can work in harmony with scientists from multiple specialties. Such individuals may not always easily be found: moreover, they cannot be selected on the basis of seniority. The counterpart can be trained, but training abroad can either lead to frustration that back home he has not much, or may need superhuman patience and courage to set up what he learnt abroad.

Research Promotion

One way by which the IAEA assists its member states is by promotion of research. However, research comes after one has instituted nuclear medicine.

A co-ordinated research program is developed around a specific scientific topic and institutes from different countries are invited to work together on an integrated research to achieve the aims of this program. Research contracts or research agreements are awarded on the basis of project proposals submitted by these institutes. The basic role of the IAEA is to define the program of work, secure its financial backing and ensure that the various efforts forming that program are properly co-ordinated. To this end and to ensure proper integration, research meetings are usually held at intervals of 15-18 months. One representative from each participating institute is invited to the meeting. Research contracts or agreements are awarded to research institutes for a period of one year renewable up to a total period of 3-5 years.

Research contracts provide modest financial support for a project on a cost-sharing basis which is usually used for a purchase of small items of equipment and supplies. Priority is normally given to contract proposals from institutes in developing countries. On the other hand, research agreements do not provide financial support and are usually awarded to

institutes in developed countries with expertise in the area of interest. In return, the agreement holder participates in exchange of information and is invited to attend the research co-ordination meetings.

Research contracts are sometimes awarded on other topics than those of co-ordinated projects depending on the availability of funding. However, preference is normally given to proposals that fall within the scope of a co-ordinated research program.

Many clinical nuclear medicine protocols currently accepted in various countries may not be suitable for the developing countries. There is a necessity to simplify the procedures and work with relatively less sophisticated instruments and with easily obtainable radiopharmaceuticals. This process of adaptation of techniques to suit the needs of the laboratories in developing countries is what the Agency programs encourage. The approach recognizes the fact that advanced methods cannot be adopted de novo but need to be adapted to existing realities in developing countries. With this kind of adaptive process in mind, the Agency has set up Co-ordinated Research Programs on subjects specific for the problems prevalent in most of these countries. In addition, simplification of technology would lead to a wider spread of these techniques in more hospitals. Over 100 laboratories are receiving support in the area of health-care at any one time.

The Agency's research program has to be tuned to nuclear medicine's current trends in the world and to the perception of what future role it will be playing in the overall management of patients.

Equipment

Putting instruments in a developing country is a risky business. You work to keep the instrument busy. You work to keep the instrument healthy. Provision of instruments to a developing country is thus a big decision financially. It is easy to initiate a radioimmunoassay facility; financial outlay required is small and manageable. For imaging, scanner is no longer easily available in the world market from an international firm. Gamma camera is too expensive. Without a computer the gamma camera also does not make sense. Both together adds to nearly US $ 300 000 investment with an attendant anxiety whether it would work or not. Even if it is satisfactorily installed, how long will the bliss last?

Finally we work out a compromise between the funds and the demand. However, the funds are always less than the demand and many who deserve and qualify cannot be provided for.

Training courses

For more than a decade, the Agency has organized periodic interregional and regional training courses and study tours in nuclear medicine for participants from developing

countries. They are usually of short duration and can be attended by 20-30 participants depending on the facilities of the host institution. Regional training courses can specially be geared to conditions prevailing in the countries of the regions. At present the practice is to select junior medical doctors for these courses, and provide a basic introductory overview of current clinical nuclear medicine techniques. Participants are provided with theoretical and practical training in the common diagnostic and therapeutic applications of radionuclides, safe handling of radionuclides, general principles of radiopharmacy, analysis, interpretation and quality control of "in vitro" and "in vivo" nuclear medicine procedures, organizational aspects of nuclear medicine service in a hospital, and a general background education in physics related to radiation and radioactivity. In addition, the Agency frequently organizes various short-term training courses and workshops throughout in different parts of the world on special topics in nuclear medicine - for example, quality control of imaging instruments, radioimmunoassays, and maintenance of nuclear medicine equipment.

