IANCAS BULLETIN

RADIOPHARMACEUTICALS
&
NUCLEAR MEDICINE

A Publication of

INDIAN ASSOCIATION OF NUCLEAR CHEMISTS AND
ALLIED SCIENTISTS

Editorial

One of the important non-power applications of nuclear energy is the use of radioisotopes in medicine. This discipline is known as nuclear medicine. It utilises the properties of radionuclides as tracers and the advantages are manifold. Radionuclides in minute quantity do not perturb the physiological processes and offer high detection sensitivity. Also, suitable chemical manipulation enhances their organ specificity. These advantages make possible, in vitro assay of trace level hormones, drugs etc. as well as in vivo monitoring of biochemical reactions. Today, innovations in radiopharmaceuticals, clinical trials and technological developments in instrumentation have together made it one of the finest non-invasive techniques to study a number of disorders in human body. In this issue, we have attempted to provide a comprehensive information on various aspects of this discipline.

I put on record my sincere thanks to Dr. N. Ramamoorthy, who is the guest editor for this issue. Also, I thank all the authors who constitute a spectrum of specialists from reputed organisations.

Our objective is to present a quality bulletin on a variety of interesting areas in science and technology. To maintain high quality and regularity, we need your active support. This support can come in the form of articles as well as comments. The latter is very important because your feedback will go a long way in helping us achieve our objective. Our dream is to see the metamorphosis of the bulletin into a scientific journal of high standard. Today's dream can be made a reality tomorrow!

P.K. Pujari
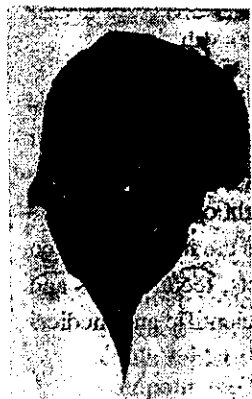
CONTENTS

Radioimmunoassay and Related Techniques

Dr. M.R.A. Pillai is presently the Head, Radiopharmaceuticals Section, Isotope Division, BARC. Graduating from the BARC Training School in 1976, he obtained his M.Sc and Ph.D degree from the Bombay University. One of the early researchers in RIA in India, he is responsible for the introduction of many RIA kits in this country. By organizing the first training course on Radioimmunoassay and by continuing as a faculty member, he has contributed immensely to the popularization of this technique in India. He has worked as a Visiting Professor at the University of Missouri-Columbia, USA and was a technical co-operation expert for IAEA. He is a recognised guide for M.Sc and Ph.D of the Bombay University and has co-authored two books, chapters in several books and has a number of publications to his credit. At present he is the treasurer of IANCAS.

Introduction

One afternoon in 1977 the author was sitting in the ante-room of a well-known endocrinologist of a leading hospital in Bombay and watching him examining his patients. One of the physical examinations done by him was to ask the patients to stretch their hands and fingers and wait for a while. The doctor later informed that he was diagnosing hyperthyroidism, a disease caused by the excessive secretion of the thyroid hormones by an overactive thyroid gland. A hyper thyroid patient's fingers tremble when kept stretched. A simple test indeed for the diagnosis of a complicated endocrine disorder. The major problem, however, was that the fingers tremble due to several other reasons unconnected with hyperthyroidism leading to a number of false positives. But as of then there was no biochemical investigation available to the doctor for measuring the precise concentration of the thyroid hormones in a blood sample, a more sure test to detect the disease. Hence, the diagnosis was mainly based on physical symptoms such as the one described above not only for thyroid disorders but also for most of the other endocrine diseases. However, the diagnosis of the endocrine disorders in India took a dramatic turn at the end of the seventies, thanks to the availability of ready to use radioimmunoassay (RIA) kits from the Bhabha Atomic Research Centre.

The RIA technique, which was first described by Rosalyn Yalow and Solomon Berson in 1960, has now become an extremely popular technique for the measurement of biologically important substances present in nanomolar and picomolar levels in body fluids. Ready to use RIA kits for almost all the hormones, a variety of vitamins, drugs, viruses etc. are now available from commercial suppliers and RIA investigations are an inevitable part of the modern day clinical diagnosis. This article is aimed at giving an idea about the principle of RIA and many related assays which are commonly referred to as immunoassays.

What are immunoassays?

Immunoassays are assays in which the specific reaction of an **antigen** with an **antibody** is used either for the detection or for the quantification of the antigen.

Before getting into more details, let us see what is an antigen and an antibody. The antigen is referred to as a substance which can react with its antibody. The antigen can be a simple molecule with a molecular weight of a few hundred Daltons to a complicated protein molecule with molecular weight of over a million Dalton. The antigen can thus be a small steroid or thyroid hormone or can also be a macromolecule such as a virus or a bacteria. Since, our intention is to measure the concentration of the antigen, the antigen is the analyte. Antibodies are biomolecules synthesized within a living system in order to nullify the effect of a foreign attacking substance. For example, if a living system encounters a new virus capable of causing disease, it will try to resist the virus. One of the mechanisms of resistance is by activation of the immune system to synthesize antibodies. These antibodies bind the antigen, in this

case the virus, to form an antigen-antibody (AgAb) complex. Once bound to the antibody, the virus is no more active and it is metabolized and excreted from the body. The reaction of the antigen with the antibody is highly specific ie. an antibody produced against a particular antigen will normally react only with that antigen. This is often compared to a 'lock and key' system. In addition, the reaction between the antigen and the antibody has a very high energy, the equilibrium constant K of these reactions varies from 10^9 - 10^{12} L/M. The antigen-antibody reaction has been harnessed to develop a series of highly specific and sensitive assays known as **immunoassays**. Some of these assays are capable of measuring analytes in nanomoles (10^{-9}) to femtomoles (10^{-15}) concentration in the presence of closely related analogues of the analyte.

Immunoassays based on precipitation

The use of antibody as a reagent for the detection of various antigens is more than a 100 year old technique. If a large concentration of the antigen is reacted with a similar amount of antibody it will result in the formation of a precipitate. Based on this, the Immunoprecipitation technique for the detection of the presence of analytes was developed. The test for identification of blood group is based on this principle. Further modification of the precipitation technique led to other immunoassays like immunodiffusion technique (diffusion of the antigen and antibody in a gel medium), electro-immunodiffussion technique (immunodiffusion under the influence of an electric field) etc. Various detection techniques like nephlometry, turbidimetry etc. are used to quantitate the antigen-antibody complex formed. Many of the above assays are widely practised even now. However, these assays suffer due to poor sensitivity.

Use of radioactivity in immunoassays

Genesis

In the fifties Dr.Rosalyn Yalow a Ph.D in nuclear physics started a collaborative research with a medical doctor, Dr.Solomon Berson. They were interested to study the invivo metabolism of the hormone insulin in diabetic and non-diabetic patients. Being a nuclear physicist, it was Yalow's idea that insulin labeled with ^{131}I could be used for

this purpose. They expected non-diabetic patients to show a slower metabolism of insulin compared to diabetic patients. To their surprise, the results were reverse. They observed that diabetic patients who were receiving insulin injections showed maximum retention of the labeled insulin. Through a very careful invitro analysis of the blood collected from these patients, they found that in insulin treated diabetic patients, the labeled insulin was bound to a macromolecule and hence retained longer in the body. The macromolecule binding the labeled insulin was later identified to be an antibody. They reported the findings of this study ie. presence of insulin antibody in insulin treated diabetic patients to a leading journal. The peer review of the paper resulted in its prompt rejection. As per the understanding of that time, a small molecule like insulin cannot produce antibody. Unmoved by the rejection, they went on and demonstrated that not only insulin can produce antibodies, but these antibodies can be used for the quantification of insulin in serum samples, thus giving birth to the radioimmunoassay technique. The Nobel prize for medicine was awarded to Yalow in 1977 for the invention of the radioimmunoassay technique. (As Nobel prizes are never awarded posthumously Solomon Berson was not a recipient.)

Principle of Radioimmunoassay

Radioimmunoassay is a denovo technique, its principle varies from all the known analytical techniques. In a conventional assay, in order to measure an analyte, it is reacted with an excess concentration of a reagent to form a product. The amount of product formed is a direct measure of the analyte. For example, to estimate Ba^{++}, an excess of H_2SO_4 is added and from the amount of $BaSO_4$ formed, the concentration of Ba^{++} is calculated. Two important criteria in all the analytical techniques are: (a) an excess of reagent is used and (b) the product formed is measured. RIA differs markedly in that both the above criteria are violated. RIA is a 'limited reagent' assay. The reagent, antibody, is used in much lower concentration than that of the analyte. The assay is based on a competitive reaction as shown below.

$$Ag^{*} + Ab \rightleftharpoons Ag^{*}Ab$$

$$Ag + Ab \rightleftharpoons AgAb$$
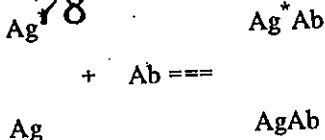
The analyte or antigen (Ag) and a radioactively labeled antigen (Ag^{*}) compete for a limited and fixed amount of antibody. Both Ag^{*} and Ab are taken in fixed and limited concentration, and the concentration of Ag is varied. It can be visualized that, in the absence of Ag, all the Ab will be used up by the Ag^{*} to form the complex $Ag^{*}Ab$. As we increase the concentration of Ag, a part of the antibody will be used by the Ag to form the complex AgAb and correspondingly the $Ag^{*}Ab$ concentration will decrease. The amount of $Ag^{*}Ab$ formed and hence the amount of radioactivity associated with the complex is inversely related to the concentration of Ag. By using known amounts of Ag as standards, we can set up a standard curve. Concentration of an unknown sample can be measured from this standard curve (Fig.1).
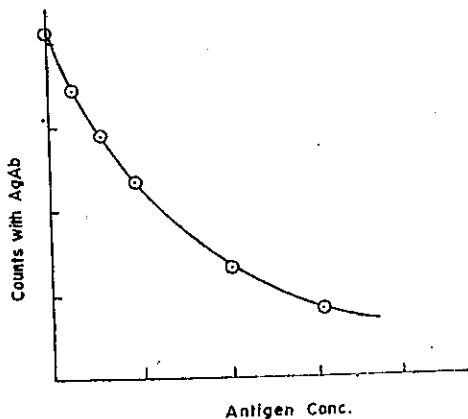

Fig. 1   A Radioimmunoassay Standard Curve

Advantages of RIA

RIA offers several advantages. The first and foremost is its remarkable sensitivity. Very small quantity (less than one tenth of a mL) of the sample is needed for measurement. Analysis by RIA does not require any sample preparation or prior extraction of the analyte. Samples can be sent from remote places to RIA laboratories in the cities for analysis and hence patient movement is not necessary. Being an invitro technique no radioactivity enters the patients body.

Requirements for a radioimmunoassay

Radioimmunoassay is a multicomponent system. It needs an antibody (the reagent), a labeled antigen (Ag^{*}) and an unlabeled antigen (Ag) of known concentrations (standards). Also needed is a separation system to separate the antigen-antibody complex from the free antigen. A brief description of the above reagents is given below.

Antibody (Ab)

Antibody is the key reagent in radioimmunoassay and the quality of the assay will depend upon the antibody used. The antibody molecules come in a broad category known as immunoglobulins. They have molecular weight of nearly 150,000 Daltons. Antibodies are prepared by injecting the antigens to laboratory animals. The animals preferred are rabbits, guinea pigs, goats, sheep etc. The antigen against which antibodies are to be produced are emulsified with a mineral oil and injected to the animal. Secondary injections or booster doses are given after three weeks to one month intervals. Antibodies of good quality are often produced after three to four such injections. The blood of the immunized rabbits are collected and screened for antibody response. The antibodies are present in the serum, a white yellow liquid left after removal of red blood cells from the blood sample. The serum is assayed for the antibody content and its quality.

The antibodies prepared are also checked for its specificity. This is done by reacting the antibody with antigens having similar structures. For example, if we have prepared an antibody for progesterone, its reactivity with most other steroid hormones will be checked. Strictly speaking, to be usable in RIA a progesterone antibody should react only with progesterone and not with any other steroids. Antibodies showing cross reactivity will over-estimate the analyte concentration.

Labeled antigen (Ag)*

Labeled antigen is also called tracer. This is prepared by introducing a radioactive isotope to the antigen. The isotope most commonly used in

radioimmunoassay is ^{125}I which offers several advantages. ^{125}I can be obtained with very high specific activity and with almost 100% isotopic abundance. It has a convenient half life of 60 days and hence the tracer will have a long shelf life. Iodine can be easily introduced into many molecules. ^{125}I decays by electron capture emitting a low energy gamma ray (35 keV) and X-rays. The combined emission of low energy photons makes ^{125}I an isotope which can be counted with very high efficiency in a small solid scintillation counter.

Standards

The radioimmunoassay is a comparative method. The response of the unknown is compared to that of a set of standards of known concentration. The standards are prepared by diluting a pure preparation of the analyte. Since most of the analytes of interest are present in nanomole to picomole level, a very high dilution is necessary. The standards are prepared in a serum matrix to make them identical to the unknown samples.

