RADIO-ACTIVE ISOTOPES IN MEDICAL SCIENCE

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1. NATURE AND MEASUREMENT OF RADIO-ACTIVE ISOTOPES

The phenomenon of radio-activity has always excited the interest and imagination of the medical profession. The therapeutic value of radiations emitted by uranium and radium was recognized soon after the discovery of these elements at the turn of the present century. Hevesy realized, as long ago as 1923, that radio-active elements could be put to a different use in biology. The substance known as Radium D is, in fact, a radio-active form of lead and Hevesy used it to study the uptake and distribution of lead in plants and later in animals, tracing the element by virtue of its radio-active emanations.[1] Further applications of this method were limited by the fact that, although over fifty radio-active isotopes occur naturally, nearly all are heavy, rare metals of little biological importance. The artificial production of radio-active forms of common, light elements—first achieved by Irene Curie and Joliot in 1934—extended the range of biological problems which could be investigated by tracer techniques. With the splitting of the uranium atom and the construction of the first atomic pile at the University of Chicago in 1942 radio-active forms of all elements in undreamt of variety became available for biological and other uses.

The application of these radio-active isotopes to physiological, biochemical, and clinical problems has proved so fruitful that knowledge already gained through their use and their potential applications to diagnosis, therapy and research must now command the interest of every medical man. It is, however, difficult for those not familiar with isotope techniques to assess the significance of 'tracer' investigations and to visualize applications of value in their own fields of interest.

The present paper has been written in an attempt to provide general orientation concerning the nature and properties of radio-active isotopes and the methods commonly employed for their measurement. In the second part of this communication the general scope of the isotope technique in medical science will be outlined.

NATURE OF RADIO-ACTIVE ISOTOPES

Structure of the atom[2]

The atomic nucleus which contains almost the entire mass of the atom consists of protons which carry unit positive charge and neutrons which are uncharged. The electrons in the uncharged atom are equal in number to the protons, carry unit negative charge, and move in definitely placed orbits around the central nucleus. The number of protons determines the chemical properties of the element and is referred to as the atomic number (Z) written as a subscript before the chemical symbol, e.g. $_1H$, $_6C$. The atomic mass (A) is the number of protons and neutrons and is written as a superscript either before (British convention) or after (American convention) the chemical symbol. It is apparent that the atomic number and chemical symbol are synonymous and the former is usually omitted, e.g. the various forms of carbon are written ^{12}C, ^{13}C, ^{14}C (British) or C^{12}, C^{13}, C^{14} (American).

Stable Isotopes

It has long been known that certain elements exist in different forms which, although identical in every chemical and in most physical characteristics, do not have the same atomic weights—for example, there are 3 forms of lead having atomic weights of 206, 207 and 208. To such elements Soddy in 1913 gave the name isotopes, indicating atoms which are identical in regard to the number of protons (and electrons) and hence in regard to their chemical properties, but different in regard to their number of neutrons and their atomic weights. Many elements have only one stable form (e.g. ^{23}Na, ^{31}P), but the majority consist of mixtures of stable isotopes and this explains why many atomic weights are not whole numbers (e.g. magnesium 24·32, chlorine 35·457).

Study of the atomic configuration of the elements reveals that there are only certain specific combinations of protons and neutrons which give rise to stable atomic nuclei. Thus, for example, the nucleus of carbon, which contains 6 protons, is stable only when associated with 6 or 7 neutrons—hence the 2 stable isotopes of carbon found in nature—^{12}C and ^{13}C—of which the latter comprises 1·1% of naturally occurring carbon.

Radio-active Isotopes

A radio-active isotope differs from the stable forms of the element in that the neutron to proton ratio is unstable. The atomic nucleus tends to return to a stable configuration by rearrangement of its particles and this is associated with radio-active emission. Thus, for example, carbon atoms containing 6 protons and either 5 or 8 neutrons are unstable—hence the radio-active isotopes of carbon, ^{11}C and ^{14}C.

Radio-active isotopes are artificially produced by the interaction of a small number of fundamental particles with the stable nuclei of the elements. In the cyclotron the target nucleus is bombarded with charged particles which are accelerated to high velocity by an oscillating electromagnetic field. By this means, for example, $^{23}_{11}Na$ (stable sodium) can be made to combine with a deuteron particle (*d*), which consists of a neutron and a proton (*p*). This compound nucleus retains the neutron, but immediately ejects a proton so that $^{23}_{11}Na$

is converted to the radio-active isotope of sodium, $^{24}_{11}$Na. Such reactions are conventionally written in abbreviated form

$$^{23}_{11}\text{Na (d, p) } ^{24}_{11}\text{Na},$$

i.e. the target nucleus is written first followed in parenthesis by the reacting particle and the emitted particle, and finally by the product nucleus. The production of radio-active isotopes in the cyclotron may involve transmutation of elements. Bombardment of $^{23}_{11}$Na with alpha particles (α), which consist of 2 neutrons and 2 protons, leads to the production of a compound neucleus from which a proton is immediately lost, leaving a radio-active isotope of magnesium

$$^{23}_{11}\text{Na } (\alpha, \text{ p}) \, ^{26}_{12}\text{Mg}.$$

The practical advantage of such transmutation reactions lies in the fact that the product is chemically different from the target and can be separated from it. By this means it is possible to obtain isotopes of high specific activity—that is, containing a high degree of radio-activity per unit mass—or even 'carrier-free', which means that all the atoms in the product element are radio-active.