Training fellowships

The ultimate aim of setting up nuclear medicine facilities in any country is to have a fully trained local person to take up the clinical and technical responsibilities of the nuclear medicine unit. For this purpose, a local person with the requisite academic credentials is sent abroad for comprehensive training to a suitable institution for periods ranging from a few months to a few years. Training a cadre of trained persons is a risky road. They might serve as an initial engine to pull the nuclear medicine train or lead to a group of frustrated people.

The Agency, thus supports the transfer of know-how through granting these training fellowships. However, training in advanced countries - in general - is relatively expensive for developing countries and the level of training programs may not always be entirely suitable for trainees from the developing world who may, in addition, have communication problems and other difficulties which make it hard for them to adapt to the local institution in a developed country. If they do adapt, there is always the danger of failing to return home after training. On the other hand, training in a developed country may provide trainees with the possibility of having valuable contacts in the country concerned.

In this respect, one way to partially overcome some of the problems encountered in training abroad would be, if possible, to establish centres of excellence for training in different regions.

Expert Assistance

One of the most vital aspects of training is on-the-spot instruction in a developing country by a recognized expert. The expert may cover some or all aspects of nuclear medicine. A mission may be of a few months duration or may extend to a whole year. When complex

equipment is supplied to a country, the project usually includes the visit of an expert to train local staff in the operational and technical aspects of the instrument.

The expert provides a new kind of work culture to the whole of the laboratory. The entire staff is exposed to him. Selection of the expert is a rather delicate matter. His guidance is tentative because many a times he comes from a far away glorious country and is not quite aware of the working conditions in a developing country. The developing countries also are at fault. They would not usually accept an expert from another developing country but would always aim at very famous names.

Maintenance of equipment.

Gone are the days of single scintillation probe, a small manual well counter, a rectilinear scanner and a small pocket slide rule to do some of the simple calculations. The present day nuclear medicine is all big machines: gamma camera - that too rotating if you can afford it, dedicated computer, automatic gamma counter for multiple samples hooked to a data processing system and a host of radiopharmaceuticals. Anyone who plans to set up nuclear medicine in a comprehensive way in a hospital will have to plan on an investment of at least half a million dollars in capital funds and $ 50 000 - $ 100 000 annually in running expenses. Thank God that in spite of all these high sounding numbers, amongst most of the imaging disciplines nuclear medicine is still considered as relatively inexpensive and cost-effective.

Doctors and patients both are tuned to this high cost, high technology medicine. The problem arises when these machines do not work, cannot be repaired and then the total investment in them is considered as dead loss. We all know in international organizations that sometimes more than 50% of the equipment in a hospital of the developing countries are defunct, out of order or producing results which are less than optimum. This situation is intolerable and should not exist.

For want of a proper site preparation prior to the installation of equipment, many of the costly equipment have withered away in the customs shed or in the hospital warehouse. Dust and humidity have worked as deadly enemies of the instruments which have no immunity against their attack. In developing countries, it may be possible to get capital funds in a large bounty but rather difficult to get a spare part which may not be costing more than few dollars.

Not one but many international organizations are interested in solving this problem of equipment maintenance and repair. All of them consider this problem as a top priority. If you have a plan to resolve this problem, money is there a plenty to implement the plan. What we are short of is ideas, workable pragmatic proposals and innovative thinking. I feel that the greatest task facing us is to generate ideas and to promote creative thinking.

It is not that we do not realize what are the problems. What eludes us is the way to solve them. For years we have been promoting a programme of preventive maintenance.

As medical doctors, we realize that an ounce of preventive maintenance is better than kilograms of cure. Prevention starts right from the purchase of the equipment. How to decide which is the simplest and the most easily maintainable?

The necessity of proper environment for the working of the instrument is well recognized. Comfortable temperature, low humidity, dust-free rooms, stable power supply - everyone knows that these are essential, but for some reason or other, these simple precepts of maintenance are the most neglected elements in the developing countries.