Separation system

The end point in RIA is the separation of the antigen-antibody (AgAb) complex from the free antigen. There exists a large difference between the above two molecules. The antibody being a macromolecule, the complex (AgAb) significantly differs from the free antigen (Ag). Hence a variety of physicochemical methods can be employed for the separation of the two. The most widely used method is precipitation of AgAb by using a reagent like polyethylene glycol. New innovations in the separation techniques have made RIA simple to perform. One such method is the solid phase separation technique. In the solid phase assay, the antibody is coated ie. physically or chemically attached to the sides of the reaction test tube to which the standard or unlabeled antigen and the tracer is added. At the end of the reaction, the reaction solution is decanted leaving behind the AgAb complex intact in the test tube, which is assayed for radioactivity.

RIA kits

All the reagents needed for performing RIA are supplied in the form of a kit by several manufacturers. These kits also include a few quality control samples to monitor the performance of each assay. A 100 tube RIA kit will be sufficient for setting up a standard curve and for the analysis of at least 35 samples in duplicate, along with a few quality control samples. The assays using RIA kits are simple to perform and often don't take more than 5-6 hours per assay.

### Further Developments in the Immunoassay technology

The radioimmunoassay technique as originally developed by Yalow and Berson has been modified by various researchers. Some of these assays are capable of giving higher sensitivity than the RIA technique. Some of these assays are discussed below.

Immunoradiometric assay (IRMA)

About eight years after the introduction of the RIA technique, Miles and Hales proposed an alternative technique called the 'immunoradiometric assay' capable of giving higher sensitivity than RIA. IRMA uses an excess concentration of labeled antibody (Ab*) as the marker instead of labeled antigen (Ag*) used in RIA. Hence IRMA is an 'excess reagent' technique unlike RIA which is a 'limited reagent' assay.

Principle of the IRMA technique

In IRMA, as originally proposed an excess labeled antibody (Ab*) is allowed to react with the analyte (Ag) either from the standard or from the sample.

$$Ag + Ab^* \rightarrow AgAb^* + Ab^*$$
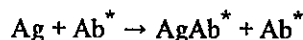
At the end of the reaction, the antigen bound (AgAb*) and the free antibody (Ab*) are separated. The antigen-bound antibody fraction is assayed for radioactivity. The activity associated with this fraction is directly proportional to the concentration of the antigen. The concentration of the unknown can be read from a standard curve (Fig 2).

The immunoradiometric assay as originally proposed didn't catch up mainly for two reasons. The first and foremost was the difficulty in the preparation of ^{125}I labeled specific antibody. The antibody available at that time was polyclonal in nature, hence it contained a number of other molecules other than the specific antibody
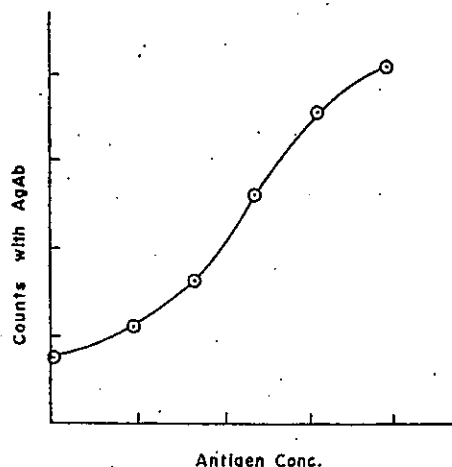
Fig. 2   An IRMA Standard Curve

Fig. 3   Illustration of the Principle of 'Two Site IRMA'

molecules. It was difficult to separate the specific antibody from other non specific molecules. The non availability of a suitable separation system was the second major problem. In the case of RIA, there exists a large difference between the properties of the free antigen and the antibody- bound-antigen. Hence the antibody bound fraction in RIA can be precipitated in preference to the free fraction. However, in IRMA there exists very little difference between the free antibody and antigen-bound-antibody, so that none of the above technique could be used for separation of the two fractions.

The above two problems were solved by two independent developments, viz. the introduction of the 'hybridoma technique' by Kohler and Milstein and the 'two site IRMA' technique. The hybridoma technique is used for the preparation of monoclonal antibodies with high affinity and purity. These antibodies are extensively used for the development of IRMAs.

'Two Site IRMA'

'Two site IRMA' uses two antibodies specific to the same antigen, out of which one is presented as solid phase. Principle of 'two site IRMA' is illustrated in Fig.3. Excess concentration of an antibody (Ab₁) is coated to the sides of the assay tubes. The standard or sample is added to this solid phase antibody, resulting in a reaction between the solid phase antibody and the analyte. As there is an
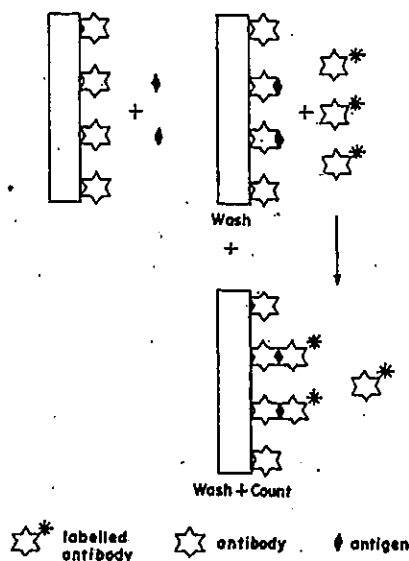
excess of solid phase antibody compared to the antigen, all the antigen reacts with the solid phase. This is then followed by the addition of a radioiodinated monoclonal antibody (Ab_2^*). The labeled antibody binds only to the antigen, which is already extracted by the first antibody. After reaction the unreacted labeled antibody is removed by decanting the solution. The solid phase containing $Ab_1AgAb_2^*$ is assayed for radioactivity. The amount of radioactivity associated with the solid phase fraction is directly proportional to the concentration of the analyte.

$$\text{Solid phase-}Ab_1 + Ag \rightarrow \text{Solid phase-}Ab_1Ag$$
$$+$$
$$\text{Excess }Ab_2^*$$
$$\downarrow$$
$$\text{Solid phase-}Ab_1AgAb_2^* + \text{Free }Ab_2^*$$
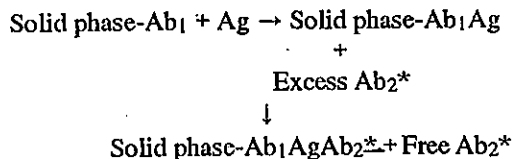
Advantages of IRMA

Being an excess reagent technique, IRMA offers higher sensitivity than RIA. The use of two antibodies make the 'two site IRMA' more specific

than the RIA technique. The IRMA being an excess reagent technique, the reaction is driven to equilibrium much faster than in RIA. This gives a significant advantage in that, the IRMA technique is much faster than a corresponding RIA technique.

Non-Radioisotopic Immunoassays

Both RIA and IRMA uses ^{125}I for labeling the antigen or the antibody. While radioisotopes offer several advantages as a tracer, several alternative labels other than radioisotopes have been suggested for immunoassays. From among all the alternative labels suggested, immunoassays with enzymes, chemiluminescent chemicals and fluorospores are becoming popular and hence these techniques are discussed briefly.

Enzymeimmunoassays

In enzyme immunoassays, the enzyme is tagged to the antigen or the antibody. The entire procedure remains the same like RIA or IRMA. However, the final quantification is done by estimating the enzyme activity in the complex. The enzyme is allowed to react with a substrate to give a colored product, which is quantified by a spectrophotometer. In this reaction the enzyme merely acts as a catalyst. Though the color measurement by itself is insensitive, the catalytic property of the enzyme makes the enzyme labels as sensitive as radioisotopic labels.

The enzyme-substrate systems most commonly used in EIAs are horseradish peroxidase along with H_2O_2 and o-phenylene diamine (OPD) as the substrate; or alkaline phosphatase with p-nitrophenyl phosphate (pNPP) as the substrate. The final color formed is measured at a suitable wavelength where the product has the maximum absorbance.

As in the case of RIA and IRMA, the enzyme can be used for labeling either the antigen or antibody. Hence we have both **enzymeimmuno-assay (EIA)** and **immunoenzymatic assay (IEMA)**, comparable to RIA and IRMA. Most of the enzymeimmunoassays use a solid phase bound antibody; hence the generic name **enzyme linked immunosorbent assay (ELISA)** is used for both antigen-labeled and antibody-labeled enzyme immunoassays.

Chemiluminescence Assays

Chemiluminescence is the phenomenon of emission of light photons when a chemical reaction occurs. In such chemical reactions the product formed is in an excited state and comes to the ground state with the emission of a photon. A molecule capable of undergoing a chemiluminescence reaction is used as the label in chemiluminescence immunoassays. One of the common luminescent systems used is the acridinium ester which in the presence of H_2O_2 gets converted to a vibronically excited molecule. The latter gets deexcited with the emission of a photon.

Chemiluminescent labels are capable of giving assays with higher sensitivity than the isotopic labels. In an isotopic label, only a very small portion of the label decays within a given time. A simple calculation using the decay equation shows that 10^7 atoms of ^{125}I are needed to obtain one count per second, whereas a chemiluminescent label is theoretically capable of giving one photon per label during the chemiluminescence reaction.

In practice, the sensitivity of a chemiluminescence immunoassay depends upon the quantum yield (i.e. the number of photons emitted per mol of the luminescent label during its reaction) and upon the efficiency of detection of these photons. The measurement of luminescence is done by using luminometers specially designed for commercial luminescent immunoassays.

Fluoroimmunoassays

Fluorescence is the phenomenon in which a molecule absorbs photons of a higher energy (shorter wavelength) and emit photons of lower energy (longer wavelength). The difference between the wavelengths of the absorbed and emitted radiations is called 'Stokes shift'. The quantum yield in fluorescence is the ratio of the emitted photons to the absorbed photons. In fluoroimmunoassay a fluorescent probe is used to label the antigen or antibody. For a fluorescent probe to be suitable as a label for immunoassay, both Stokes shift and the quantum yield should be high.

Fluorescence, unlike chemiluminescence, is induced by excitation of the molecules by an external source. The fluorescence life time is generally not

more than a millisecond and hence the same molecule can get excited and deexcited repeatedly. This in turn tells us that a single molecule of the label is capable of emitting several photons as against one photon per chemiluminescent label or one photon per 10^7 labels in the case of the radioisotopic label ^{125}I. The capability of the same label or molecule to emit several signals makes the fluoroimmunoassays extremely sensitive compared to isotopic and even chemiluminescence immunoassays.

Some of the labels used in the initial development of fluoroimmunoassays were fluorescein isothiocyanate and dansyl chloride. However, the assays with these probes suffered from high background fluorescence as many of the constituents of serum and plastic tubes used in the assay are also capable of giving fluorescent radiation. This problem is now fully solved by the use of rare earth chelates and by developing a technique called 'time resolved fluoroimmuno-assay'. This assay utilizes the difference in the decay time between the fluorescence from the label and that of the background. This is analogous to trying to count a radioisotope in a mixture of two. If the half life of one of the isotopes is very short compared to the other, one can allow sufficient time for the complete decay of the short lived isotope and then quantitate the other. The typical decay time of the background fluorescence is about 10 ns, but chelates of rare earth metals like europium and terbium have fluorescent decay time varying from microseconds to milliseconds. In time resolved fluoroimmuno-assay, fluorescence is induced by a pulsed radiation of a very short duration. After excitation, all the fluorescent molecules start decaying, but the background fluorescence from the serum constituents decays much faster than that of the specific fluorescent probe. The detection system, a suitable photon counter, is set up in such a way that it starts counting only after a time gap after excitation with a pulsed radiation, so that only the desired fluorescence is counted (Fig. 4).

The time resolved fluoroimmunoassays based on labeled antibodies (immunofluorimetric assays) are becoming a very powerful tool for the development of extremely sensitive assays capable of measuring femtomoles (10^{-15} moles) of analytes.
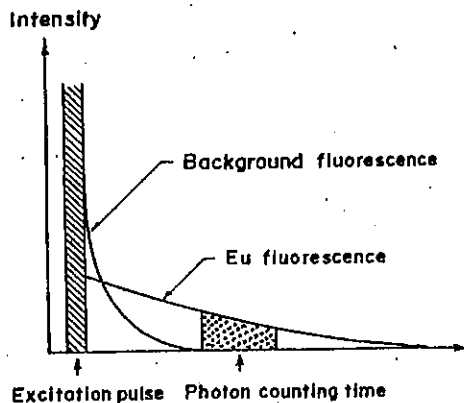

Fig. 4   Principle of fluorescence Counting in 'Time Delayed Fluorescence Immuno- assay'

Applications

The advent of radioimmunoassay had a revolutionary effect on clinical medicine. RIAs have been developed for the measurement of hundreds of hormones, vitamins, drugs, viruses, enzymes etc. present in human body. RIA has made endocrinology one of the hottest areas of medical research. Thanks to RIA, major advances in the diagnosis and treatment of the body's major hormone systems, including thyroid function, growth and fertility were achieved.

