RADIOACTIVE ISOTOPES IN MEDICINE: A REVIEW

BY

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The following account is very selective: no attempt is made to deal exhaustively with radioisotope applications. In particular, practically no mention is made of treatment using, e.g., ^{131}I , ^{198}Au , or ^{90}Yt , or of the literature dealing with the localizing of tumours, much of which is unprofitable. Circulatory dynamics, e.g., cardiac output, are ignored, as are most biochemical investigations which have no direct clinical bearing as yet. Radiation dosimetry for individual tracer quantities and the organs most affected are to be found in the quoted references; recommendations and data for maximum doses are to be found in "Code of Practice for the Protection of Persons Working with Ionizing Radiations in National Health Service Hospitals" (1957). London, H.M.S.O.

Logical division into subject groups is almost impossible, and topics are discussed under the headings of counting techniques, haematology, chemical pathology, thyroid metabolism, and general considerations.

The Radiochemical Centre, Amersham, is the source of most radioactive materials, many of them processed, e.g., ^{58}Co -labelled vitamin B_{12} , and publishes stock and price lists which are readily available.

Counting Techniques

Geiger Muller Tubes.—These are both cheap and versatile. A large range of sizes, shapes, and characteristics is obtainable commercially, and some special modifications are made for specific purposes. "End window" counters with a specially thin window of material, which minimizes absorption of weak β rays, have been in use for some years. Much of the early work with ^{35}S and ^{14}C was done with these counters, and provided the amount of activity present in a sample (which is dried on to a disc or planchette of standard size) is of a reasonable order, nothing better is required. Sensitivity is very low, since apart from self-absorption considerable absorption occurs even with very thin mica windows (less than 1 mg./cm.^2), and this is the greater with decreasing energy of emission. Tritium (^3H) cannot be counted for this reason. The technique has been reviewed (Libby, 1947).

Improved counting efficiency (three- or four-fold) is obtained by placing the sample within the counter.

Self-absorption problems remain, but absorption due to intervening air and window is no longer a factor. Geometrical conditions are also improved. The correct pressure and quench gas filling must be maintained: a suitable apparatus is some form of gas flow proportional counter (Simpson, 1947; Taylor, 1953) where gas is allowed to flow through the chamber at atmospheric pressure to maintain the correct gas conditions in spite of leakage. Certain modifications, some with automatic sample changing devices, are available commercially. Expense is increased by the high amplification needed, but the apparatus is simple to use, and, for many purposes, satisfactory.

A useful modification of Geiger Muller tubes is the liquid sample β counter (Veall, 1948), in which a thin-walled tube is surrounded by an outer glass wall which contains the liquid sample to be measured. The geometrical conditions for counting are good, and give the most efficient means of counting energetic β emitters such as ^{42}K and ^{32}P . They are particularly useful for counting samples containing mixed isotopes in multiple isotope techniques.

Although Geiger Muller tubes are inefficient counters of γ rays, this does not matter provided there is enough activity present. Geiger Muller tubes can therefore be used for a number of purposes where they replace the more costly crystal scintillation counters. Rings of Geiger Muller tubes, made to accommodate, e.g., bottles containing 24-hour urine saves or cartons containing whole faecal specimens (Veall and Vetter, 1952; Veall, Lowe, and Whyard, 1955b), are most useful in the laboratory.

Scintillation Phosphors.—The standard γ -ray counting equipment in most laboratories are the well crystal and directional counter. The former consists of a block of phosphor with a central well into which a sample of a few millilitres contained in a glass tube is placed. Photons emitted from the phosphor are converted to electrical signals and amplified in a photomultiplier valve (Anger, 1951). The latter is of the same basic construction, but the crystal is surrounded by any desired shape of lead shielding, so that the counter "sees" a roughly cone-shaped volume of radioactive material (usually the tissues of a patient). The directional counter is used in many kinds of body surface counting, especially when activity in a particular organ is to be measured.

The most sensitive crystals are of thallium-activated sodium iodide, but their cost is very high. For precise differentiation of narrowly separated energy peaks in mixtures of isotopes this type of crystal is the best

and may be necessary in some multiple tracer techniques (Kahn and Lyon, 1953), but for most purposes the plastic phosphors now available are good enough. These are produced in many sizes and shapes and are very much cheaper than sodium iodide crystals. Availability and cheapness make possible more sensitive counters than Geiger Muller tube rings for large volume work, together with photomultipliers which have also been enlarged to give increased areas of light-sensitive cathode.

But the problem of very weak β emitters remains. Tritium, in particular, cannot be counted using these methods, and the very small quantities of ^{14}C used, by reason of its long biological and radiological half-life, in human tracer studies cannot be measured accurately by the counting methods already mentioned. Other techniques have therefore been developed.