Hospital based equipment should have minimum of downtime. For this, it is necessary that we have a stock of spare parts or we have a reserve fund in foreign exchange from where necessary spares can be ordered almost by a telegram. We all recognize the need for spare parts but most of the time it is the most difficult single component of maintenance. There are customs regulations, import licenses, permissions and sanctions, but why this maze of rules and regulations to strangulate the working of an equipment?

It is customary to lament the lack of trained personnel for maintenance. At the same time, it is also recognized that the modern electronic equipment is a proprietary item and can be serviced best by the manufacturing company itself. Many of these companies are notorious, in developing countries, to promise the sky at the time of the purchase but show total disdain for all maintenance needs after the warranty period or even before that.

Regular quality control of instruments is well emphasized by all nuclear medicine specialists. I do not want to belittle the importance of quality control, but quality control without the means of correcting the performance of the equipment, is meaningless. All quality control programs should be backed by good maintenance programs. We need to have the proper skills to handle the medical, radiopharmaceutical, computer as well as the other complex engineering aspects of equipment. This can only be accomplished by proper training and the choice of dedicated persons. The IAEA is deeply involved in solving the problems of maintenance and quality control of nuclear medicine equipment.

Dissemination of information.

This is another prominent mechanism of the IAEA to assist its Member States. This is usually accomplished through meetings of various formats, designed for exchange of ideas between scientists from various countries. Each year, the IAEA organizes a number of symposia and seminars at which specialists from both developed and developing countries review their progress and present their latest findings on a specific subject. Almost all of such meetings are organized in cooperation with our sister organization, the WHO.

In 1985, the IAEA had organized an international symposium on "Nuclear medicine in developing countries". Another symposium on "Dynamic functional studies in nuclear medicine" and a seminar on "Training in nuclear medicine in developing countries" were held

in 1988. In 1990, a seminar was held on the "Application of nuclear techniques in the early diagnosis of cancer in developing countries". In 1991, the IAEA is organizing an international symposium on "Radioimmunoassay and related procedures: perspective in developing countries" at its headquarters in Vienna.

In addition, the Agency convenes smaller meetings to examine particular topics in-depth to plan future programs or to review results achieved in research activities. Information of these different meetings, including proceedings of symposia are being published and distributed. Last year, the Agency published as a technical document an "Atlas of Liver Imaging" which was most helpful to practitioners in developing countries serving a rather vital educational need of these countries. Publication of this Handbook is also an effort on the part of the Agency in the same direction.

Trends and new directions.

Three decades ago, nuclear medicine started as a tracer investigation for the function and flow. The advent of scanners and the development of new radiopharmaceuticals localizing in various organs directed the attention of nuclear medicine physicians to imaging of various organs. For years, apart from radiology, nuclear medicine was the only technique available for such imaging. Now that no longer holds true. Various competing imaging modalities are available, such as ultrasound, computerized tomography, and magnetic resonance imaging. Each one has its own virtues and limitations. A hospital administrator is faced with alternatives. What kind of imaging services should be established in his hospital? A physician also is faced with a similar dilemma as to what is the most appropriate investigation for which he should refer the patient.

There is a gradual awakening of an awareness that the primary role of nuclear medicine is not to seek anatomical defects or search for structural abnormalities. Its main forte is the study of function with respect to time or, in other words, to study a radiopharmaceutical's changing distribution in the body during a certain time period. This kind of study yields important information about the function of various organs. Nuclear medicine's future is intimately tied up with development in the field of radiopharmaceuticals. In all other imaging modalities, one needs only the instrument and the patient. In nuclear medicine, one also needs a suitable radiopharmaceutical. It is possible to develop a wide variety of new radiopharmaceuticals each tracing one or another function of the body. This helps to enhance the versatility of nuclear medicine. This realization has led to special attention to the study of cardiovascular, renal and cerebral functions, where in the last few years nuclear medicine has brought about a dramatic surge of new data. So far, such data has been obtained with the use to special instruments such as PET and cyclotron-produced radiopharmaceuticals. Now it appears that new cerebral and myocardial radiopharmaceuticals labelled with $^{99}Tc^{m}$ have started becoming available in the developing countries, and that it would be possible to do "in vivo" functional brain and heart studies with conventional gamma cameras.