Large-scale production of radio-active isotopes only became possible with the development of the nuclear reactor (atomic pile), in which the target element is bombarded by neutrons. When ^{235}uranium absorbs a neutron it undergoes fission into 2 smaller fragments and, at the same time, liberates a number of neutrons, which may be used to split further uranium atoms (chain reaction) so that the number of available neutrons multiplies progressively. Some of these neutrons may be allowed to escape from the reacting mass and be used for target bombardment. The neutron is uncharged and hence experiences no repulsion on approaching a target nucleus. The possibility of neutron capture is dependent mainly on the time it lingers in the vicinity of the nucleus and this is related inversely to the velocity of the neutron. In the atomic pile neutrons which are liberated at high velocity during uranium fission are slowed by passage through materials of low atomic weight—such as deuterium or graphite—before they are allowed to reach the target element.

Neutron capture may transform the target element into a radio-active form of the same element, e.g.

$$^{37}_{17}\text{Cl (n, } \gamma) \, ^{38}_{17}\text{Cl},$$

or lead to transmutation, e.g.

$$^{14}_{7}\text{N (n, p) } ^{14}_{6}\text{C}.$$

Such reactions have made it possible to produce at least one radio-active isotope of every element. At the present time over 1,200 isotopes are available for the 96 common elements; about 80% are radio-active and the great majority of these are artificially produced.[3]

Decay of Radio-active Isotopes

As indicated above the phenomenon of radio-activity is exclusively a property of the atomic nucleus. Unstable combinations of neutrons and protons tend to undergo rearrangement towards a stable configuration. This process which is often referred to as nuclear disintegration or radio-active decay may be accomplished by:

1. Emission of a negative beta particle from a neutron, leading to transformation of the neutron into a proton. An example of this form of decay is provided by radio-active ^{32}phosphorus which emits negative beta particles and is converted to stable sulphur:

$$^{32}_{15}\text{P (radio-active P)} \rightarrow ^{32}_{16}\text{S (stable S)} + \beta^-$$

The atomic weight is unchanged by this decay process, since beta particles have negligible mass equivalent to that of the electron. These particles leave the nucleus at a velocity approaching that of light and are thought to arise from the atomic nucleus only during disintegration. Beta particles vary considerably in their energy, but can never penetrate more than a few mm. through tissues. It follows that in biological work β radiations can be measured in blood and tissue samples, but cannot be detected at the body surface when emitted from deep, internal structures.

2. Emission of a positive β particle (positron), leading to the transformation of a proton into a neutron; e.g.

$$^{11}_{6}\text{C (radio-active C)} \rightarrow ^{11}_{5}\text{Bo (stable Bo)} + \beta^+$$

The positron differs from the β^- particle in that it carries positive charge and has only an ephemeral existence, rapidly undergoing combination with a negative electron, as a result of which both particles are annihilated and 2 gamma rays are emitted at 180° to each other.

3. Combination of a proton with an electron, leading to transformation of the proton into a neutron. This is referred to as K electron capture and is followed by a rearrangement of the remaining electrons in their orbital shells—this process being associated with X-ray emission; e.g.

$$^{37}_{18}\text{A (radio-active A)} \rightarrow ^{37}_{18}\text{Cl (stable Cl)} + \text{X-rays}$$

4. Emission of an alpha particle. This occurs only in elements having an atomic number greater than 82 and is of little importance in biological work. Alpha particles consist of 2 neutrons and 2 protons, are about 8,000 × the mass of beta particles and can penetrate only a few microns through body tissues.

5. Emission of gamma rays. Alpha or beta particle emission may leave the atomic nucleus in an excited energy state so that the further emission of one or more gamma rays is required to restore the ground state. Gamma rays consist of electromagnetic quanta (photons) which are uncharged and travel at the speed of light. They originate within the nucleus, but are otherwise similar to X-rays and have great penetrating power. For this reason gamma-emitting isotopes lodged in deep-lying tissues are readily detected *in vivo* by recording equipment placed at the body surface.

There are thus radio-active isotopes which are pure alpha, beta or gamma emitters; in addition, many combine various types of radiation—such as beta-gamma emitters (Table I). Both the type of emission and the energy of radiation are characteristic for each radio-active isotope.

Rate of Radio-active Decay

It is apparent from the above discussion that radio-active atoms continually undergo disintegration, during which process radiations of various types are emitted and atoms of stable nuclear configuration are formed. A fundamental attribute of this decay process is that its rate cannot be influenced by either chemical or physical means. The number of disintegrations occurr-

ing per unit time is proportional to the number of radio-active atoms present and is not dependent upon the age of any individual atom.