By using RIA, physicians could separate diabetics into two groups. Those who typically lose the ability to make insulin during childhood and those who produce insulin but during adulthood lose the ability to use it. The treatment differs in the two cases. The first group needs insulin injections, whereas in the second group diet control or medication will be sufficient.

Before RIA, little was known about human growth hormone. Essential for bone development and growth, it is secreted by the pituitary gland to stimulate the liver to produce growth factors. Dwarfism in children can be caused by insufficient growth hormone or by a variety of other factors. The former could be detected by RIA and rectified by injection of the hormone. RIA of growth hormone is also useful to find out whether abnormal bone growth is caused by over secretion of growth hormone.

Perhaps the most important application of RIA is in the management of thyroid disorders. Estimation of the thyroid hormones triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) is used for the differential diagnosis of thyroid disorders. In addition, RIA is used for screening new born babies with under active thyroid glands. The symptoms of hypothyroidism are undetectable until a baby is more than three months old. By then brain damage would have occurred. Estimation of the thyroid stimulating hormone in a few drops of blood collected on a filter paper by pricking the heel of a baby can diagnose neonatal hypothyroidism in time to prevent irreversible brain damage.

RIA has helped in the better understanding and solving problems associated with child bearing and infertility. Estimation of human chorionic gonadotrophin (hCG) is used for the early detection of pregnancy. RIA of luteinizing hormone (LH) and follicle stimulating hormone (FSH) is used for identifying the causes of infertility. Estimation of estradiol and progesterone is used for finding out the best time for artificial insemination.

From heroin in drug abusers to steroids in athletes and antibiotics in patients, RIA can pinpoint the concentration of drugs in blood, urine and saliva. RIA is used in pharmacology for the estimation of bioavailability of drug formulations. In forensic sciences, RIA is used for investigating deaths due to intentional overdosing of drugs. RIA is used for screening donors blood for hepatitis-B antigen, a virus capable of causing liver infections.

The immunoassay techniques have contributed in a big way in the detection and management of cancer. RIA is used for the measurement of a number of molecules secreted or associated with cancer, which are called 'tumor markers'. They are released into the circulatory system where their presence or concentration may indicate the existence of the tumor. RIA of tumor markers serve as valuable tools for monitoring the course of therapy and the detection of recurrence.

The applications of RIA and other related techniques are increasing manifold and the higher sensitivity offered by the fluoroimmunoassay technique should bring out many more new applications in the future.

Conclusion

It is often said that great discoveries are made by accidents and the same is attributed to the RIA technique also. The detection of the presence of antibodies in diabetic patients by using labeled insulin was an accident. The inverse, ie. the idea to use antibody to measure insulin was by no means an accident, but the ingenuity of two great individuals, Sol and Ros as they were known to their friends. Coming from two unconnected branches of science, together they created a new technique which has now become an inevitable part of the modern day clinical medicine thereby helping in the well being of millions of needy patients the world over.

Suggestions for further reading

1. 'Measurement of Hormones- Radioimmunoassay' by Solomon Berson and Rosalyn Yalow, North Holland Publishing Company, Amsterdam.

2. 'Alternative Immunoassays'. Ed: W.P.Collins, 1985. John Wiley and Sons, New York.

3. 'Immunoassays, An Introduction' by Ray Edwards, 1985, William Heinemann Medical Books, London.

4. 'An Introduction to Radioimmunoassay and Related Techniques' by T.Chard, 4th edition, 1990, Elsevier, Amsterdam

5. 'Nobel Prize Women in Science' by S.B.McGrayne, Carol Publishing group, New York, 1993.

6. 'Radioimmunoassay, Principles and Practice' by M.R.A.Pillai and S.D.Bhandarkar, 2nd edition, 1994, Bhabha Atomic Research Centre, Bombay.

Radiopharmaceuticals

Dr. N. Ramamoorthy is presently the Senior Manager, Radiopharmaceuticals Programme of BRIT. He is a graduate of the 15th Batch of BARC's Training School and obtained his Ph.D. from Bombay University. He has made major contributions to the development and production of a number of radiopharmaceutical products, especially Tc-99m compounds and cyclotron based products. He has participated in a number of IAEA's Coordinated Research Projects, worked as technical expert of IAEA, served as invited faculty in several national and international training courses and workshops and was a Visiting Assistant Professor at University of Missouri-Columbia. He is a recognised teacher of University of Bombay for DMRIT Course. Also, he has been an active member and office bearer of IANCAS and Society of Nuclear Medicine (India).

Introduction

The applications of radioisotopes for medical purposes constitute one of the earliest and important developments in nuclear science. Formulations of radioisotopes administered to humans for diagnosis or therapy were generally called special medical products for a long time and in mid-sixties, the term radiopharmaceutical was introduced. Radiopharmaceuticals can be described as a special class of radiochemical formulations of high purity and safety, suitable for administration to humans orally or intravenously, to carry out organ investigations in-vivo by studying its localisation, uptake, excretion and distribution; or in certain cases to bring about a therapeutic effect. The elegance of the concept of radiotracers cannot be perhaps more amply demonstrated than through the use of diagnostic radiopharmaceuticals, while it is the utilisation of the radiation energy deposited that forms the basis for use in therapy. Nuclear reactors and particle accelerators like cyclotrons are the main sources of radionuclides used for medical purposes (Table-I). Reactor produced radioisotopes are more routinely used thanks to their easy, abundant availability at reasonable prices.

Evolution of Radiopharmaceutical Products and Applications

In the early days, radiotracer of an element of interest was used, perhaps without much specific reference to its nuclear characteristics, since only relatively a small amount of activity was administered and estimation of the gross activity in the organ/tissue of interest was the basis for investigation e.g. ^{24}NaCl, NH$_4$ ^{82}Br, ^{42}KCl Na$_2$ ^{51}CrO$_4$ and Na^{131}I. The type of investigations could be as simple as administering orally 10-25 μCi of ^{131}I as NaI in a capsule and monitoring the uptake in thyroid as a function of time with a NaI(Tl) detector and a spectrometer. One could also carry out a simple procedure like isotope dilution analysis for determining the blood volume by injecting a known aliquot of a non-diffusible tracer, and after allowing for adequate time for equilibration, withdraw a sample of blood and estimate the activity in it for comparison with the injected dose. Among these products Na^{131}I and Na$_2$ ^{51}CrO$_4$ are useful even today, the former for thyroid uptake studies & investigations of certain thyroid disorders like organification defect and the latter for studies on RBC. The clinical utility of other such products has been rather limited.

Rather than determining the gross concentration, the radiotracer could be better utilised by mapping its distribution over an organ/regions (scintigraphy). The evolution of the rectilinear scanner with organ specific products has enabled scans of the organs to be obtained e.g. ^{131}I-Rose Bengal, ^{198}Au colloid for liver and $^{197/203}$Hg-chlormerodrin (Neohydrin) for kidney. Rectilinear scanning procedure, however, takes relatively long time for completion of a scan and is also incompatible with the dynamic nature of physiologic processes.

The introduction of Gamma Camera which enables simultaneous mapping of the radiotracer distribution over large area (30-40 cm), covering one or more organs, was a major turning point in the development and utilisation of radiopharmaceuticals. The compatible radiotracer should emit 100-200 keV gammas in high abundance (since 6-10mm thick NaI(Tl) crystal is used in cameras) apart from having other requisites like short half-life and absence of particulate emission. ^{99m}Tc discussed later in this article proved to be an ideal match and led to high resolution images of organs (spatial resolution) for clinical diagnosis. Various ^{99m}Tc products specific for different organs have been developed. The addition of computer technology to the gamma camera has been a crowning point in that it made possible the dynamic function imaging procedures, i.e. mapping the changes in the tracer concentration over region(s) of interest (ROI) as a function of time (temporal resolution). This is a clear distinguishing feature of nuclear medicine from other imaging modalities. Also the quantitation aspect would not have been feasible but for the computer aid. The advent of computerised tomography (CT) led to the development of Single Photon Emission Computed Tomography (SPECT) which uses a rotating head gamma camera or an array of these cameras.

Another important development based on positron emitters, is Positron Emission Tomography (PET). This system consists of many detector rings, each containing arrays of bismuth germanate (BGO) scintillation crystals for coincidence detection of the positron annihilation photons and an advanced computer system for image reconstruction.

The well-known relation between iodine and thyroid physiology enabled the early realisation of treatment of certain thyroid disorders using Na^{131}I. Therapeutic products, formulations containing particulate radiation emitters, would require very high specificity for target tissue, e.g. iodine for thyroid cells and phosphates for bone/bonemarrow.

Basic Concepts and Product Features

Radiopharmaceuticals can be classified into products used for imaging applications (static, dynamic, quantitative study), for non-imaging applications and for therapy. The products used for imaging can be grouped into 3 categories: reactor based, cyclotron based and generator based. Radiopharmaceuticals are marketed as finished products in ready-to-use form as well as those to be formulated at the hospital end in the radiopharmacy (generator based products). A list of important radionuclides used in radiopharmaceuticals, their radiation characteristics, product chemical forms and application(s) is given in Table I and II. The absorbed radiation dose to patients from most of the diagnostic radiopharmaceuticals administered in μCi to mCi levels is generally less than or at the most comparable to that in conventional radiological investigations e.g. chest X-ray.

The action of the radiopharmaceuticals is based on their chemical form which dictates the biological behaviour in-vivo.

a) Simple inorganic chemicals e.g. Na^{131}I, ^{133}Xe gas, Na^{99m}TcO$_4$

b) Labelled compounds, in turn divided into:
True/ isotopically labelled compounds e.g. Hippuran-^{131}I
Foreign/ non-isotopically labelled compounds e.g. HSA-^{131}I

c) Particulate formulations e.g. radiocolloids of ^{198}Au/^{99m}Tc

d) Coordinate complexes of radiometals e.g. ^{111}In/^{99m}Tc-DTPA

The important parameters for any product are: radionuclidic purity (a measure of the extent of the exclusive presence of the stated radionuclide), radiochemical purity (the direct index for the biological efficacy), chemical purity, pH, radioactive concentration, specific activity (the activity per unit mass), particle size distribution in the case of particulate formulations, biological efficacy (by bio-distribution tests in test animals like rats or mice) and pharmaceutical purity, i.e. lack of toxicity or any reactions and in the case of injectables, sterility and apyrogenicity.

Generally the shelf life of these products is dependent almost exclusively upon the half-life of the radionuclide used in the product and is always much shorter (hours to days) than in the case of conventional pharmaceuticals. Hence, in most of the cases it is not possible to carry out all the quality

control and assurance tests on all the products before releasing for use. This particularly applies to the biological controls, namely, time consuming tests for sterility and apyrogenicity. Physicochemical control tests are, however, always done in part or fully before the release/actual use of the products. In the case of the generator based products, controls are possible only on the generator (mostly one time check on a few important performance parameters) and the 'cold' (non-radioactive) kits at the producer's end, since the actual product for administration to patients will be formulated in the user's end only.

Basis of Action of Radiopharmaceuticals

Compounds linked to/ containing a suitable radionuclide can be tailored for concentration by a particular organ, or following a physiologic process, by exploiting a variety of mechanisms such as active transport (of iodide to thyroid, glucose or its analogues to brain), phagocytosis (of Tc-sulphur/Tc-tin colloid) for liver imaging, capillary blockade (of Tc-HSA macroaggregates) for lungs imaging, compartmental localisation (of Tc-RBC) for blood pool imaging etc. For disease specific products, factors such as vascularity of tumours, cell permeability (in tumour or infection sites), substances expressed in the lesions etc. dictate the course of the radiopharmaceuticals e.g. infection imaging with ^{111}In/^{99m}Tc-leucocytes (specific) and ^{67}Ga-citrate (non- specific). In general, the factors influencing the distribution of an intravenously administered tracer are: molecular weight, size, structure & charge, nature of functional groups, specific activity, lipid solubility, serum protein binding, blood flow to organ/lesion, stability at physiological pH and at large dilution, stereochemical aspects, similarity to endogenous substances etc.

One can identify two groups of products, one, where the biodistribution is substrate dependent, and the other, where the characteristics of the whole compound determine the biodistribution pattern. In the former group, the radiolabel is truly a marker, a beacon e.g. radiolabelled particulates (^{99m}Tc colloid), biologicals (*M-RBC/WBC, *M-proteins/peptides) and biochemical/pharmaceutical compounds (*I-fatty acids/phenylalkylamines). Coordinate complex compounds of radiometals like ^{99m}Tc and ^{111}In

belong to the second group, wherein suitably designed ligands are used to chelate the metal in an appropriate oxidation state. Some of the resultant coordinate complexes have shown desirable biological properties for medical use. Optimisation of biological properties is done by modifying the ligand backbone without affecting its main chelating environment. In order to tap the potential of radiolabelled biological compounds, the use of bifunctional chelating agents (BCA) has been developed. BCA contains both a chelating group for complexation with a radiometal and a second functional group for covalent coupling to the biologic compound of interest.