Another new direction - also related to radiopharmaceuticals - is the development of specific monoclonal antibodies labelled with an appropriate radionuclide and targeted towards an antigenic site either for imaging or for therapeutic purposes. In addition, tumour markers for diagnosis and management of cancer patients and the detection of antigens and antibodies in infectious diseases are two of the most potentially fertile areas in this respect. The new inexpensive personal computer based analysis, display and storage systems make it possible to carry out practically any kind of program desired. The Agency is aware of all these new applications and is seriously promoting their uses in its developing member states.

These new trends in nuclear medicine also generate an impression that the frontiers of nuclear medicine are advancing. Nuclear medicine's glory will be on the functional studies based on positrons and cyclotrons or on new yet expensive radiopharmaceuticals. The gulf between what the developing countries can do and what we can provide them and what is current in the world is widening. The IAEA has to support the on-going process of evolution and facilitate the wheels of progress.

Nuclear medicine is an integral part of health care structure of all countries especially industrialized ones. However, the third world as a whole is slowly working up to the realization that science and technology are what distinguishes the third from the first. The field of nuclear medicine, like other technologically advanced medical specialties has undergone considerable changes. These changes have come about as a result of the technological developments in instrumentation including computers, and the availability of new radiopharmaceuticals. It is therefore expected that the role of nuclear techniques in medicine will continue to make important contributions towards the identification, treatment and management of diseases in the years to come. Just as yesterday's research is today's practice; today's research is tomorrow's practice.

In the field of nuclear medicine, the interests of international organizations such as the IAEA and the WHO overlap. Many Agency activities in nuclear medicine are done in cooperation with the WHO and many future activities of these two bodies will have to be in close collaboration when they relate to nuclear medicine.

As I stated at the beginning of this presentation, one objective of the IAEA is "in accelerating and enlarging the contribution of atomic energy to peace, health and prosperity"; promotion of nuclear medicine is one step forward in this direction.

What I have tried to describe to you is the ways and means by which the IAEA helps the developing countries in the promotion of nuclear medicine. I have spent more time on describing where the Agency's assistance get bogged down with innumerable uncertainties and anxieties. It is still a small wonder that we succeed somewhere, sometime. My basic philosophy has always remained that it is always better to light even one candle than to curse the darkness.

CHAPTER 37

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Chapter 38

FUTURE OF NUCLEAR MEDICINE

R.D. Ganatra

When it comes to setting up nuclear medicine in a developing country, there is a group of people, who feel that such high technology has no place in a developing country. Their nihilistic argument runs as follows:

The boom of the sixties is over for nuclear medicine in many countries, even those which are not "developing". The new imaging devices, starting with the gamma camera with computer on line and going on to SPECT and PET, are beyond the reach of many countries, which in the sixties were full of rectilinear scanners and enthusiasm. Obtaining up-to-date radiopharmaceuticals is also a big problem, as many of the radionuclides used today are not easy to produce locally, even in countries which have spent large sums on nuclear research. Many of the developing countries, depend on few commercial suppliers, from the advanced countries, for their supply of radiopharmaceuticals. In some of these countries, customs regulations are so cumbersome, that clearing radionuclides through customs represents a real achievement.

The current global economic situation has kept health expenditure to almost zero growth in many countries. Many countries spend only 10 - 50 US dollars per inhabitant/year for total health care, a good majority of the developing countries spend less than $10. This amount on health care is totally inadequate, particularly when the developing countries are committed to:

(a) provide potable water for a large percentage of the population;

(b) organize a primary health care network;

(c) vaccinate all children against the prevalent diseases;

(d) fight against diarrhoeal disease using oral rehydration and against other specific endemic diseases, such as malaria, schistosomiasis, amoebiasis, etc.;

(e) improve maternal and child care, as well as nutrition;

(f) provide essential drugs;

In this context, the decision to purchase, for example, a SPECT machine is not easy to justify. Justification for SPECT is even more difficult when competing alternatives offered are more cost/effective. For US $50 000 you can have a large variety of ultrasound diagnostic machines, real time or more complex with Doppler facilities, with image recording systems, measuring possibilities, guidance for interventional procedures, etc.