When the intensity of radiation has dropped to half its initial value, the number of radio-active atoms is reduced to half the number originally present. The time required for this diminution is termed the half-life ($t_{\frac{1}{2}}$). Each isotope has a characteristic half-life (Table I) and there is considerable variation, even among isotopes of the same element—for example,

TABLE I. CHARACTERISTICS OF SOME RADIO-ACTIVE ISOTOPES COMMONLY EMPLOYED IN BIOLOGICAL WORK

Element	*Radio-active Isotope*	*Type of Radiation*	*Half-life*
Hydrogen ..	^{3}H (tritrium)	beta	12·1 year
Carbon	^{11}C	positron	20·5 min.
	^{14}C	beta	5,700 year
Sodium	^{22}Na	positron, gamma	2·6 year
	^{24}Na	beta, gamma	14·8 hour
Phosphorus ..	^{32}P	beta	14·3 days
Sulphur	^{35}S	beta	87·1 days
Chlorine	^{36}Cl	K capture, positron, beta	10^{6} year
	^{38}Cl	beta, gamma	37 min.
Potassium ..	^{42}K	beta, gamma	12·4 hour
Calcium	^{45}Ca	beta	180 days
Iron	^{59}Fe	beta, gamma	47 days
Cobalt	^{60}Co	beta, gamma	5·3 years
Iodine	^{131}I	beta, gamma	8 days

various isotopes of cobalt have half-lives varying between 18 hours and 5 years. The rate of radio-active decay is obviously a matter of considerable practical importance in biological experiments. Isotopes having extremely short half-lives (e.g. ^{128}iodine—25 minutes; ^{18}fluorine—112 minutes) can be used only by laboratories close to the source of production and for experiments of short duration. On the other hand, isotopes having very long half-lives (e.g. ^{14}carbon—5,700 years) are convenient from the analytical point of view, but carry the danger for the recipient of prolonged tissue irradiation. Unfortunately, radio-active isotopes of the commonest organic elements are either very short-lived or very long-lived. Thus, the two radio-active isotopes of carbon have half-lives of 20 minutes (^{11}C) and 5,700 years (^{14}C), radio-active hydrogen (tritrium—^{3}H) has a half-life of 12½ years and the radio-active isotopes of nitrogen and oxygen decay too rapidly to be of any practical use. For this reason the rare stable isotopes of these elements are frequently used in biological work, their presence being detected by means of the mass spectrometer.

MEASUREMENT OF RADIO-ACTIVITY

The quantitative determination of radio-activity is based upon measurement of the ionization produced in media traversed by radiations. The most generally useful methods are those which measure the ionization produced in gases. Passage of charged particles through a gas leads, by electrostatic interaction, to the production of ion pairs consisting of heavy positive ions and negative electrons. Uncharged gamma and X-rays produce ionization only through collision mechanisms and for this reason detection of these rays by ionization tubes is less efficient than for beta particles.

Gaseous ions are unstable and have a marked tendency to recombine. However, if the ionization occurs in an electric field the electrons are accelerated rapidly towards the anode and the heavy positive ions move more slowly towards the cathode. The imposed electric field may be of such strength that only those electrons liberated by passage of the ionizing particle are collected and measured. In such an instrument, which is known as an ionization chamber, the total amount of ionization within the sensitive volume of the counter is measured. If the strength of the imposed electric field is increased (Fig. 1), the electrons initially liberated by the ionizing

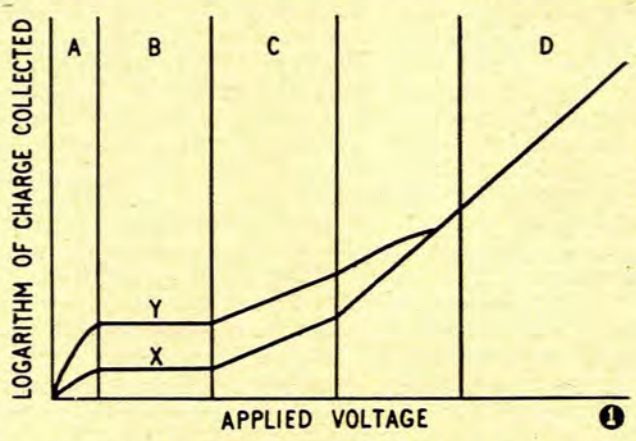

Fig. 1. Diagramatic representation of the relationship between the applied voltage and charge collected at anode in a counting tube. Curve X—result obtained when ray or particle produces a small number of ion pairs. Curve Y—result obtained when a large number of ion pairs are produced by ray or particle.
Region A—some ions lost by re-combination.
Region B—all the ions initially formed are collected and measured (ionization chamber).
Region C—multiplication of ions proportional to initial ionization and applied voltage (proportional counter).
Region D—multiplication of ions unrelated to initial ionization (Geiger-Muller counter).

radiations are accelerated to such a degree that they cause ejection of other electrons, leading to multiplication of the initial charge by as much as 10 orders of magnitude. Voltages leading to such multiplication of the initial charge are used in Geiger-Muller and proportional counters, which are widely employed for the measurement of beta particles.