In most cases of the applications of radiopharmaceuticals, the normally functioning tissues take up the tracer and the defective non-functioning areas are consequently seen as cold spots. Examples of this type (organ specific products) are thyroid imaging with radioiodine, imaging of blood flow to myocardium with ^{201}Tl, liver imaging with radiocolloids of ^{99m}Tc etc. The converse is true in some cases i.e. where the tracer is taken up only by the defective tissues, but not by the normal one and thus the abnormal areas are detected as hot spots. Examples of this type (disease specific products) are imaging of infection with ^{111}In/^{99m}Tc-leucocytes, certain tumours with ^{67}Ga citrate and myocardial infarcts with ^{99m}Tc pyrophosphate. The images obtained non-invasively show not just the morphology (structure) - size, shape, location of the organ/ lesion, but provide vital functional information of organ/ metabolic pathway. Serial images obtained and processed yield information/ data on dynamic process(es) e.g. kidney function and cardiac function. At times, the data are augmented by interventional procedures using drugs, stress etc. The access to functional information of dynamic physiological processes is the hallmark of application of diagnostic radiopharmaceuticals. Since functional deterioration precedes any gross structural changes in the tissues, the applications of radiopharmaceuticals serve to ring "early alarm bells". Similarly, while monitoring the response to therapy, the recovery of organ function is earlier and reliably recognized using these products and thereby the efficacy of a certain treatment mode is determined much early. The applications of diagnostic products are: presence and

confirmation of a disease, staging of the disease, differential diagnosis, monitoring the response to therapy, detection of remnant disease/ recurrence and evaluation of a therapy regimen.

^{99m}Tc radiopharmaceuticals

^{99m}Tc occupies a pre-eminent position as the ideal tracer for in- vivo applications with over 80% of all diagnostic procedures being carried out using ^{99m}Tc products. The favourable nuclear properties of short half-life of 6 hours and decay by isomeric transition with only about 10% internal conversion result in low absorbed radiation dose with as high as 30mCi administered to patients. The high compatibility of the 140keV energy of ^{99m}Tc gammas with NaI(Tl) crystal thickness (6-10mm) of the gamma camera yields images/data of high quality. The easy availability of ^{99m}Tc at relatively low cost from a ^{99}Mo-^{99m}Tc generator and the versatile coordination chemistry of technetium (a second row transition metal) enabling the preparation of a variety of compounds are further merits providing the basis for an important area of medical diagnosis.

^{99}Mo-^{99m}Tc Generator

The kinetics of the radioactivity growth and decay in the ^{99}Mo(66h)-^{99m}Tc(6h) system result in the establishment of transient equilibrium. The time required for the daughter nuclide to attain the maximum activity (t_{max}) in this case is about 23 hours, enabling daily separations of ^{99m}Tc using the generator. The most common method in use for the radiochemical separation of ^{99m}Tc is chromatography over an acid alumina column loaded with fission produced ^{99}Mo of high specific activity.

Apart from the world-wide use of such commercially available column ^{99m}Tc generators, another technology adapted in countries like India compatible with ^{99}Mo of low specific activity (100-500mCi/g) obtainable from the local reactors has been based on the selective extraction of ^{99m}TcO$_4^-$ into methylethylketone (MEK) from an alkaline solution of sodium/ potassium molybdate - ^{99}Mo. The advantages and disadvantages of the two systems are obvious. The column chromatography generators are preferred due to user-friendly nature,

assured performance, pharmaceutical safety and product quality. The merits of the solvent extraction system are compatibility with ^{99}Mo of low to medium specific activity, very high radioactive concentration of ^{99m}Tc and adaptability to an individual hospital unit as well as to a centralised radiopharmacy. The disadvantages stem from the nature of the wet complex operations involved and the consequent need for well-trained and experienced personnel apart from appropriate infrastrucutural facilities at the-user's end.

Formulation of ^{99m}Tc Compounds - Role of Kits:

Sodium pertechnetate-^{99m}Tc obtained from the generator can be processed further using 'kits' - pre-mixed reagents - to form other useful technetium compounds. Kits contain the active ingredient, e.g. sodium phytate, methylenediphosphonic acid etc., stannous chloride as the reducing agent and at times preservatives. They are usually lyophilised to protect the stannous ions from hydrolysis and aerial oxidation. Upon addition of the generator produced pertechnetate in required volume to the kit vial and mixing, the technetium compound of interest is formed in high yield and purity. Today ^{99m}Tc products are applied in almost all nuclear medicine procedures and also new compounds are being added every year.

Cyclotron Based Products

The modes of decay of neutron deficient radionuclides, electron capture and positron emission, both provide good basis for use in medicine due to gammas accompanying the former and the annihilation photons of the latter. Particle accelerators have been an important source of radionuclides of the neutron deficient type in general and the short-lived positron emitters in particular. The cyclotron has remained the most common type of accelerator used for medical purposes including neutron therapy. The accelerator based products are technologically more difficult to produce in large quantities, prone to higher levels of radionuclidic contaminants, relatively more expensive and require automated processing facilities.

The range of products includes (Table-II) specific formulations of radioisotope compounds such as gallium citrate-^{67}Ga and thallous

chloride-^{201}Tl, gamma emitting versatile tracers like ^{111}In and ^{123}I used for preparation of many chemical formulations and labelled compounds, parent nuclides for radioisotope generator systems yielding gamma emitters (^{81m}Kr, ^{195m}Au) and positron emitters (^{62}Cu, ^{68}Ga, ^{82}Rb). Another important group of products obtained are short-lived positron emitting tracers (^{11}C, ^{13}N and ^{15}O) of the biological elements carbon, nitrogen and oxygen and their analog ^{18}F. Such products require the deployment of in-house medical cyclotron. ^{18}F and ^{11}C have the features for relatively wider applicability.

Being versatile tracers, ^{123}I (despite the high cost and constrained availability) and ^{111}In products are likely to dominate in future. The clinically proven products ^{67}Ga-citrate and ^{201}TlCl are, however, likely to be replaced by superior substitutes due to certain drawbacks in their nuclear and biological properties. Most of these products are available from commercial and other sources.

The successful harnessing of the merits of the positron decay by coincidence detection of the annihilation radiation with PET has made feasible quantitative studies of bio-chemistry in-vivo and also precise quantitation of the concentration of the radiopharmaceutical. While this has provided an exquisite modality for probing biochemistry in-vivo and in turn contributing to medical knowledge, the prohibitively high cost to be paid for availing this technology has confined the benefits to the developed nations. Slow entry into regular clinical practice in a few specific areas in developed countries has occured, e.g. glucose metabolism in brain, heart and tumours (with ^{18}F-fluorodeoxyglucose, FDG), blood flow (with ^{13}NH$_3$) and volume (with ^{11}CO), neuroreceptor involvement (^{11}C/^{18}F-spiperone, ^{11}C-carfentanil) etc. Another important development is the attempts at such studies with gamma emitter tracers (^{123}I, ^{111}In, ^{99m}Tc) and SPECT, that would help patients to avail these benefits in a relatively less expensive manner.

Radiopharmaceuticals for Therapy

There is a definite role of radiopharmaceuticals for therapy, wherein the utilisation of the radiation energy from the product deposited onto the target cells for their destruction forms the basis of action.

Therapeutic products are characterised by demands of a much higher radiochemical purity, in-vivo stability and specific activity. In addition to very high target specificity, other features required in this case are rapid transport and adequate concentration in target tissue, rapid and quantitative clearance from other tissues, appropriate radiation dosimetry aspects and availability of a large quantity of the radionuclide at affordable cost.

Treatment of hyperthyroidism by administration of 5mCi of Na^{131}I to suppress the hyperactivity of the thyroid gland is an efficacious procedure for patient management. In the case of patients of differentiated thyroid cancer, administration of 100-250mCi of Na^{131}I (in specially equipped wards in hospitals) after the thyroidectomy helps in selectively destroying the cancer cells at the metastatic sites.

Many patients of cancer, especially of prostate, breast and lung, develop extensive metastases in bones and suffer intractable bone pain. For those who do not respond to other forms of treatment, palliative treatment for suppression of bone pain has been successfully done in recent years using the bone seeking therapeutic agents, ^{89}SrCl$_2$, Na$_3$^{32}PO$_4$, ^{186}Re- EHDP and ^{153}Sm-EDTMP.

An emerging area of great importance for cancer detection and monitoring treatment is radio-immunoscintigraphy (RIS) based on the localisation of radiolabelled monoclonal antibodies specific to tumor associated antigens present at the tumor and metastatic sites. A promising mode of treatment is also evolving, called radioimmunotherapy (RIT) involving the administration of large doses of radiotoxic nuclide labelled (beta/alpha emitter) monoclonal antibodies raised against these tumor associated antigens for selective destruction of the cancer cells.

Manufacture and Utilisation of Radiopharmaceuticals

There are many international companies involved in the manufacture of radiopharmaceuticals in USA, Canada, Europe and Japan. Also national centres, under the atomic energy programme, produce these products in a number of countries. The basic philosophies are similar to the conventional pharmaceuticals in terms of applicability of Good

Manufacturing Practices (GMP) but allownaces are made for the radiation protection measures and occasional conflicts in the requirements for pharmaceutical and radiation safety aspects. In India, the Bhabha Atomic Research Centre (BARC) and since 1989 the Board of Radiation and Isotope Technology (BRIT) have been responsible for the development, regular production, quality assurance, regulation and distribution of these products.

In India work in this field began very early with the commissioning of APSARA reactor in 1956 and CIRUS reactor in 1960. The first planned radiopharmaceutical laboratories were built in the Radiological Laboratory (RLG) builiding and were in use for well over a decade till 1983. Since 1984, an exclusive facility called ISOPHARM has been in operation at the BARC Vashi Complex in New Bombay. Presently about 25 products are regularly processed and over 40,000 consignments are supplied annually to about 120 centres. 50 centres spread all over India provide diagnostic facilities using ^{99m}Tc products, while nearly all the centres provide other services including treatment of hyperthyroidism. Treatment for thyroid cancer can be availed from 10-12 centres which have set up the special facilities required. Radiation Medicine Centre of BARC located in the Tata Memorial Hospital provides comprehensive nuclear medicine services.

It is estimated that in India over 250,000 patient investigations (in-vivo) are carried out annually using these products, while over a million investigations using ^{99m}Tc products have been carried out during the last five years.

Conclusion

From the early days of primary radiochemicals like ^{24}NaCl, NH$_4$ ^{82}Br, Na ^{131}I etc. & radioiodine (^{131}I) labelled compounds to later generator produced ^{99m}Tc and cyclotron based products, from simple primary inorganic chemicals & radiolabelled particulate formulations to labelled organic compounds / biological molecules & coordinate complex compounds of radiometals, the radiopharmaceuticals branch has grown over the last 30 years into an exclusive speciality. This field is an outstanding example of the success of a multi-disciplinary approach in science. The chemists, radiochemists, biochemists and pharmacists take care of the product design, development and formulation, the physicists and instrumentation specialists contribute to the effective utilisation of the products with appropriate imaging devices, the radiation biologists handle the dosimetry aspects especially for therapeutic products, the doctors tackle the clinical end and so on. The early products used for eliciting information were of a wide nature: for understanding their behaviour, general curiosity and attempts to prove a certain hypothesis etc. It has given way to the products of regular clinical utility. The subsequent developments have brought forth unique exquisite applications which would not have been otherwise possible. Target specificity has been moving down from organs to cells to molecules. The future holds an unfathomable reservoir of potentials and promises - getting down to the molecular level of disease processes and consequently creating sophisticated approaches to therapy. Therapy which was the early aim of use of radioisotopes in medicine has thus been re-discovered to be a major trend of nuclear medicine in future.