Gas Counting.—The sample is converted to a gas by combustion and the gas then led into a Geiger Muller tube or ionization chamber (Banks, Crawhall, and Smyth, 1956; Miller, 1947; Brownell and Lockhart, 1952).

The technique is laborious, requires considerable skill in operation, and has a complicated vacuum system for transferring samples. The sample is destroyed in preparation, and rather complicated chemical preparation may be necessary. On the other hand, counting efficiency is almost 100%, due to elimination of absorption losses. As an ordinary laboratory tool, however, the disadvantages are too great.

Liquid Scintillation Counting.—The sample is dissolved in the same liquid as soluble plastic phosphor. Once again absorption losses are very small, the radioactive atoms being intimately mixed with the phosphor. Efficiency depends then on optics and the photomultiplier. This is an oversimplification, and there are numerous snags. Unless the sample is soluble in the organic solvent which dissolves the phosphor, its volume is limited to the amount which can be got into solution using, e.g., a water-alcohol-solvent system. Any pigment in the sample diminishes light transmission, and proteins tend to be precipitated, producing opalescence which again interferes with light transmission. (Increased solubility of various substances can be attained by using some quaternary amines, e.g., hyamine (Radin, 1958).) The molecular structures of the compounds to be counted may themselves produce a quenching effect (Kerr, Hayes, and Ott, 1957). A further difficulty is the "noise," adding to background, of the dark current produced by the photomultipliers. In the United States commercial equipment is available using photomultipliers "in coincidence." No signal is accepted by the counting circuit unless it arises simultaneously in the two photomultipliers, i.e., the signal arises due to the advent of a photon in the phosphor and not to a random impulse in either photomultiplier. In addition, the whole apparatus is refrigerated since the

dark current increases with temperature. The resulting equipment is efficient, with very low backgrounds, for tritiated water and other ^3H -compounds, but is enormously costly and bulky.*

Recent developments with single photomultiplier systems have shown that similar efficiency is obtainable by using an improved photomultiplier of small light-cathode area, and by paying close attention to optics. Equipment is much less bulky, does not require refrigeration, and is only a fraction of the cost. Efficiency is about equal, and quantities of about 0.1–0.2 millimicrocurie of ^3H per sample can be counted.

In addition to the small volume scintillation counters described above, large volume detectors have been developed. These are mainly used for whole body counting, large plastic phosphors being arranged in batteries, or a large annular tank, filled with liquid scintillator and capable of accommodating, e.g., a human adult, is used (Hayes, 1956; Bird and Burch, 1958). Although whole body counters using Geiger Muller tubes in suitable geometrical form (Fowler, 1957; Veall, Fisher, Browne, and Bradley, 1955a) are quite adequate in some metabolic turnover studies, their sensitivity is low, and spectrographic separation of γ rays is not possible. Large volume scintillation counters, however, are capable of measuring, e.g., the body content of naturally occurring ^{40}K .

Ionization Chambers.—For routine counting in the laboratory ion chambers are not widely used, although, since the chamber can be large, and the gas filling is not critical, accurate measurement of very low specific activity gas samples, e.g., of ^3H and ^{14}C , can be counted. However, extremely high quality small current measurements are necessary, using, e.g., a vibrating reed electrometer, and the apparatus is not cheap. Nevertheless, the ion chamber is widely used for radiation monitoring, and most radiotherapy laboratories use them. If available, an ion chamber is extremely useful for measuring quantities in the order of tens or hundreds of microcuries, and thus tracer doses of various isotopes can be measured and checked simply in a way which is not possible with other routine laboratory counting devices (Veall, 1952).

Haematology

Blood Volume and Red Cell Survival.—The use of ^{51}Cr as a label for red cells (Gray and Sterling, 1950) has largely superseded methods involving other radioactive labels, such as ^{32}P . The labelling process, using the patient's own blood, is simpler, incubation is unnecessary, and the number of washings reduced (Mollison and Veall, 1955). The half-life of ^{51}Cr , 27 days, is more nearly that of the red cells, and the rate of loss of the label from the cells is less rapid and more

* For an exhaustive discussion of liquid scintillation counting problems see *Liquid Scintillation Counting* (1958). C. G. Bull, Jr., and F. N. Hayes (Editors). Pergamon Press, London.

predictable (Hughes Jones and Mollison, 1956). ^{51}Cr from old cells is not reincorporated into new cells. The mechanism of labelling is thought to depend on the fact that hexavalent chromium can pass through the red cell membrane, after which it is reduced to the trivalent form and bound on to cell protein: on breakdown of the cell the trivalent chromium thus released cannot pass through the membranes of new cells.