Ionization in a Geiger-Muller tube (Fig. 2) is followed by a period known as the 'dead time' during which the counter cannot respond to a second ionizing particle; this introduces an error at counting rates of above 5,000 per minute. The dead time can be reduced by filling the tube with a mixture of a monatomic and polyatomic gas, the latter being referred to as a quenching gas. Argon and ethanol are commonly used, with the latter supplying about 5% of the total pressure 5-40 cm. mercury. Such tubes have a finite life because with each discharge some of the polyatomic gas is broken down into smaller particles which do not have 'quenching' properties.

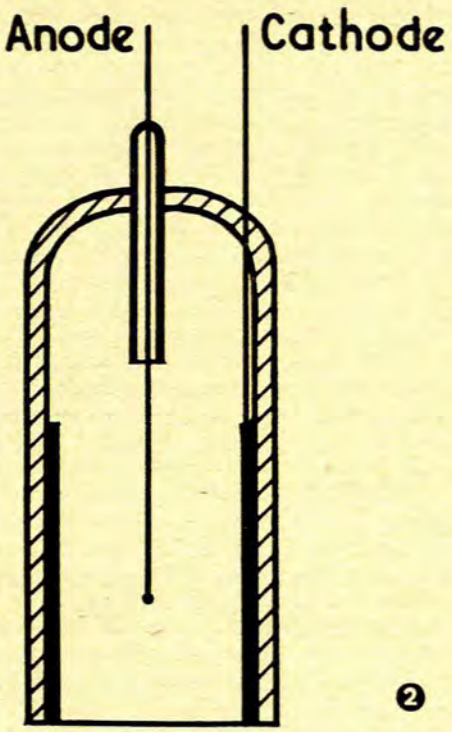

Fig. 2. Diagram of Geiger-Muller tube having a thin end-window permitting the entry of low-energy beta particles.

Proportional counters are operated at lower voltages than Geiger-Muller tubes and have the advantage of a very much shorter 'dead time', so that counting rates of up to 100,000 per minute are possible. In addition, they are operated at atmospheric pressure, so that the sample can be conveniently introduced into the counter before the counting gas is pumped into the sensitive volume of the tube.

Scintillation counting is dependent upon the production of fluorescence by the ionizing particle or ray. Substances having the property of fluorescing in the presence of radio-activity are called phosphors. Lord Rutherford used the method of counting flashes of light in a zinc-sulphide phosphor to obtain the data on which was based his theory of the atom. In modern scintillation counters a sodium iodide-thallium crystal is commonly used as the phosphor. In addition, a variety of organic phosphors are now available in crystalline, plastic or liquid form; in the last-mentioned the radio-active sample can be dissolved in the phosphor itself. With the development of the photomultiplier tube, which enables light flashes emitted by the phosphor to give rise to detectable electrical impulses, scintillation counting became an effective and convenient method of radio-active assay especially valuable for counting gamma radiations.

The electrical discharges from counting tubes or from the photomultiplier of the scintillation counter are too rapid to operate a mechanical register directly. For this reason the pulses are first passed through an electronic scaling circuit which records a known number of impulses before activating the mechanical register, which may respond to only 1 out of 1,000 discharges from the counter.

Corrections in Radio-active Assay:

All methods for the assay of radio-active isotopes are sensitive to the ionization produced by cosmic rays and the natural radio-activity of the earth's surface. These sources of ionization produce what is called a background counting-rate, which is measured by operating the counter without exposure to a known source of radio-activity; this value is subtracted from the gross rates obtained with samples. The background limits the sensitivity of the instrument and can be reduced by suitable shielding of the counter, with lead.

The radio-activity of samples analysed is also corrected, on the basis of the half-life of the isotope, for physical decay occurring during the course of the experiment. Any observed reduction in radio-activity is then attributable only to such biological processes as dilution of the labelled compound by the body 'pool' or catabolism of labelled molecules and replacement by newly-synthesized unlabelled ones.

Since a radio-active source emits radiations in all directions it is apparent that only a portion of the ionizing particles or rays will enter the counter. This proportion is determined mainly by the geometrical relationship of the sample to the counter. In addition, in beta decay some particles travelling away from the counter are reflected back from the material on which the sample is mounted and may enter the tube (back scattering). Other beta particles will be absorbed in the window of the counter, in the air between counter and sample, or in the sample itself. The last-mentioned is known as self-absorption and is dependent on the energy of the beta particles and the weight of the sample per unit area. Samples are said to be of 'infinite thickness' when particles emitted by the lower parts are completely absorbed in the upper so that only the top layers contribute towards the observed counting-rate. Under these circumstances self-absorption is constant and can be neglected.

It is apparent that the radio-activity of samples must be measured under strictly constant conditions during the course of any experiment. By measuring standard samples of known radio-activity under the same conditions it is possible to determine the efficiency of the method and convert measured counting-rates into units of radio-activity.

Units of Radio-activity

The original unit of radio-activity was called the curie and was defined as the quantity of radon in equilibrium with one gram of its parent radium. Later the definition was changed to indicate the absolute radio-activity of one gram of radium ($3{\cdot}7\times10^{10}$ disintegrations/sec.). With the advent of artificial isotopes the curie was adopted as the unit for all radio-active sources and now indicates the amount of a radio-active isotope which contains $3{\cdot}7\times10^{10}$ disintegrating nuclei/sec. This unit is too large for tracer experiments and has therefore been subdivided into the milli-curie ($3{\cdot}7\times10^{7}$ disintegrations/sec.) and the micro-curie ($3{\cdot}7\times10^{4}$ disintegrations/sec.).