Please see **Fig. 7.1** which refers to different imaging modes and shows the current trends and predicts the course of events in the next few years.

Looking at nuclear medicine during the sixties and early seventies, this imaging technology was widely applied in investigations of thyroid, liver, brain, kidneys, lungs, bones, pancreas, etc., particularly for space-occupying lesions, and in limited cases for function testing. The new imaging modalities available have changed this situation: ultrasound has almost taken over the field of liver and gall bladder imaging, kidney and urinary bladder, pancreas and is now entering the area of thyroid, brain lesions in infants, etc.; computed tomography has superseded nuclear medicine in brain imaging, is gradually becoming more accurate in bone metastases evaluation and is starting to compete with nuclear medicine in the area of heart.

Even in the advanced countries, every one does not feel that future of nuclear medicine is so bright. There are cries of despair and disappointment.

Nuclear medicine is a speciality based on a technology. Any scientific discipline tied to a specific technology or a disease cannot be everlasting. For instance, specialities related to tuberculosis will vanish as soon as a cure for tuberculosis is discovered. The moment a technology is found that does what nuclear medicine does in a better way, is more sensitive and more specific, less invasive in terms of cost or adverse effects, the nuclear medicine as a speciality would face a bleak future.

Fear of radiation is not going to disappear easily from the minds of public. NMR has to camouflage itself as MRI. That fear is not entirely irrational, and there is every reason to fear that in years to come, it will increase and not diminish.

Specialities like cardiology or nephrology do not face this problem, because heart or kidneys are not going to disappear from the human body. "Who does What" disputes between cardiologists and nuclear medicine physicians are not likely to be settled in our favour. Our claim is based on the premise that a radiothallium is used for cardiac diagnosis. That, by itself, does not give us a right over any territory. A nuclear medicine physician can not be a cardiologist and a nephrologist and a neurologist - a super specialist. If we do Nuclear Cardiology, we can not avoid being subservient to cardiologists.

In vitro nuclear medicine is considered our prerogative because radioimmunoassays form a principal part of in vitro work. As soon as a non-radioactive label is as successful as a radioactive label, we are going to lose that ground to clinical chemistry which has no reason to fear extinction.

There is no denying the extremely useful role that the radiotracers play in medical research. It will always remain unique and unsurpassed but then that does not make nuclear medicine. The radiotracers would certainly survive in the research setting. The role of nuclear medicine as a diagnostic service facility is recent and likely to be short lived.

FUTURE OF NUCLEAR MEDICINE

In advanced countries, nuclear medicine is trying to expand in new directions, like PET and immunoscintigraphy. They would remain largely as research areas for years to come but not likely to form a major part of diagnostic nuclear medicine.

Wide spread use of radiolabelled monoclonal antibodies in the detection, let alone the treatment of malignant disease, is doubtful. The "magic bullet" is still going to remain a dream only. Monoclonal antibody research may produce interesting and useful radiopharmaceuticals such as white blood cell labelling agents and will probably produce in vitro tests for the presence of tumour. The problem is that there is no particular reason why the latter should require radioactive labels.

Nuclear medicine claims that its unique strength is its ability to do functional studies, yet very few are being done. WHY? They are not likely to be possible without significant development of a new generation of radiopharmaceuticals.

Nuclear medicine will survive. The scientific and intellectual activities of nuclear medicine will thrive. The party is over for nuclear medicine as a large volume prosperous speciality of wide diagnostic application. What nuclear medicine needs is an investigation like chest X-ray or blood count which can be ordered by any physician uncritically for each and every patient that attends a hospital. There is nothing on the horizon like that. Nuclear medicine is not going to be a money spinning speciality, which is usually the yard stick, by which success or failure is measured, in the advanced countries.