Table I : Important Radionuclides - Characteristics and Method of Production

Radionuclide	Characteristics			Major method(s) of Production
	$T_{1/2}$	Decay mode	Energy (MeV)	
Reactor based products				
^{32}P	14.3 d	β^-	$E\beta^-$ 1.7	$^{32}S(n,p)$
^{51}Cr	27.8 d	EC	$E\gamma$ 0.32	$^{50}Cr(n,\gamma)$
^{89}Sr	50.5 d	β^-	$E\beta^-$ 1.5	$^{88}Sr(n,\gamma)$, $^{235}U(n,f)$
^{99}Mo	2.7 d	β^-	$E\beta^-$ 1.33	$^{98}Mo(n,\gamma)$, $^{235}U(n,f)$
^{99m}Tc	6 h	IT	$E\gamma$ 0.14	^{99}Mo-^{99m}Tc generator
^{131}I	8 d	β^-	$E\beta^-$ 0.6 $E\gamma$ 0.364	$^{130}Te(n,\gamma)$ $^{235}U(n,f)$
^{133}Xe	5.3 d	β^-	$E\gamma$ 0.081	$^{235}U(n,f)$
^{153}Sm	46.8 h	β^-	$E\beta^-$ 0.81 $E\gamma$ 0.103	$^{152}Sm(n,\gamma)$
^{186}Re	90.6 h	β^-	$E\beta^-$ 1.07 $E\gamma$ 0.137	^{185}Re (n,γ)
Cyclotron based products			$E\gamma$ (keV)	
^{57}Co	271 d	EC	122	Ni(p,x)
^{67}Ga	78 h	EC	93, 180	Zn(p,xn), $^{65}Cu(\alpha,2n)$
$^{81}Rb/^{81m}Kr$	4.6 h/13 s	EC	191	Kr(p,xn), ^{81}Rb-^{81m}Kr
^{111}In	67 h	EC	173, 247	Cd(p,xn); $^{109}Ag(\alpha,2n)$
^{123}I	13.3 h	EC	159	$^{124}Xe(p,2n)^{123}Cs \rightarrow ^{123}Xe \rightarrow$
^{201}Tl	73 h	EC	135, 167 Hg X-ray 69-80	^{203}Tl (p,3n)$^{201}Pb \rightarrow$
^{11}C	20 min	β^+	511	$^{14}N(p,\alpha)$
^{13}N	10 min	β^+	511	$^{16}O(p,\alpha)$
^{15}O	2 min	β^+	511	$^{14}N(d,n)$
^{18}F	110 min	β^+	511	$^{18}O(p,n)$ $^{20}Ne(d,\alpha)$

Table II - Important Radiopharmaceuticals and Applications

Products	Application(s)
^{32}P - Sodium phosphate	Bone pain palliation
^{51}Cr - Sodium chromate	RBC mass & survival studies
^{57}Co - Vitamin B$_{12}$	Differential diagnosis of anemia
^{133}Xe - gas / in saline	Brain & lung imaging
^{67}Ga citrate	Tumors & infection imaging
^{201}Tl chloride	Myocardial perfusion imaging
^{131}*I Products*	
Sodium iodide	Treatment of hyperthyroidism and thyroid cancer
Sodium iodide (capsule, low dose)	Thyroid uptake studies
Hippuran	Probe renography
MIBG	Neuroendocrine tumors imaging and therapy
^{123}*I Products*	
Hippuran	Renography
Iodophenyl fatty acid	Myocardial metabolism
MIBG	Tumors & myocardial imaging
Iodoamphetamine	Brain perfusion imaging
Monoclonal antibody	Tumors imaging
^{111}*In Products*	
In - leucocytes	Infection imaging
In - DTPA	Cisternography
In - Monoclonal antibody	Tumors & myocardial infarcts imaging
In - Peptides	Tumors & infection imaging

Table II (Contd)

Products	Application(s)
^{99m}Tc Products	
Tc - S colloid & Tc - Phytate	Liver imaging
Tc - Mebrofenin	Hepatobiliary function imaging
Tc - MDP	Bone imaging
Tc - Pertechnetate	Thyroid & brain imaging
Tc - DTPA & Tc-Glucoheptonate	Renal function and imaging
Tc(III) - DMSA	Renal cortical imaging
Tc - MAG3	Renal tubular function imaging
Tc(V) - DMSA	Medullary thyroid cancer imaging
Tc - RBC	Cardiac function studies
Tc - MIBI, Tc - Teboroxime & Tc - Tetrofosmin	Myocarial perfusion imaging
Tc - Human serum albumin aggregates/microspheres	Lung perfusion imaging
Tc - Aerosol / Technegas	Lung ventilation imaging
Tc - Exametazime (Tc- HMPAO) & Tc - Bicisate (Tc - ECD)	Brain perfusion imaging
Tc - Leucocytes	Infection imaging
Tc - Human Immunoglobulin	Inflammation imaging
Tc - Monoclonal antibody	Tumors imaging
PET Products	
^{11}C - Palmitate/Acetate	Myocardial metabolism
^{11}C - Amino acid	Oncology
^{11}C - Spiperone & others	Neuroreceptors
^{13}N - Ammonia	Blood flow
^{15}O - Oxygen	Oxygen metabolism
^{18}F - 2-Fluoro-2-deoxy glucose (FDG)	Glucose metabolism in brain, heart & tumors

Application of Radionuclides in Clinical Practice

Dr. A.K. Padhy is an Additional Professor in the Department of Nuclear Medicine of AIIMS, New Delhi. He is a renowned nuclear medicine physician of the country and his special interest is in the field of applications in nephrourology. He is a co-editor of two volumes on the role of nuclear medicine in nephrourology. He has also a number of publications to his credit. He has served as IAEA's technical expert and has also worked as a visiting scientist at St. Bartholomew's Hospital, London.

Nuclear Medicine is defined as a clinical speciality devoted to diagnostic, therapeutic and research applications of internally administered radionuclides. Diagnostic implies both invitro and invivo uses. There is hardly any branch of medicine in which there is not even one nuclear medicine application. On the other hand, in modern times there is hardly any medical research where radioactive tracer is not used in some form or other.

Diagnostic Nuclear Medicine

Nuclear Medicine is primarily diagnostic, its reputation being mainly because of the all pervasive and non-invasive nature of its investigations. The nuclear medicine investigations can be broadly classified into two categories: 1. In-vivo studies and 2. In-vitro studies (Radioimmunoassays). The in-vivo studies may be further classified into imaging studies (organ scanning) and non-imaging functional studies (e.g. thyroidal radio iodine uptake, Cr-51 RBC survival studies, GFR estimation, ERPF estimation etc). In most instances the nuclear medicine studies provide functional information of the system/organ studied unlike CT,US or MRI, which provide predominantly structural or morphological information.

The Nuclear Medicine diagnostic studies need radiopharmaceuticals as well as instruments to produce results. The radiopharmaceutical of choice must be safe, non-toxic for human administration, the radiations from the radionuclide of choice must be easily detectable by the nuclear medicine instruments and the radiation dose to the patient should be minimal and within acceptable range. These radiopharmaceuticals should be sterile and pyrogen free and undergo all quality control measures required of a conventional drug. Further details about radiopharmaceuticals may be found in

the article on Radiopharmaceuticals by Dr. N.Ramamoorthy elsewhere in this issue.

The basic imaging equipment in nuclear medicine today is a gamma camera. Basically the gamma camera is a device 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 30-40 cm in diameter with thickness varying from 6 to 12 mm. Behind the crystal there are 37 or more photomultiplier tubes arranged in a specific way to detect the position signals. In front of the crystal is a heavy collimator with a large number of holes to channelise the path of gamma photons. Whenever a gamma photon interacts with detector, it produces three signals: X, Y and Z. The first two are the position signals and define 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 map of the distribution of the radiopharmaceutical in the organ (1).

A computer is absolutely essential for dynamic functional studies. To-day most of the gamma cameras are sold as an integrated gamma camera-computer system.

Single Photon Emission Computed Tomography (SPECT) now offers images in tomographic format leading to improvement in detection of smaller lesions. At present SPECT has already proven its usefulness in the field of neurology, oncology, bones and joints and cardiology.

Clinical Applications:

A detailed discussion on various clinical applications of radionuclide methods is beyond the scope of this communication. However, efforts will be made to briefly outline some of the important and commonly used applications.

Endocrinology

Thyroid disorders may manifest as abnormalities of anatomy or function of the gland. The in-vitro thyroid function tests measure the level of thyroid and related hormones in circulation and the thyroid hormone binding ratio. The in-vivo thyroid function tests include radioactive iodine uptake (RAIU) and thyroid scan. Radionuclide scan of thyroid is an important investigation in the management of patients with thyroid nodules, thyroid ectopia and cancer of thyroid. Radionuclide scanning helps in the characterisation of the nodule, its function, vascularity, and multiplicity and autonomocity. It also helps to detect functioning metastases from differentiated thyroid cancers (2,3).

Thallium-technetium subtraction imaging has proved very helpful in the localisation of hyperfunctioning parathyroid tissue (Fig.1). This technique offers a sensitivity of 80-85% in the detection of parathyroid adenomas (4). Labeled precursors of adrenocortical hormones like (I-131
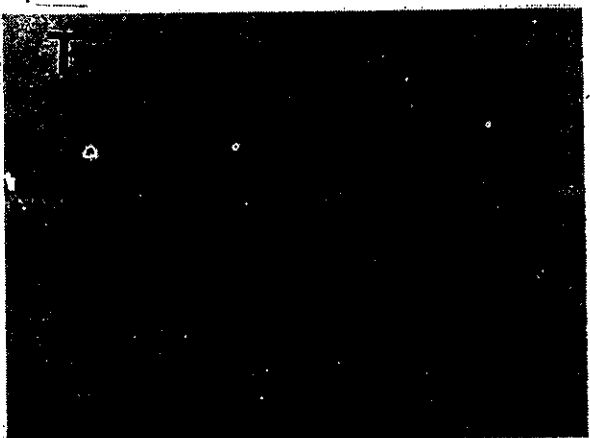

Fig. 1 Tl-201 and Tc-99m subtraction imaging showing a parathyroid adenoma of the left superior parathyroid gland (Arrow). Top left and Bottom left: Imaging of anterior neck done with Tc-99m pertechnetate. Bottom right: Imaging of anterior neck with Tl-201. Top right: Tl-Tc subtraction scan showing parathyroid adenoma (Arrow).

norcholesterol or Se-75 Selenocholesterol) help in the functional evaluation of adrenal cortex like adrenal hyperfunction, determination of its cause and localisation of a neoplasm if present (5). Advent of I-131/123 labeled metaiodobenzylguanidine (MIBG) has significantly improved the management (both diagnosis and treatment) of pheochromocytomas and other neuroendocrine tumours (6).

Nephrology & Urology

The availability of gamma camera imaging devices and high quality radiopharmaceuticals such as Tc complex of MAG3, DTPA, DMSA and I-123/I-131-hippuran has greatly facilitated radionuclide studies of the kidney and urinary tract. Renography, renal scanning, clearance studies and isotope voiding cystography provide valuable information. These procedures are non-invasive, sensitive, rapid and non toxic. Using these techniques it is possible to assess differential renal function in various renal and urinary tract disorders, evaluation of kidney size in advanced azotemia, detect presence of kidney which are poorly functioning, evaluate renal perfusion, diagnose upper urinary dilatation, detect intra-renal space occupying lesions and scarring (Fig.2,3), monitor renal function in renal failure, evaluate renal transplant function and diagnose vesicoureteric reflux (7).

Cardiovascular System

Nuclear Medicine imaging and data processing technique provide accurate, repeatable evaluation of cardiac structure, perfusion and function. These approaches have application in patients with coronary artery disease, congenital and valvular heart disease and cardiomyopathy. Gated images of the labelled cardiac blood pool (MUGA study) evaluate ventricular function and regional ventricular wall motions. Myocardial perfusion is studied by Thallium-201 chloride imaging which can provide information about myocardial blood supply, myocardial cell viability and integrity of membrane sodium potassium transport system in the heart. Tc-99m labelled pyrophosphate concentrates in the regions of the damaged myocardium and is a sensitive and accurate means of diagnosing acute myocardial infarction (8,9).
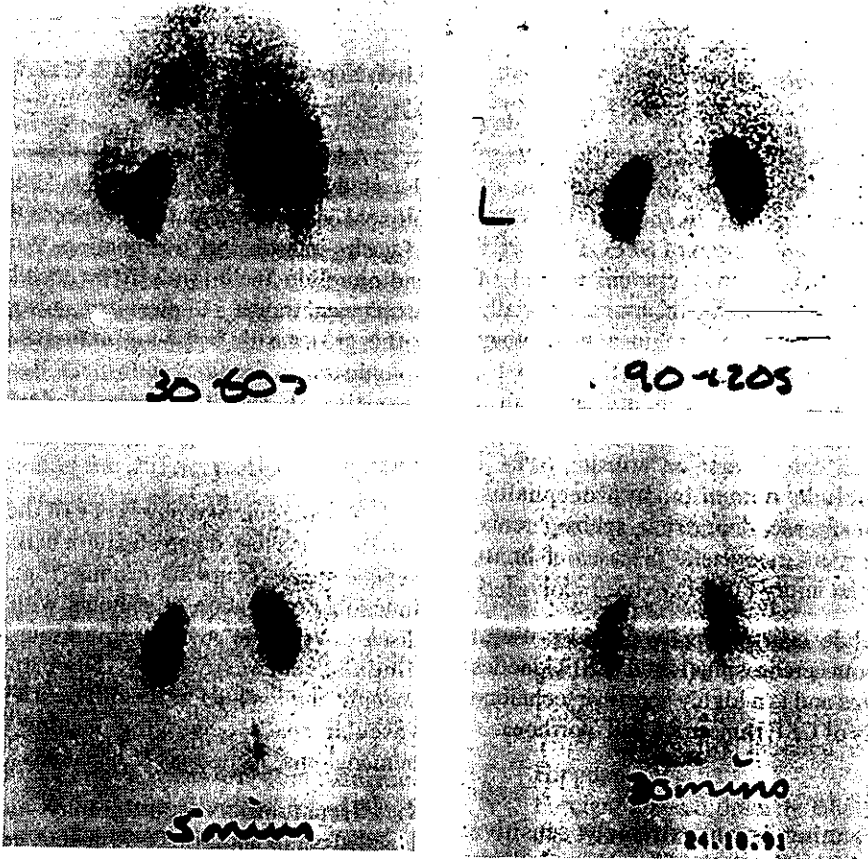
Fig. 2 Tc-99m DTPA Renal study. Posterior views. Sequential images obtained at 1 min, 2 min, 5 min and 30 min. Normal study showing good perfusion, function and drainage in both kidneys.
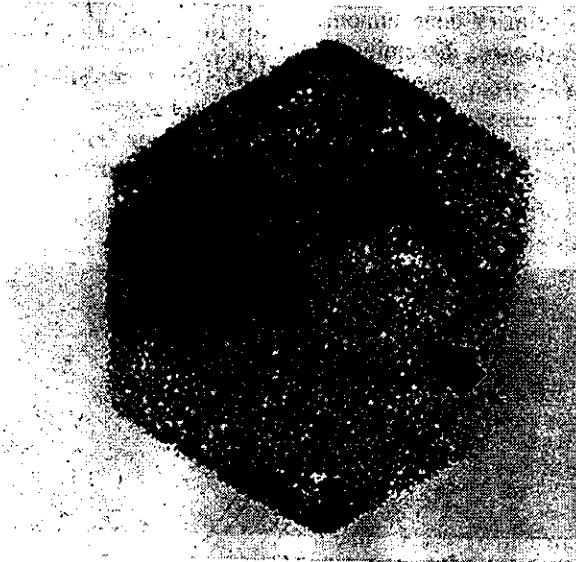