Another unit, the rutherford (10^6 disintegrations/sec.) has been proposed but has not met with general acceptance.

PREPARATION OF ISOTOPES FOR BIOLOGICAL WORK

It is necessary for biological purposes that the isotope be administered in appropriate chemical form and, in some cases, with the labelled element in a specific position in the molecule. In most instances conventional chemical methods are used for synthesis. However, many compounds of biological importance are prepared by means of biosynthesis—for example, plants exposed to $^{14}CO_2$ are used for the preparation of labelled sugars and pharmacological compounds such as nicotine and morphine, while algae grown in an atmosphere of labelled CO_2 are a source of various organic compounds including proteins and amino acids. Another method of preparing labelled compounds consists in the bombardment of the unlabelled substance with neutrons in the atomic pile. This procedure is usually unsuccessful because the energy liberated in nuclear reactions leads to rupture of chemical bonds within the molecule. However, it has been possible by this procedure to label the cobalt atom of vitamin B_{12} without destroying the biological activity of the vitamin.[4]

At the present time a great variety of labelled compounds of biological importance are available from the Atomic Energy Commission in the USA and the Radiochemical Centre in England. In the current catalogue of the latter, for example, more than 120 ^{14}carbon-labelled compounds are listed and the range of available products is constantly being extended.

II. GENERAL SCOPE OF THE ISOTOPE TECHNIQUE

The fundamental assumption underlying the use of radio-active isotopes is that, apart from the emission of radiations, their physical and chemical behaviour is identical with that of naturally occurring forms of the elements. This assumption is not always completely valid and observed differences are referred to as 'isotope effects'. These differences are concerned mainly with the energies of activation and the velocities of chemical reactions,[5] but are relatively minor in extent and for practical purposes can usually be ignored in biological work.

Methods employed for radiation detection respond to the decay of single atoms, so that as little as 1/1,000th microgram of a radio-active element may be sufficient for analysis. This remarkable sensitivity makes it possible to employ extremely small 'tracer' doses of isotopes and so to study the fate of labelled compounds in the organism without disturbing the natural equilibrium. It is not surprising that an analytical method having these unique properties should have found application in every branch of biology and taken a place among the standard procedures of clinical medicine.

In the consideration of the general scope of the isotope technique in medical science applications of the method will be divided into 4 categories dependent upon:

1. Localization of the labelled compound.
2. Measurement of dilution of the labelled compound.
3. Measurement of incorporation of labelled compounds into bodily constituents and 'turn-over' of such labelled constituents.
4. The therapeutic value of radiations.

LOCALIZATION OF LABELLED COMPOUNDS

The distribution of specific elements and compounds among various tissues of the body has been established by classical methods of chemical analysis. Thus, for example, potassium is known to be localized predominantly within cells, sodium and chloride are mainly extracellular, while certain elements such as iodine are highly concentrated in specific tissues. Radio-active isotopes by virtue of the sensitivity and ease of their measurement provide a convenient method of studying the distribution of compounds in the body. In addition, tracer methods constitute a unique means of investigating dynamic exchanges between tissues under conditions which do not disturb the natural equilibrium.

The chemical exchanges which occur across capillaries and the endothelium of the gastro-intestinal tract as well as between cells and their fluid environment have been extensively studied with the aid of tracer methods. It has been found in many instances that previously observed net exchanges are associated with an extremely rapid transfer of materials in both directions. Thus, the well-known 'chloride-shift' which occurs in erythrocytes during gas transport is superimposed upon a rapid exchange of this ion in both directions across the red-cell membrane.[6] During absorption of sodium and chloride from the gastro-intestinal tract these ions also pass freely from the circulating plasma into the intestinal lumen.[7] One of the most elegant applications of the isotope technique has been the demonstration of the fact that passage of a nerve impulse is associated with an increased movement of potassium outward and of sodium inward across the axon membrane leading to a transient accumulation of sodium within the nerve fibre.[8, 9, 10]

The tracer method has revealed that exchanges across capillary walls between intra- and extra-vascular compartments occur with extraordinary rapidity. A volume of water equal to the total amount present in the body is lost from the plasma every twenty minutes and is replaced by water from extra-vascular fluid, while 60% of the sodium and chloride present in the circulation is exchanged each minute.[11, 12] Plasma proteins pass out of the plasma more slowly, about 5% of circulating albumin leaving the blood-stream each hour.[13, 14] Traumatic shock does not appear to be associated with a generalized increase in capillary permeability, but there is considerable leakage of plasma protein in damaged areas,[15, 16] while injured cells become freely permeable[17] and may accumulate a large proportion of the body sodium, which is then unavailable for exchange with other tissues.