Of other isotopes used in red cell labelling, ^{32}P (Hevesy, Köster, Sørensen, Warburg, and Zerahn, 1944; Reeve and Veall, 1949; Chaplin, 1954) has many comparative disadvantages, but can be used when simultaneous volume or short-term survival measurements are made, since it can be differentiated by physical means from ^{51}Cr . It also has the advantage that it can be measured by Geiger Muller counting and crystal scintillators are not necessary. ^{42}K can be used in the same way as ^{32}P (Yalow and Berson, 1951); it has the advantage of a very short half-life (12.4 hours) when repeated red cell volume measurements over a short period are needed.

In spite of the simplicity of ^{51}Cr labelling, the technique requires care, and a standard procedure should be used. There is evidence that the subsequent elution rate from injected labelled cells is dependent on the method of preparation, and, in addition, chromate in high concentration is toxic to cells (Hughes Jones and Mollison, 1956).

Blood Volume.—Injected ^{51}Cr -labelled cells are presumed to be distributed throughout the vascular space in a short time. Ideally, several samples are taken over a period of, e.g., 30 minutes, and the slope obtained extrapolated back to zero time and theoretical instantaneous mixing (Sterling and Gray, 1950). Loss of label by elution is quite small over the period, and the slope is usually quite flat after an initial mixing period of about five minutes in the normal, so that commonly only one sample is taken at 10 minutes and extrapolation ignored. Equilibration may be delayed in some abnormal circulatory states, e.g., shock and cardiac failure (Noble and Gregersen, 1946). In these circumstances simplified procedures may not give valid results.

Counting is done on whole blood using a well type scintillation counter, and the red cell volume, and, indirectly, the whole blood volume, obtained using the venous haematocrit value, to which a small correction is usually applied for trapped plasma (Chaplin and Mollison, 1952). From simultaneous measurement of red cell volume and plasma volume, using a different indicator for each, it has been shown (Chaplin, Mollison, and

Vetter, 1953) that the venous haematocrit ratio is not representative of the whole body R.B.C./plasma ratio. (Capillaries contain comparatively more plasma than do larger vessels (Everett, Simmons, and Lasher, 1956; Gibson, Seligman, Peacock, Aub, Fine, and Evans, 1946). In general, whatever the venous haematocrit, there is a relationship between it and the whole body R.B.C./plasma ratio, so that multiplication by 0.91 gives a valid correction. It is stated that the exception is in the patient with anaemia and splenomegaly, in which case the whole body R.B.C./plasma ratio may be greater than the venous haematocrit ratio (Verel, 1954; Rothschild, Bauman, Yalow, and Berson, 1954).

Recent work, simultaneously using ^{51}Cr -labelled cells for red cell volume and ^{131}I -H.S.A. (human serum albumin) for plasma, has demonstrated that when the blood volume is rapidly increased by using "plasma expanders," such as dextran and albumin, the body R.B.C./plasma ratio may vary widely from the venous haematocrit. Indeed, it appears that in some circumstances parts of the vascular volume become inaccessible to the label, and this inaccessibility may vary in different ways for plasma and red cells, the absolute red cell volume appearing to shrink while the plasma volume expands (Hayter and Clapham, 1960). It may therefore be necessary, in some abnormal circulatory states, to have reservations about the applicability of the "whole body haematocrit" correction factor.

The haematologist is usually specifically interested in the red cell volume, but direct measurement of plasma volume may be needed, for instance, when values for total plasma iron are required (see Iron Metabolism), or when simultaneous measurements of red cell and plasma volumes are needed to give more precise measurement of total blood volume. ^{131}I -H.S.A. has the advantage of relative simplicity in the measurement of the indicator concentration in the plasma, and ^{131}I or ^{132}I -labelled human serum albumin provides a suitable indicator for plasma volume measurement. Compared with dyes, ^{132}I -H.S.A. combines this with repeatability, the short half-life of the ^{132}I (2.3 hours) preventing the build-up of high concentration of indicator from estimation to estimation. Iodinated human serum albumin can be conveniently prepared in the laboratory, although it can be obtained ready made from the Radiochemical Centre, Amersham. Laboratory preparation is mandatory for ^{132}I -H.S.A., of course, because of its short half-life (Veall, Pearson, and Hanley, 1955c; Cook, Eakins,

and Veall, 1956). About 10% of a given dose of human serum albumin disappears from the circulation in an hour (Pritchard, Moir, MacIntyre, and Inkley, 1955) so that when mixing time is expected to be prolonged it is even more important to extrapolate through several readings.