Fig. 3 Tc-99m DMS. . Renal study in a Patient with a tumour (Hypernephroma) of right kidney (Arrow)

Central Nervous System

With the advent of CT and NMR imaging which offer better sensitivity and specificity, there has been a dramatic decline in the volume of conventional brain scanning in the last 15 years. However, with the introduction of SPECT and PET technology, there has been a gradual revival of radionuclide studies in imaging the brain, especially with respect to measurement of regional physiology particularly regional cerebral blood flow (rCBF), glucose metabolism and oxygen utilisation in a spectrum of clinical conditions. The clinical conditions which can be studied utilising SPECT technology include dementia, hydrocephalus, cerebrovascular disease, depression, schizophrenia, movement disorders, neoplastic diseases of brain, epilepsy and head injury (10,11).

Radionuclide cisternography provides useful information about cerebrospinal fluid (CSF) kinetics in hydrocephalus and is a highly sensitive technique in the diagnosis of CSF rhinorrhea and otorrhoea.

Bones & Joints

Bone scanning is an extremely sensitive diagnostic tool in the diagnosis of focal bone lesions. One of the commonest clinical applications of bone scanning is detection of metastatic bone diseases. Besides, it has a spectrum of other applications like, evaluation of patients with primary bone tumour, osteomyelitis, fracture, prostheses, disseminated tuberculosis and arthirits (12).

Liver, Hepatobiliary (HB) tract & Gastrointestinal (GI) System

Adaptation of nuclear medicine technique to the study of aboral and retrograde movement of luminal contents is a major advance in the evaluation of gastro intestinal motor function. With the help of radionuclide techniques it is possible to study esophageal transit and clearance, gastro esophageal reflux (13), gastric emptying, entrogastric reflux and colonic transit. Radionuclide methods are extremely sensitive in the diagnosis of gastrointestinal bleeding, Meckel's diverticulum and protein losing enteropathy (14).

Hepatic scintigraphy is one of the most widely used and time tested investigation in the detection of hepatic space occupying lesions (Fig. 4) and in the functional evaluation of patients with diffuse liver disease. With the advent of ultrasound its role has diminished considerably. Nevertheless it has specific applications in the differential diagnosis of vascular space occupying lesions of liver like hemangioma, metastasis & hepatomas.

Hepatobiliary scintigraphy using Tc-99m labelled compounds of Tc-99m-IDA complexes like Tc-99m-Mebrofenin offer excellent functional information about hepatocellular function, help in the differential diagnosis of jaundice, choledochal cysts (Fig.5), detect biliary leak and aid in the follow up of cases after bilio-enteric by-pass surgery (15,16).
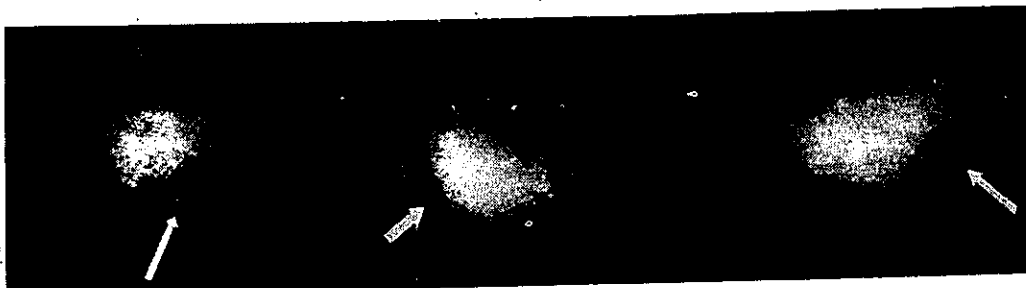

Fig. 4 Tc-99m Sulphur Colloid Liver Scan in a patient with abdominal trauma. Anterior, posterior and right lateral views showing a filling defect in the postero-superior aspect of right lobe of liver (Arrow). Final Diagnosis: Haematoma.
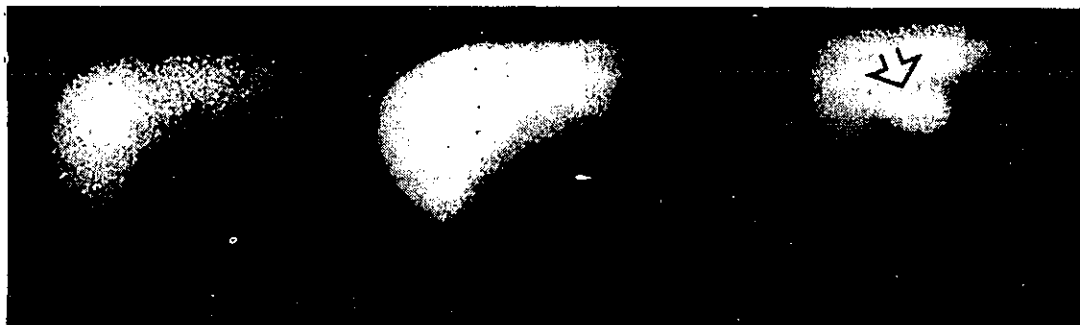
Fig. 5Tc-99m DISIDA Hepatobiliary Scan in a child with a choledochal cyst (Arrow). Anterior view images of liver at 5, 15 and 60 min (from left to right) following intravenous injection of radiotracer.

Applications in Oncology

The sensitivity & specificity of available radionuclide imaging methods in detecting primary neoplasms and their metastases varies with tumor type and location. In some conditions radionuclide imaging may provide the best available means of documenting the presence or absence of disease, as by Iodine-131 scintigraphy in patients with differentiated thyroid cancer with functioning metastases (3), Iodine 123/131-labeled MIBG scanning for pheochromocytoma and other neuro endocrine tumors (6). Radiopharmaceuticals like Ga-67 citrate and Thallium-0 chloride are used in the detection of primary and secondary malignancies. Bone scanning provides a highly sensitive means of detecting bone metastases. Radionuclide lymphoscintigraphy is a safe reproducible and easily performed technique for staging various malignancies. Recently, monoclonal antibodies labeled with appropriate radionuclides provide the potential for specific delivery of radionuclides to a particular cancer for detection and treatment.

Applications in haematology

By using simple techniques it has been possible to label various elements of blood like RBC, WBC and platelets with radionuclides and use them in the study of various haematological disorders. Blood volume, plasma volume, red cell mass, RBC life span, ferrokinetics etc can be accurately studied by nuclear medicine techniques. Labeled leukocytes help in imaging infection.

Pulmonary system

Ventilation-perfusion (V/P) scans of the lung provide accurate diagnosis of pulmonary embolism which continues to be a major health problem. The problem in diagnosing pulmonary embolism relates to the non-specificity of clinical signs and symptoms and to the relatively invasive nature and high financial costs associated with the definitive diagnostic procedure of pulmonary angiography. The radionuclide lung scan is a highly sensitive test for diagnosis of pulmonary embolism. It is widely used because it is safe, readily available, easily performed and provide clinically important information. Ventilation-perfusion scan can also be used to evaluate non-embolic pulmonary disorders, including restrictive lung disease, lung carcinoma and obstructive airway disease. The V/P study can provide quantitative data on relative whole lung and regional lung function that may be useful to the surgeon in determining how much lung to resect and which regions are significant contributors to pulmonary function in patients with compromised pulmonary function (17).

## Radionuclide Therapy

Therapeutic application of internally administered radionuclides has been limited. Nonetheless some procedures are in common use, the major application being the use of I-131 for the treatment of thyroid disorders, both of a benign and malignant nature. I-131 has been in use for the treatment of hyperthyroidism for more than 40 years. It has been shown to be an effective, safe and

econo.nical method of treating hyperthyroidism (18,19). On the other hand most well differentiated thyroid cancers can be treated effectively with a combination of surgery, radioiodine and thyroid hormones (3). P-32 has been in use for the treatment of polycythemia vera rubra and to-day it remains the best therapy for p vera in otherwise uncontrolled patients (20). P-32-phosphate and more recently Strontium-89 chloride and Samarium-153-phosphonate are being used for the alleviation of bone pain in patients with metastatic bone disease. I-131 labeled MIBG has already added a new dimension to the treatment of patients suffering from pheochromocytomas and other neuroendocrine tumours.

References

1. Parker RP, Smith PHS, Taylor DM. Basic Sciences of Nuclear Medicine (2nd ed). New York, Churchill Livingstone, 1984, 167-271

2. Werner SC, Ingbar SH (Eds). The Thyroid: A Fundamental and Clinical Text (2nd Ed.). Hagerstown, MD, Harper & Row, 1978

3. Hurley JR, Becker DV. The use of radioiodine in the Management of thyroid cancer. In: Freeman LM and Weissmann HS (eds) Nuclear Medicine Annual 1983. New York, Raven Press, 1983, 329-384

4. Winzelberg GG. Parathyroid Imaging. Ann Intern Med 1-987; 107: 64-70

5. Lieberman LM, Beierwaltes WH, ConnJW et al. Diagnosis of adrenal disease by visualisation of human adrenal glands with I-131-19 iodocholesterol. N Eng J Med 1971; 285: 1387-1393

6. Hoefnagel CA, Vout PA, de Kraker J, Marcuse HR. Radionuclide diagnosis and therapy of neural crest tumors using I-131 metaiodobenzylguanidine. J Nucl Med 1987; 28: 308-314

7. Gopinath PG and Padhy AK (. eds) - Essentials of Nuclear Nephro- uroloqy, Bombay, Himalaya Publishing House, 1988

8. Iakandrian AS. Nuclear Cardiology Imaging: Principles and applications, Philadelphia, FA Davis Co, 1987

9. Rozanski A, Berman D. Efficacy of cardiovascular nuclear medicine studies, Semin Nucl Med 1987; 17: 104-120

10. Magistretti PL (ed). Functional radionuclide imaging of the brain. New York, Raven Press, 1983, 1-368

11. George MS, Ring HA, Costa DC, Ell PJ, Kouris K, Jarritt PH (eds). Neuroactivation and neuroimaging with SPET. London, Springer-Verlag, 1991

12. Fogelman I (ed). Bone Scanning in Clinical Practice. New York, Springer-Verlag, 1986

13. Padhy AK, Gopinath PG. Sharma SK et al. Gastroesophaqeal reflux in children suffering from recurrent lower respiratory tract infection: experience in a referral centre in northern India. Indian J Nucl Med 1990; 5: 14-18

14. Padhy AK, Gopinath PG, Chirala SK, Sharma S, Shah P, Amini AC. Tc-99m Human Serum Albumin abdominal scanning in the diagnosis of protein-losing enteropathy. Indian J Gastroenterol 9:313, 1990

15. Padhy AK, Gopinath PG. Evaluation of neonatal jaundice using Tc- 99m BULIDA. Clin Nucl Med 7; 1982: 334

16. Padhy AK, Gopinath PG, Basu AK, Upadhyaya P. Hepatobiliary scintigraphy in congenital cystic dilation of biliary tract Clin Nucl Med 10: 703, 1985

17. Neumann RD, Sostman HD, Gottschalk A. Current status of ventilation-perfusion imaging. Semin Nucl Med 1980; 10: 198-217

18. Beierwaltes WH. The treatment of hyperthyroidism with I-131,. Semin Nucl Med 1978; 8: 95-103

19. Sridama V, McCormick M, Kaplan et al. Lonq term follow up study of compensated low dose I-131 therapy for Graves Disease. N Eng J Med 1984; 311: 426-432

20. Berlin NI. Polycythemia vera rubra, leukemia, and P-32 therapy. In Principles of Nuclear Medicine, Wagner HN (ed) Philadelphia, WB Sanders, 1968, pp 443-452

Instrumentation In Nuclear Medicine
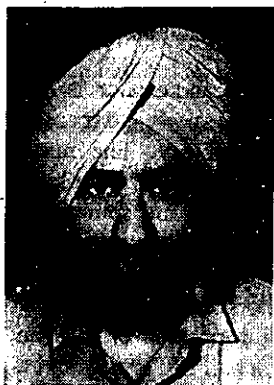
Dr. P.S. Soni is presently in-charge of the Medical Physics Section of Radiation Medicine Centre (RMC), BARC. Dr. Soni is a leading scientist in the field of nuclear medicine technology and qulity control of imaging instrumentation. A post-graduate in physics, he joined RMC/BARC after obtaining the PG Diploma DMRIT from the first batch of the course conducted by RMC and subsequently completed his Ph.D. He has been responsible for developing and promoting the use of radioactive aerosol inhalation unit for lung ventilation imaging and quality control surveillance of imaging instrumentation. He has been a participant in IAEA's coordinated research projects and has also served as IAEA's technical expert. He is a recognised teacher of University of Bombay for DMRIT Course.