Following the administration of gamma-emitting isotopes the distribution of radio-active material can be traced by means of counters placed at the body surface. This technique forms the basis of several procedures which have proved to be of considerable clinical value. The simplest test of thyroid function using radio-iodine is the determination of the percentage of the isotope taken up by the gland within 24 hours of administration.[18] By means of an automatic scanning counter the size and functional activity of the thyroid can be mapped after injection of ^{131}iodine, the functional status of thyroid nodules determined, and metastatic thyroid tissue demarcated.[18] Circulatory efficiency in vascular disease can be assessed by comparing the counting rate over the affected region with that of an apparently normal area after the intravenous administration of radio-active sodium or labelled serum-albumin.[19] In addition, measurement of the rate of blood flow between two defined points or the rate of removal of radio-active material from intramuscular or subcutaneous sites provide a hitherto unobtainable over-all picture of circulatory efficiency.[20] Similar methods can be used to test the circulation in pedicle skin-grafts[21] and to determine the cardiac output.[21]

Rapidly proliferating cells may take up certain radio-active compounds more rapidly than normal tissues. There have been many attempts to make use of this phenomenon in localizing tumours of the liver[22, 23] and brain.[24] However, localization by means of gamma-emitting isotopes is liable to considerable error and with cerebral tumours the positron emitter, ^{72}arsenic, is likely to prove of greater value.[25] As stated previously, the positron undergoes an annihilation reaction when it combines with an electron and during this process two gamma rays are produced which emerge at 180° to each other. The use of a counter sensitive only to these coincident radiations allows the axis on which the tumour lies to be defined and by mapping two axes the site of the tumour can be fixed with some certainty.[26, 27]

ISOTOPE DILUTION

The isotope-dilution technique, which was first employed by Hevesy to measure the lead content of an ore, is a convenient and accurate means of determining the total amount of a specific substance present in a complex chemical mixture. A labelled form of the material to be determined is added in known amount and after complete mixing a small quantity of the material is isolated and purified. The degree of dilution of the tracer isotope provides a measure of the amount of the material originally present.

This method has been used in the analysis of many compounds of biological importance. Thus for example, the amino-acid content of haemoglobin,[28] B-lacto-globulin[29] and various enzymes[30] has been measured by isotope dilution. The same principle has been extensively used to estimate the volume of various fluid compartments of the body and the mass of specific chemical substances present in the living subject.

A basic requirement of such estimations is that isotope dilution should be calculated at a time when the label is uniformly mixed throughout the compartment or chemical substance under investigation. This may require prolonged observation and in some cases may be impossible to achieve. The volume of dilution of ^{82}bromine, for example, which is thought to be confined to extracellular fluid, expands significantly between 4 and 24 hours after administration to healthy adults, so that measurements of the extracellular space based on shorter periods of observation are erroneously low.[31] Moreover, it is probable that complete mixing of labelled bromine with the extracellular fluid is even slower in oedematous subjects.[32] Injected labelled-sodium is rapidly diluted by the sodium in the plasma and extra-cellular fluid and then for a period of 24 hours undergoes a further progressive dilution by exchange with the sodium of bone, which contains some 30% of the total quantity in the body. Thereafter no further dilution occurs, despite the fact that the isotope has exchanged with only about 45% of the total bone sodium. Thus, the isotope-dilution technique provides a measure of body sodium which excludes about half of that present in the skeleton;[33] this quantity is usually referred to as the 'total exchangeable sodium'. The dilution of radio-active potassium at 24 hours accounts for about 98% of the potassium present in the body, and excludes only a small amount in erythrocytes and bone which exchanges relatively slowly.[33] An accurate estimate of the total plasma-protein content of the body is difficult to obtain because labelled protein molecules enter the extravascular compartment relatively slowly and do not attain a stable and uniform distribution between plasma and lymph.[34]

A second basic requirement of the method is that the degree of isotope dilution should be measured at a time when the labelled compound is confined to the compartment or chemical substance under investigation. The rapid exchanges which occur among components of the body's metabolic pool make this difficult to achieve in many instances. Labelled glucose, for example, is rapidly converted into those chemical intermediates which lead to the formation of glycogen or oxidation to carbon-dioxide and water, so that estimates of the 'glucose pool' are considerably in excess of the total amount of free glucose present in the body.[35, 36] The use of tritrium(^{3}H)-labelled water[37] over-estimates the total body-water by about 2%, because the label exchanges with the hydrogen of the hydroxyl groups of carbohydrate and peptide bonds of protein.