In the initial days of nuclear medicine, there was no imaging. Scientists were not thinking in terms of imaging. It was an excitement and enthusiasm of doing tracer studies. Hevesy called his book "adventures with radioisotopes" with a sub-title "their applications in Biochemistry, Physiology and Pathology". The word imaging does not even appear in the Index. Nuclear medicine did not start with imaging. It was there even before imaging became possible. It will be there even after imaging goes away from nuclear medicine.

The gamma camera was developed in 1954 but became the instrument of choice, only when Tc became available, after a decade or so. For us, in nuclear medicine, the tracer, the radiopharmaceutical has always remained of paramount importance. The detector has always remained secondary.

"I am carrying the ocean on my shoulders and looking for the shores."

Put down the ocean, put down the burden of imaging and you will find the shores you are looking for. Only when you get rid of the burden of the preconceived notions, you can find the shores. The last thing to be discovered about science, is what the science is about.

"To carry the burden of the instrument, count the cost of the equipment and never to know that it is for music, is the tragedy of life's deafness."

Nuclear medicine is not only about imaging. It is about detecting the ever changing distribution of a radioactive substance in the body measured in quantitative terms, in relation to time. In CT, density does not change with time; dead or alive it is same. In US, the acoustic impedance does not change. In MRI, the nuclear spin does not change. You can have beautiful images - frozen flower vases, exquisite, but motionless.

If one wanted to count the shortcomings of nuclear medicine as an imaging modality, it will be like that famous Belafonte song "there's a hole in the bucket". First of all, the nuclear medicine imaging needs radiopharmaceuticals as well as the instruments to produce an image of an organ; the radiopharmaceuticals are short-lived and need to be imported periodically, or rather every few days from abroad; the photon yield from the radiopharmaceuticals in an organ is not of the same order of magnitude as that arising in the case of a radiograph; moreover, the image is a two dimensional display of a three dimensional distribution of the radiopharmaceuticals in an organ!

However, the radiograph and the nuclear medicine image of the organ differ as much as a 'photograph' and a 'mirror': a photograph shows a physical image, where time is static, a moment of time frozen in a frame; mirror shows a physical image where the time element is dynamic, all the antics of the subject can be visualized, while they are happening! Oscilloscope becomes a mirror of functional changes happening inside the body. Who would like to tell the flying bird not to fly? Who wants to freeze the ever changing dynamic processes in the body? We are not photographers. We are like a mirror, tracing every moment of the body, without freezing the motion. A photograph confines you to the frame of the observer, the mirror is limited only by the antics of the tracee. We reconstruct anatomy out of the function. We conceptualize abnormality rather than visualize it. In nuclear medicine, time is our third dimension.

93% of the American Hospitals with more than 100 beds have gamma cameras. There is an 8% rise in the nuclear medicine investigations annually even now. Every third patient in an American hospital undergoes a nuclear medicine investigation. Third world countries have a lot to do to catch up. Our instrument companies have realized this. Low cost of nuclear medicine is going to be its redeeming feature. Do not mention the still lower cost of ultrasound. As long as the US pictures look like a lunar landscape, we have some hope.

"A dew drop knows the sun in its own tiny orb."

What kind of spectrum we see there for the nuclear medicine? Biochemistry of the brain. Thyroid, anyway, is our private preserve. There is no other better way of detecting pulmonary embolism. Radionuclide scan is still an investigation of choice for liver imaging in most of the developing countries. Renogram is a superb test for screening patients with hypertension. Bone pathology is still best detected by bone imaging. Nuclear Cardiology can not be bypassed, if coronary insufficiency is suspected. Anything that moves during the course of time, we can image. Time domain may be slow in a dynamic functional study like RBC survival; or very rapid as in Nuclear Cardiology. Time is our third dimension.

RIA is also a pharmacokinetic distribution of a labelled substance in a test tube in its interaction with a clinical isolate, in the same way as a colloid distributes in vivo in the presence of a liver. RIA done repeatedly gives a functional profile of an organ.

To challenge the function of any organ adds a special dimension to nuclear medicine studies. It is not enough to have a servant, the servant must respond to the command. It is not only possible to show the function of an organ in nuclear medicine, but also the functional reserve can be studied by the challenge tests.