Introduction

The evolution of nuclear medicine instrumentation during the past three decades has been a major contributing factor in the rapid growth that has occurred in both the basic science and clinical areas of nuclear medicine. The primary development that has provided the foundation for modern imaging equipment occurred when one could couple a scintillation crystal and photomultiplier tube (PMT) with a recording device to create the first effective imaging apparatus. Using scintillation detector (thallium- activated sodium iodide crystal; NaI(Tl)) , a great variety of moving (rectilinear scanner) and stationary detectors (planar gamma camera) have been developed subsequently. Recent improvements include the 3D image reconstruction by SPECT, PET, refinements in solid state detectors, collimators design and phototubes etc. The operation of a scintillator detector depends upon the detection of fluorescent radiation emitted from the phosphor following absorption of radiation. Further, the scintillation is detected by a photomultiplier coupled optically to the scintillator. The function of the photomultiplier is to convert light photons into photoelectrons and to do electron multiplication. The resultant electronic pulse is directly proportional to the energy of incident radiation. It is this latter property that facilitates energy discrimination using scintillation detectors. The improvements in crystal manufacturing, crystal packaging, PMT performance and optical coupling of phototube with crystal have profound effect on the overall performance characteristics. Till now, the scintillator detector remains one of the most versatile and sensitive detectors at our disposal in nuclear medicine.

Types of Instruments

The procedures of nuclear medicine fall into four broad categories: in-vivo counting (thyroid uptake by single probe); static scintigraphy (scanning done by either moving detector-rectilinear scanner or stationary detector-gamma camera); dynamic studies with either multiprobes (renogram) or gamma camera (such as kidney, nuclear cardiology etc.); in-vitro measurements (T-3 resin uptake, Schilling test, etc.). Virtually all counting systems for gamma radiation measurements in-vivo/ vitro are based on scintillation detectors embodying NaI(Tl). Associated electronics in all counting systems comprises of amplification, pulse-height analysis and counting of the pulses from the detector assembly. This article deals primarily with those instruments using scintillation detector and in regular clinical practice.

Thyroid/Renogram Probe

While numerous radioisotope studies are concerned with the actual localisation of the radioisotope depositions, in many cases pertinent information may be obtained by recording the rates of accumulation, or clearance of the tracer. For example, one of the first applications of external gamma ray counting for in-vivo tracer studies in medicine was the measurement of the uptake of ^{131}I by the thyroid gland at a specific time following the administration of a known dose. The sensitivity of the detector depends on the crystal size and energy

of the radiation. For medium energies, a crystal 50 mm in dia. and 25 mm thick is satisfactory. Lead shielding around the detector reduces its response to room background and environmental radiation, and a simple lead collimator (usually flat field) is mounted to confer the necessary directional characteristics and this collimated detector is called a probe.

In the case of renogram probe, the flat field collimator with two probes (one each for the two kidneys) are mounted on the unit and output of each probe is fed to scalars & analogue rate meters. The dynamic data of the latter is recorded by a strip chart recorder. Now, the utility of this instrument is limited because the study with renogram probes is time consuming and it is cumbersome to position the probes exactly on the kidneys in order to get dynamic data. Also, certain studies like regional glomeular filtration rate (GFR) are not possible with the renogram probes. With the advancement in camera and clinical software (e.g.deconvolution) most of the probe renogram units are now phased out.

Rectilinear Scanner

In nuclear medicine, a rectilinear scanner is an instrument designed to produce a two-dimensional image of distribution of radioactivity over a region or organ by scanning point-by-point in successive rectilinear passes made with a shielded and collimated scintillation detector (NaI(Tl)). The output electrical pulses from the detector is further amplified, passes through pulse-height analyser and are fed to one or more display devices rigidly connected with the scanner head, such that they follow its movement. The output of scanner is recorded as a distribution of monochrome or coloured marks produced by an electromechanical tapper on paper or as shades of gray produced by a flashing light source on photographic film (photo display). Recently, ECIL has made an attempt to interface the scanner to PC and obtained the output on printer. The crystal of scanner is usually of 7.5 cm or 12.5 cm in diameter and 5.0 cm thick. Interchangeable collimators, usually of lead, are provided for use in different clinical situations. These collimators are of the multi-hole, focussing type. The important performance parameters for focussing collimators are the spatial resolution, which expresses the ability to perceive details in the

distribution of radioactivity in the focal plane and the depth of focus. These characteristics depend primarily on geometrical design i.e. number of holes, septal thickness etc.

The advantage of rectilinear scanner is 1:1 unmagnified image that can be directly superimposed on the chest X-ray. The main disadvantage is its slow speed. One requires at least 30-45 min to scan most of organs and sometimes patients may move during the scanning. Also, it is not possible to do dynamic studies due to the limitation of mechanical factors and it is absolutely impossible to get required information of a organ where radiotracer's biological half life is very short.

Scintillation (Anger) Gamma Camera

A superior alternative concept to rectilinear scanner is a stationary gamma camera. The first stationary gamma camera was reported by Anger (1) in 1958 with a large NaI(Tl) crystal in order to see whole organ at a time. The main difference of camera over scanner is the much reduced imaging time, usually seconds or a few minutes. The shorter exposure time has important clinical advantages. Firstly, it is compatible with the dynamic nature of physiological processes. Secondly, dynamic studies are possible and may give additional information of hemodynamics. Thirdly, multiple views of an organ from various angles can be taken in a short period of time and more number of patients can be studied per day.

The scintillation camera is an imaging device utilising a thin but large diameter NaI(Tl) crystal viewed by an array of photomultipliers as the radiation detector. Photons emitted by the radionuclide in the patient reach the crystal after passing through a lead collimator. Most collimators are of the parallel- hole, diverging, converging, slant-hole or pin-hole type. Earlier version of camera had 11" diameter crystal with 19 PMT. NaI(Tl) crystals are generally available in two diameters, corresponding to a small field-of-view (300 mm) and a large field-of-view (400 mm), as well as in several thickness ranging from 3.2 to 12.7 mm. The crystal is viewed from its back surface, either directly or through a light guide, by the photomultipliers (usually the numbers of PMT are 37, 61, 91 in circular detector and 55, 75 in rectangular detector),

which are all fed from a common high voltage supply, the voltage or gain being slightly adjustable at each tube. All PMT must have matched amplification (gain) in order to provide a uniform count density when the crystal is flooded with a uniform flood source.

The PM tubes are connected to an electronic network, which has been designed so that each tube contributes a fixed portion of its output signal generation of a set of four position signals. The amplitude distribution of the pulses from all the PMT in the array due to a single photon interaction contains positional information. Further, the pulses are amplified and pass through PHA and registered on the cathode-ray tube. The analogue image information produced by the scintillation camera normally consists of three signals - the X and Y signals representing the position of the photon interaction in the crystal and the energy of the interaction is defined by the amplitude of a Z pulse obtained by summing the outputs of all the PMT. The older version of cameras are analogue systems and information accrued from such a system was only qualitative in nature. With the advancement of computer technology, the clinical value of radionuclide images has increased. Additional hardware required for camera-computer system is an analogue-to-digital converter (ADC) which converts the analogue to digital data which the computer is able to manipulate and an image display.

Semiconductor Detector Systems

Recently, a lot of work has been done to use semiconductor detectors (2) in place of scintillation detector in scintiscanners or gamma cameras. For this purpose a semiconductor detector is incorporated in place of the NaI(Tl) detector, with the data processing equipment remaining essentially unchanged. The semiconductor detector most commonly used in scanning is lithium-drifted germanium [Ge(Li)] and detectors as large as 7 cm diameter and 1 cm thickness have been used. High energy resolution (approx. 20 times better than NaI(Tl) for 140 keV) and small physical dimensions are some of the main features of the detector.

For semiconductor camera two basic design of semiconductor cameras have been tested to date: the

orthogonal-strip (3) and polar-coordinate designs (4).

In the first case, a single slice of semiconductor material in which parallel insulated grooves are cut through the p and n surfaces. When a gamma ray interacts in the intrinsic region between the p and n layers, the charge created is collected by a single strip from each surface (p and n) of the detector, and the position of the event is determined by the x_i, y_i coordinates of two orthogonal strips. Polar coordinate Ge(Li) gamma camera uses a co-axial semiconductor detector and is provided with insulated grooves cut radially into its n-type outer surface to divide the detector into isolated pie shaped individual sectors. The position of the point of interaction of a photon is identified by the sector number in which it is occurred and the depth along the sector where the event occurred and computer converts the polar to cartesian coordinates.

Semiconductor detector has also been used for fluoresence scanning (5). The technique involves irradiation of stable iodine in the thyroid gland by a gamma source (5 Ci of ^{241}Am; 60 keV) and detection of flourescent radiation by the moving semiconductor detector. The salient features of the technique are scanning without radioisotope, minimal radiation dose to a patient and scanning of a pregnant lady & child.

Single Photon Emission Computed Tomography (SPECT)

In the past two decades new approaches and notable advances in medical imaging have resulted from new concepts and development of computer science and applied mathematics. A major emphasis in diagnostic instrument development has been on the three dimensional description of physiological and biochemistry process in health and disease. The history of tomographic imaging dates back to 1917 to the idea of mathematical reconstruction of a two or three dimensional object from an infinite set of all its projections by the Austrian mathematician, J. Radon. In nuclear medicine, Kuhl and Edwards (6) introduced the concept of tomographic imaging, through their scanner based approach, which was basically longitudinal axial tomography in nature. After the introduction of Anger camera and the subsequent interfacing of computers with gamma

cameras, the concept of transaxial tomography has made rapid strides in nuclear medicine and now provides a rather easily accessible and relatively inexpensive tool for three dimensional physiologic measurements as contrasted with expensive, positron emission tomographs (PET) discussed later in this article.

The most commonly used SPECT system comprises of a conventional scintillation camera mounted on a special gantry and connected to an appropriate computer system. The SPECT data is obtained as a series of planar images by rotating the gamma camera through either 180° or 360° around the patient. Each planar view is made up of rows of pixels and each row contains the data from the transaxial plane in front of that row for the angle at which it is acquired.

During acquisition of data, the gamma camera moves in a circular orbit around the patient and the lesser, the radius of rotation, the better, the resolution. Elliptical orbital acquisition of data is also possible for better resolution as the detector can closely follow the body contour and thereby should give better results with cardiac SPECT. But then compared to circular orbits, elliptical acquisition requires complex motion of either the gantry or the patient table. The acquisition is usually done with step and shoot arrangement in which, while rotating the detector makes discrete stops during which time, the data are acquired.

Image Reconstruction

The simplest method of image reconstruction is by "back projection technique" in which each projection is back projected into a computer matrix along the appropriate angle at which they were acquired so that the overlap represents the original source. However, the image obtained will be of poor quality because of blurring (due to overlapping) and 'star artifact' produced during back projection. The most common method for removing the star artifact is the introduction of a filter to each individual projection. Filtering may be accomplished by the introduction of small negative values to each side of the projection (RAMP filter in object domain). When these filtered ray sums are back projected, the negative values will in effect cancel the positive background. If the filter is of an ideal design and if

enough projections are acquired, the cancellation effect might be perfect and with the result, a close representation of the original source can be obtained. A number of commercially available filter packages are available e.g. RAMP filter, with a various cut off frequencies. The function of the window is to improve signal to noise ratio during reconstruction.

Fourier Reconstruction

In this method, the data in object plane (spatial domain) is converted into frequency domain by applying Fourier transforms of the projection data and filtered by multiplying digital filter (noise reduction) and is then interpolated into a two dimensional array from which the image is easily reconstructed by simply taking the inverse Fourier transforms of the array.

Iterative image reconstruction techniques are numerical algorithms that use successive approximations (iterations) to arrive at a solution.

SPECT offers several advantages over planar imaging. Lesion contrast is improved, since the reconstruction process essentially removes the effect of tissues containing activity that overlie or underlie an area of interest. The ability of SPECT to display three-dimensional anatomy also enhances the sensitivity of diagnosis in contrast to planar scintigraphy. Another important advantage of SPECT is the volume estimation of organ of interest.