Despite these limitations, which are due either to incomplete mixing or failure of the label to remain confined to the space or chemical substance measured, the isotope-dilution technique undoubtedly constitutes an analytical method of considerable clinical value—and one which is particularly useful in studying the constitution of the body fluids (Table II). Its application

TABLE II. BODY CONTENT OF WATER AND TOTAL EXCHANGEABLE SODIUM, POTASSIUM, CHLORIDE AND ALBUMIN, MEASURED BY ISOTOPE DILUTION IN HEALTHY HUMAN ADULTS

Total Body Water	44-70% of body-weight[24]
Extracellular Fluid	14-17% of body-weight[32]
Potassium	24-54 mEq./kilo body-weight[24]
Sodium	24-46 mEq./kilo body-weight[24]
Chloride	34-54 mEq./kilo body-weight[38]
Albumin	2·1-4·5 g./kg. body-weight[13]

in pathological states has emphasized the fact that it is frequently impossible to assess the body content of a component from its plasma level. Serum-sodium concentration, for example, is frequently low at the time when the total body-sodium is increased, and the reverse relationship is often true of potassium.[33]

INCORPORATION AND TURN-OVER STUDIES

The use of isotopes has undoubtedly found its most fundamental application in the study of intermediary metabolism. Ingested substances rapidly lose their identity in the body and it is impossible to discover what proportion has been stored, oxidised, or used in synthetic processes. By means of isotopic labelling, however, the fate of dietary compounds can be followed and the nature of the relatively simple precursors used by the tissues for synthesizing complex organic molecules can be established. By this means it has been shown, for example, that the creatine of muscle is built up from 3 amino acids—arginine, glycine and methionine,[39] that the haem pigment of haemoglobin is derived largely from glycine,[40, 41] and that acetate is the main precursor of cholesterol, which is synthesized almost exclusively in the liver.[42] Significant progress has also been made in elucidating the pathways of hormone synthesis in the adrenal cortex,[43] adrenal medulla,[44] and thyroid gland.[45] Studies of isotope incorporation have demonstrated the remarkable capacity of tissues for building up complex molecules from relatively simple precursors and have revealed that, in general, circulating proteins and lipids are not directly used in synthetic processes.

Before the introduction of isotope techniques the chemical constituents of tissues were regarded as being relatively inert, undergoing only slow 'endogenous' processes of catabolism, while ingested 'exogenous' compounds were thought to be almost completely broken down to yield the energy required by the body. The fallacy of this concept was revealed by the classical experiments of Schoenheimer and Rittenberg,[46] who demonstrated that even in animals on a deficient caloric intake an appreciable proportion of dietary fat was deposited in the fat depots. Since there was no corresponding increase in the mass of body fat it was evident that the constant deposition of adipose tissue was balanced by an equal rate of mobilization from the fat depots. Moreover, it was evident that fat oxidised at any time must be a mixture of dietary and tissue fat which had merged together in a common 'metabolic pool'. Later experiments with labelled amino acids revealed that tissue and dietary proteins are in a similar state of dynamic exchange, the rate of which varies considerably in different organs of the body. Proteins of liver, intestinal mucosa, kidney, spleen and plasma have been estimated to be completely replaced about once every 2 weeks in man;[47] the protein of muscle and skin, on the other hand, exchanges relatively slowly, while that of collagen is almost completely inert.[48]

These findings have given rise to the concept of 'turn-over', indicating the dynamic processes of synthesis and catabolism which underlie the maintenance of steady state conditions in the body. Turn-over studies involve observation of the rate of decline of radio-activity in a particular constituent following its initial labelling by means of an isotope. The exchangeable protein pool can be labelled, for example, by the intravenous injection of a tracer dose of ^{14}carbon[49] or ^{131}iodine-labelled[50, 51] plasma-protein and the subsequent decay of radio-activity measured on serial samples of plasma (Fig. 3).

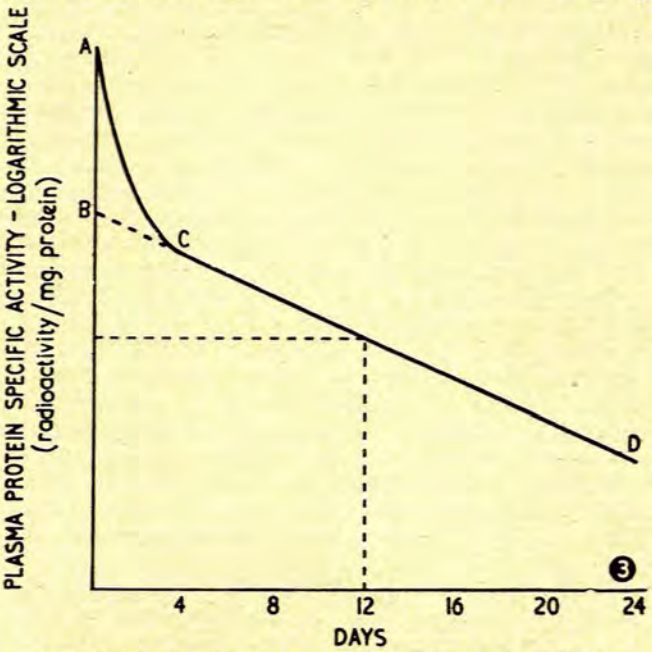

Fig. 3. Decline in plasma-protein radio-activity following the intravenous injection of a tracer dose of labelled protein.

A—plasma-protein radio-activity at 5 minutes after injection —the labelled molecules have mixed completely with the circulating blood, but have not escaped from the circulation.

AC—rapid decline in radio-activity due to mixing of labelled molecules with extravascular protein of lymph and tissue fluids.

CD—slower decline in radio-activity due to metabolic break-down of plasma protein and replacement by newly-synthesized, unlabelled molecules.