The dynamic functional studies, the epitome of the present day nuclear medicine, can be done with simple instruments also. The very first study in nuclear medicine, where circulation time was measured was done with ^{214}Bi and a cloud chamber. Thyroid uptake studies when done serially are dynamic studies, because they provide quantitative information in temporal terms. RBC survival is a dynamic study although spread out over a number of days. Radioimmunoassays done serially in the same patient also provide dynamic information.

Instrument has never been our concern. Cloud chamber, single probe, scanner, oesophageal probe. In nuclear medicine,

"we change oars, but we do not change boats."

CT and MRI are instrument based specialities. They are like escalators. It can take you that far and no further. We are not moving on the escalator. We are climbing mountains making our own way, finding our own radiopharmaceuticals and devising new instruments to suit our radiopharmaceuticals. Sometimes we move, sometimes the road moves, but we are constantly in motion.

What is there in future?

"A worm while exploring the dust never knows that there are stars in the sky."

Several new radiopharmaceuticals have become available in recently, e.g. new hepatobiliary agents, new cerebral perfusion agents, new Tc based myocardial perfusion agents, monoclonal antibodies labelled with Indium-111 - it is an unending list. Cyclotron is adding a totally new breed of radionuclides.

The Ace in our hands is, of course, the non-imaging part of the nuclear medicine, which was so far neglected and relegated to the background, but which had a marked upswing and recognition because of the Nobel award to the discoverer of the principle of the Radioimmunoassays. The achievements of the Radioimmunoassays is a glorious chapter of nuclear medicine, where without administration of any radioactivity to the patient a large number of biologically important substances can be estimated in the circulating blood.

This is what Rosalyn Yalow has to say on the future of RIA: "Those promoting

non-nuclear tests are not necessarily suggesting that these have any technical advantage, but rather that regulatory procedures and the fear of radiation at any level would make the radioisotopic label less desirable. Nonetheless, radioimmunoassay is likely to remain the method of choice for measurement of the peptide hormones, since their concentrations are well below 10^{-10} M and the sensitivity of the RIA is essential" ?

RIA is likely to remain the method of choice for the research laboratory. The use of radioisotopic label has many advantages compared to the use of an enzyme marker. Generally, iodination is simpler than the preparation of an enzyme labelled substance, especially since there has been no agreement as to which enzyme is best for substances as small as steroids or as large as viruses. In addition, there may be some change in the configuration of the enzyme or the substance to be labelled during the conjugation procedure.

Monoclonal antibodies can provide virtually unlimited amounts of homogenous antibodies against a specific antigenic site. The heterogeneous antibodies are more likely to provide more sensitive assays than the monoclonal antibodies, although assays employing the latter are likely to be more specific. The optimal choice of the antiserum may depend on whether sensitivity or specificity is required for the assays.

RIA is now used in thousands of laboratories around the world to measure hundreds of substances of biological interest. Even now, a quarter century after the introduction of RIA, there remains many additional fields that can be explored with its help.

We are not reaching a limit, we are finding that we are limitless. The rumours of the death of nuclear medicine are somewhat exaggerated. The curtain is not being brought down, it is being raised. Don't send your ocean into my pond to drown my little boat. We do not want MRI into NM.

When radionuclides were introduced first in medicine, they were heralded as a discovery as great as a microscope, a tracer technique which can be turned inward to find out what is happening inside the body. There are so many research problems of relevance to the developing countries, which can be resolved by application of radioisotopes as research tools. For example, in vitro assays can be applied for the diagnosis of the communicable diseases, absorption studies can be useful in problems related to nutrition, even myocardial ejection fraction can be studied by a single probe. Depending on the interests of the clinical group and depending on what instruments are available, a variety of clinical research problems can be tackled in the developing countries.

"In the autumn of my life, I am still announcing new dawns.
The same sun is newly born in new lands in a ring of endless dawns."

SUGGESTED READING.

[1] Radioimmunoassay - a historical perspective by Rosalyn Yalow; Journal of clinical immunoassay, Vol. 10, No. 1 (1987) 13.

FUTURE OF NUCLEAR MEDICINE