Positron Emission Tomography (PET)

The basic principle of the PET is that two 511 keV photons are emitted in opposite directions (180°) following the annihilation of a positron and an electron. Thus by positioning two detectors around a patient one could determine the line along which a disintegration occurred. The availability of physiologically interesting positron-emitting radiopharmaceuticals from the cyclotron and of suitable instrumentation, as well as the development of algorithms for image reconstruction, provide the impetus for PET. The fundamental physical difference between PET and SPECT is the use of annihilation coincidence detection or ACD, in the detection of two 511 keV photons. ACD detects only those pair of events that are detected within a narrow time interval (typically 5-20ns). Events registered in only one detector are rejected electronically and

hence, the name "electronic collimation". Although NaI(Tl) is satisfactory for most imaging systems used with ordinary gamma ray emitters, the 511 keV annihilation photons require detectors with greater stopping power for efficient detection. Bismuth germanate (($Bi_4Ge_3O_{12}$)) is currently the preferred detector material, even though its scintillation light yield is somewhat low and decay time somewhat long. All commercial systems have approximately the same characteristics and they have rings of bismuth germanate detectors individually coupled or coded to photomultiplier tubes. Employment of cross coincidence between rings results in more image levels than the number of individual rings of crystals; thus a four-ring system can give data for seven planes.

The recent trends in positron instrumentation have been in two directions (7). The first is toward high-resolution positron instruments; the second is toward the use of time-of-flight to improve image quality. Time-of-flight means estimation of position of the origin of the photons by estimating the difference between their arrival times at the two detectors. It is hoped that time-of-flight instrumentation will improve the quality of image and this concept is proposed for future instruments. High energy resolution is achieved by using many thin detectors in a ring configuration. Since PM tubes have relatively large dimensions, various coding schemes have been proposed to identify individual detectors, and this aspect of the design may become a limiting factor unless a new phototube is developed.

An advantage of PET over SPECT is its much greater efficiency (nearly 10-20 times than SPECT) for detecting radiations, because in SPECT physical collimation results in loss of many available photons. For positron emitter radionuclides it is necessary to have hospital based cyclotron or, for some positron emitters, a portable generator. By contrast, single-photon emitters, for instances, ^{99m}Tc and ^{123}I are easier to work with because of their longer half-life. The PET technique has the potential for measurement of glucose, fatty acid, amino acid and other substrate metabolism as well as receptor concentration in the body. It is a valuable new research tool for the investigation of diseases such as ageing, schizophrenia, atherosclerosis and cancer.

Future of PET lies further on advancement of instrumentation, radiopharmaceutical and mathematical modeling.

Conclusion

Anger gamma camera-computer and SPECT constitute main instrumentation in any clinical nuclear medicine unit. Use of single head or dual head probes is also not uncommon though their role is much limited. In India ECIL is the only organisation producing the probes & scanner. Recently they have started manufacturing the gamma cameras too. The level of sophistication in the SPECT & PET systems required are confined to the domain of multinationals. The advancement in computer and hardware technology has made it possible to develop state of the art digital integrated camera-computer system and anlaogue cameras are slowly phasing out. Amongst detectors, NaI(Tl) for gamma photons and BGO for annihilation photons are the major detector systems used in nuclear medicine. Recent introduction of cesium fluoride in place of BGO may offer exciting possibility of using time-of-flight techniques for PET. Though semiconductor detectors have excellent energy resolution, low efficiency for high energy gamma and stringent requirement of cooling, limit its application. Their ultimate role in nuclear medicine remains to be determined.

References

1. Anger HO: Scintillation camera. Rev Sci Instrum 29: 27, 1958

2. Hoffer PB, Beck RN, Charleston DB, et al: Use of lithium-drifted detectors for clinical radioisotope scanning, J. Nucl. Med.11:326, 1970.

3. Detko JF: A prototype, ultra pure germanium orthogonal strip gamma camera, in: proceeding of IAEA Symposium on medical Radioisotope Scintigraphy, Oct. 1972, Monaco.

4. Strauss MG, Sherman IS: Gamma ray camera using a co-axial germanium detector. JNM 13:767, 1972

5. Hoffer PB, Gottshalk A, Foster J: Fluorescent thyroid scanning (scanning without radioisotopes) initial clinical results. Radiology 99:117, 1971

6. Kuhl DE, and Edwards, RQ: Image separation radioisotope scanning. Radiology 80, 653-662, 1963

7. Reivich M, Alavi A. (eds.): Positron Emission Tomography, New York, Alan R. Liss, 1986.

INTERVIEW

"Prospects and Promises of Nuclear Medicine"

Dr. N. Ramamoorthy talks to

Dr. (Mrs.) A.M. Samuel
Head,
Radiation Medicine Centre (RMC)
BARC.

NR *At the outset how do you assess the contribution of current nuclear medicine procedures towards clinical decision making and patient management?*

AMS "Nuclear Medicine" is a unique contribution to the discipline of Medicine. Its uniqueness lies in the utilisation of the properties of radionuclides as "tracers". The minute quantities of radiolablelled compounds do not disturb the physiological processes in the body, can be "tailor-made" to suit the objective of the study, and can be easily detected by sensitive instruments. All these properties and advantages can be used to measure changes in physiology of an organ to assess structural changes in the organ and to study the kinetics of blood flow, biochemical changes, function and number of receptor on cell surfaces and many other "molecules" of the body.

Radionuclides in the form of a variety of compounds can be used "in vivo" in the patients as well as "in vitro" in a test tube (Test tube Nuclear Medicine) to study minute concentrations of hormones, drugs, bacterial antigens and antibodies, viral antigens & antibodies and a host of substances. With such a wide range of applications, there is no doubt that the techniques in Nuclear Medicine play a very important role in clinical decision making and management of patients with a variety of diseases.

NR *What will be the role of radionuclide imaging as a diagnostic modality especially in view of the considerable advances in the other imaging systems?*

AMS As mentioned earlier the "forte" of Nuclear Medicine is its ability to study physiology, function, mechanism of action of various biomolecules like receptors on cell surfaces, metabolic functions of cells like glucose and amino acid nutrients, blood flow, and many other parameters using appropriate radiolabelled compounds and detecting instruments. Sophisticated information technology using PET/cyclotron or SPECT systems gives data which can be at the level of biomolecules. The other imaging modalities like CT, MRI and ultrasound, though they are able to give exquisite details of morphology and structural aberations, they cannot give diagnostic information of physiological changes. Hence in the holistic management of a patient, all procedures which can give an overall diagnosis is useful. They should be used as complementary modalities for management of patients.

NR *It is said that nuclear medicine procedures are 'sensitive' but lack 'specificity'. What are the approaches to overcome this drawback ?*

AMS Yes it is true that Nuclear Medicine techniques are "sensitive" because of the inherent advantages of a "tracer" technology. The presently utilised tracer compounds depend on non-specific factors which are used to target or localise them in a particular organ. Blood flow to an organ is one such factor. Since blood flow may increase or decrease in a variety of disease processes, the localisation of a tracer would depend on this factor. Hence the localisation indicates changes in blood flow and cannot give a diagnosis whether the change in blood flow is due to infection, cancer or any other reasons. Apart from blood flow there are several other reasons for localisation of radiolabelled compounds which would be difficult to enumerate in this short space but blood flow was quoted as an example. Hence the "specificity" which means the possibility of giving a diagnosis of the disease process is poor. The other imaging modalities like CT, MRI are also handicapped to a certain extent with regard to ability to diagnose specific diseases.

This present drawback will continue to exist until newer compounds which can trace specific diseases are obtained. The development of monoclonal antibodies and receptor based compounds are the molecules of the future and it is hoped that specificity of diagnosis will improve.

NR *"The future of nuclear medicine is in the therapy of certain diseases" is the impression given these days. What are the exact areas of treatment wherein significant promise has been noted?*

AMS The use of radionuclides as therapeutic agents has been very limited. Since the dawn of the era of Nuclear Medicine, internally administered radionuclides as therapeutic agents has been restricted mainly to the use of $Na^{131}I$ for the treatment of thyroid cancer and thyrotoxicosis, ^{32}P orthophosphate for the treatment of polycythaemia, Radio-Gold ^{198}Au colloid for malignant effusions. This is probably because adequate efforts have not been made to open new avenues.

Recently there has been renewed interest in expanding this field.

^{89}Sr Chloride, ^{186}Re-HEDP, ^{153}Sm-EDTMP are compounds tested for the relief of pain from skeletal metastases.

^{131}I-MIBG, ^{131}I-octreotride and ^{131}I-monoclonal antibodies which can be targeted to specific malignancies are receiving attention from nuclear medicine physicians. Alpha emitting radionuclides like Astatine-211 are being considered for therapeutic applications. The future appears bright and in the succeeding years, I hope the repertoire in our armamentarium to manage onclogical diseases will increase.

NR *Turning to the Indian scene, how do you rate the level of progress in the practice of nuclear medicine in our country, especially in comparison to the developed nations?*

AMS The progress of Nuclear Medicine in our country has been slow in relation to developed countries. Although the progress has been slow, there is no doubt that in the past decade the enthusiasm and efforts made to develop this discipline is encouraging. The slow progress has been due, not to the lack of interest among physicians or to the nonavailability of trained staff or radioisotopes, but due to the fact that establishing the infrastructure and technology is expensive and not within reach of most medical colleges and hospitals.

NR *How about the radiopharmaceuticals? We did not have for a long time indigenous production of cyclotron based products. Has it not come in the way of the quality of nuclear medicine services available to our patients? Is there really a need for PET in clinical nuclear medicine or is it confined to medical research and knowledge?*

AMS It goes without saying that the lack of a Cyclotron/PET system in our country is sorely felt by the Nuclear Medicine community. Although for many years, it was believed that Cyclotron/PET technology was more research oriented and did not have a role to play in patient management, in recent years it has been shown to be very useful in cardiology, neurology and oncology. The information obtained, added to the other modalities of investigation like CT & MRI,

has changed concepts in management of diseases.

The establishment, maintenance and day to day cost of running a PET centre is prohibitively expensive even for developing nations and in view of this very serious drawback, the number of PET centres in the world will continue to form a small percentage of Nuclear Medicine Centres. However, the ingenuity of scientists is such that they will never give up on any challenges. There are frantic efforts by the chemists to develop compounds which can be labelled with ^{99m}Tc or ^{123}I or ^{111}In which could replace the positron emtitting labelled compounds. The former could be used with SPECT-Gamma cameras and could give information akin to that obtained with PET/cyclotron. I feel that a major component of PET/cyclotron applications may one day be replaced with single photon emitting radionuclides.

NR *What about the extent of practice of radioimmunoassays (RIA) in our country? Despite being a 'low cost technique' is it not still confined to cities and major towns? With the advent of non-radiometric assays, how do you rate the prospects for RIA in the next few years ?*

AMS Radioimmunoassays are simpler techniques and should have made a bigger impact among the medical fraternity. The possible causes for the rather slow progress could be due to

a) Fear of handling radioisotopes

b) Licensing procedures which might alienate the user

c) Expensive automated counting systems

d) Short shelf-life of the labelled compounds due to decay.

In the present scenario of enzyme labelled techniques, which offer advantages over radiolabelled compounds, the tendency of the user is to opt for these techniques. I feel that over the years RIA will gradually be replaced by ELISA techniques for a large number of assay procedures.

NR *How about the HRD and regulatory aspects? Are we fully geared in these directions?*

AMS Our regulatory procedures are based to a considerable extent on those practised in developed countries. The regulatory procedures are necessary from the view point of safe usage of radiations both for the practitioner, the patient and the public. It is necessary to create a greater awareness of the regulatory procedures amongst the licensed users of radioisotopes and also there should be better monitoring systems to ensure that the regulatory procedures are practised as required by law.

NR *Could you compare the contribution of DAE, Defence, Academic and Private Centres to the growth of nuclear medicine in India?*

AMS Nuclear Medicine technology as stated earlier is an expensive technology and hence the funding, maintenance and regulation of Depts. of Nuclear Medicine is mostly within the purview of agencies like DAE, Defence services rather than Private Hospitals. In the formative years of the discipline, the DAE & Defence have played a pioneering role in establishing well equipped state of the art centres, development of manpower, supply and development of radioisotopes, establishing regulatory laws and so on. However, of late the "lure of the lucre" has drawn many Private Hospitals and Clinics into the vortex of the speciality. In view of the wide applications of Nuclear Medicine in both in vivo and in vitro technologies, the ability to make money has been an important consideration and there has been a tremendous demand for developing and establishing centres of Nuclear Medicine in the many "Five star Hospitals" mushrooming all over the country.

NR *What are your suggestions and recommendiations for the further growth of this field?*

AMS My suggestions for the future growth of the discipline are as follows.

1. Encourage and fund large Medical Colleges to set up Nuclear Medicine Centres. It is the newly graduating medical doctors who should be made