B—plasma-protein radio-activity which would have been observed had the injected labelled molecules mixed instantaneously with the entire exchangeable protein pool. The biological half-life is the time taken to reach half the value B, i.e. the time taken for half the molecules in the exchangeable pool to be catabolised and replaced (in this instance, 12 days, i.e. a turn-over of 5·8% of the exchangeable pool per day).

From such observations the 'biological half-life' is readily determined, i.e. the time taken for the level of radio-activity to fall to half the initial value obtained after complex equilibration. Red blood-cells labelled with radio-active chromium have a biological half-life of between 25 and 40 days in normal subjects.[52] A considerable shortening of the red-cell half-life is observed in haemolytic states as well as in some patients in whom there is no chemical or clinical indication of increased red-cell destruction.[53] Fibrinogen and beta-lipoprotein normally have a very much more rapid turn-over than albumin or gamma globulin. It is surprising that in cases of hepatic cirrhosis the biological half-lives of plasma proteins do not appear to be significantly altered.[54] It must be emphasized that the calculation of absolute rates of synthesis and catabolism

from the biological half-life may be misleading—especially when the labelled compound enters into many different metabolic processes or is not uniformly distributed between the various compartments of the exchangeable pool.[55]

THERAPEUTIC VALUE OF RADIATIONS

Several radio-active isotopes such as ^{60}cobalt, ^{90}strontium and ^{137}caesium, which is a waste product of the atomic reactor, have been employed as external or locally implanted sources of radiation. These are to be regarded as potent substitutes for X-radiation and radium therapy—^{60}cobalt, for example, is 35 times more radio-active (gram for gram) than radium. Other radio-active isotopes employed for therapeutic purposes are administered internally—a method initiated by the work with ^{131}iodine in thyroid disease[56, 57] and ^{32}phosphorus in leukaemia and polycythaemia.[58, 59] These two isotopes have particular therapeutic value because they are selectively concentrated in specific tissues—^{131}iodine in the thyroid gland and ^{32}phosphorus in all rapidly dividing cells, including those of the bone marrow. Partial or complete ablation of the thyroid in hyperthyroidism can be achieved with radio-active iodine,[60, 61] while polycythaemia vera can be brought under effective control with ^{32}phosphorus,[62, 63] which also has a measure of usefulness in chronic leukaemia.[64]

Internally administered radio-active isotopes may expose tissues throughout the body to potentially damaging radiations. This type of therapy therefore requires a thorough assessment of dosage distribution based upon knowledge of the physical half-life of the isotope employed, the nature and energy of its radiations and the metabolic fate of the labelled compound injected. Radio-active manganese, for example, is rapidly removed from the bloodstream and accumulates transiently in the liver and pancreas before being excreted into the gastro-intestinal tract. By injecting an isotope of manganese having a half-life of only 2·6 hours it is possible to obtain emission of about 96% of the radiation in liver and pancreas. The possibility of employing this isotope in the treatment of hepatic and pancreatic malignancies is being investigated.[3]

A new experimental approach to the problem of localizing the effects of irradiation is under investigation at the Brookhaven National Laboratory, where a technique known as neutron-capture therapy[3, 65] has been elaborated. A stable element which will readily capture a neutron and become radio-active is administered by intravenous injection and the appropriate region of the body is then exposed for a short period to slow neutron bombardment in a nuclear reactor. The element rendered radio-active is one in which decay is almost instantaneous and the range of radiation produced limited to a few microns. The rare stable isotope of boron (atomic mass 10) readily absorbs a neutron and immediately thereafter emits an alpha particle. Boron is concentrated in cerebral-tumour tissue so that its intravenous administration followed by neutron bombardment of the head liberates extremely potent alpha particles confined to the neoplasm itself; a series of patients with glioblastoma multiforme have been experimentally treated in this way with encouraging results.[66]

CONCLUSION

It will be evident from the examples quoted above that isotope techniques have been of great academic and practical value in many branches of medical science. New tools of investigation are often applied with over-zealous enthusiasm and with the isotopes it is necessary to remember that more direct methods can often be used to obtain the same information. Nevertheless, isotopes will be preferred in many instances—not because they are indispensible, but on account of greater convenience and sensitivity. It should also be borne in mind that although isotopes can be measured with remarkable precision, this does not imply that the results of tracer experiments are any less fallible than those obtained by other means. Tracer studies can seldom be interpreted in isolation, and successful application of the method is almost invariably based upon information derived from other chemical and physiological techniques of investigation.

Radio-active isotopes have contributed in one unique and outstanding way to medical science. It is impossible to visualize how the remarkably rapid exchanges which occur in both directions between the components of plasma and extracellular fluid and between cells and their chemical environment could ever have been measured without the use of isotopically labelled compounds. In this field of study the isotope technique has revolutionized the concepts of biological investigators and, in the future, will undoubtedly supply new standards of clinical measurement of considerable practical value.

The author is greatly indebted to Sir Charles Harington, F.R.S., and to Dr. A. S. McFarlane, who provided generous facilities for work in the Biophysics Division of the National Institute for Medical Research, London, during the tenure of a Nuffield Dominion Fellowship.

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