Some interest has been shown in the possibilities of rapid and simple methods of measuring blood volume in emergency, e.g., in acute blood loss due to injury or post-operatively. Quick methods designed to be used, perhaps by the house surgeon, have been devised. It is doubtful whether assessment of blood volume diminution by this means offers any advantage over clinical observation. If acute cardiac failure (with which sudden blood loss may be confused) is liable to give anomalous results, there is a distinct possibility that a transfusion might be given where it is absolutely contraindicated.

Values quoted for blood volumes vary over a wide range; they are usually expressed as millilitres per kilogram of body weight. Since the distribution of blood through fatty and lean tissues is not equal, and since fat/lean ratio is so variable in humans, weight as an index is imprecise. It has been shown that when a more precise index is used (in this case "lean body mass") differences in red cell volume of quite small degree can be demonstrated (Muldowney, 1957). Further reference to this is made in the section on body fluids and electrolytes.

Red Cell Survival.—The Ashby technique of differential agglutination (Ashby, 1919), although capable of giving good information on cell survival, is difficult to perform, is very laborious if enough cells are counted to give reasonable accuracy, and, apart from demanding a proper compatibility relationship between the donor and recipient bloods, it also assumes that the donated cells will survive in the same manner as the recipient's. In addition, there is evidence that normal cells will survive less well than the patient's in acquired haemolytic anaemia (Hughes Jones and Mollison, 1956). In spite of apparent blood group compatibility, the survival is sometimes curtailed in an unexpected way, presumably due to unrecognized antibody (Loutit, Mollison, and Young, 1943; Jandl and Greenberg, 1957). Primarily, however, it is the technical difficulties which have prevented its wide use clinically. Apart from the relative simplicity of ^{51}Cr labelling and counting, cell survival study using this technique has the advantage that the patient's own cells are reinjected, so that with certain reservations their survival should be exactly similar to

that of the red cell population. In addition, tests of survival of possibly incompatible donor red cells can be performed (Mollison and Cutbush, 1955, 1958), and data can be obtained on the useful life of stored blood under different conditions (Chaplin and Mollison, 1953).

When a random sample of red cells is labelled and reinjected into a red cell pool, provided that the sample consists of a normal distribution of cell age, their survival could follow two main patterns. If the cells are assumed to have a finite life span, a constant proportion will disappear in each unit of time, until, if the percentage surviving were plotted against time, zero would be reached at a time equivalent to the cell life. In fact such a linear plot would show some tailing off towards the end, which would depend on the variance of cell lives about the mean, but extrapolation of the linear part of the curve to zero would give the mean cell life. It is this pattern which has been mainly demonstrated in normals using the Ashby technique, and which gives the accepted mean cell life figure of 100 to 120 days. On the other hand, it can be assumed that the cells are potentially immortal, and that they are destroyed by some random haemolytic process, in which case, if a constant quantity were destroyed in each unit of time, the numbers of labelled cells would diminish exponentially. In this case, the mean cell life would be that time at which 37% of the cells remain. In practice, the results obtained are something of a combination of the two processes, the former behaviour being most marked when there is an intrinsic cell defect, and the latter when there is a marked haemolytic process. The expected behaviour will be modified by a number of factors: if the age distribution of the cells in the labelled sample is abnormal (and this will be so in the case of impaired cell survival); if the cells are not uniformly susceptible to an extrinsic destructive agent; and if the rate of production of cells is fluctuating during the time of survival measurement the shape of the curve will be influenced. Theoretical consideration of the above factors is given by Dornhorst (1951).

When a sample of *in vitro* ^{51}Cr -labelled cells is reinjected into a patient, there is a rapid early loss (over two to three days) of about 10% of the label followed by a slower, predictable rate of elution (Ebaugh, Emerson, and Ross, 1953; Mollison and Veall, 1955). Corrections for these losses can be made (Hughes Jones and Mollison, 1956). The type of correction (tabulated factors for which are given in the previous reference) will depend on the type of curve. An empirical correction factor

allowing for the initial rapid loss, and relating the actual survival time to the conventional Ashby survival slope, is applied to the "linear intrinsic defect" type of slope, and simple correction for slow elution to the exponential "haemolytic" type.

Interpretation presents some difficulties. The corrections for elution do not produce the linear slope expected for normal cell survival, and although mean cell life might be obtained from the early part of the curve, the distortion produced by the initial rapid loss of ^{51}Cr makes this difficult, especially in interpreting the curves of only slightly abnormal cell survival. Mean cell life as an expression of cell survival is therefore not satisfactory. Expression in terms of half-life of the ^{51}Cr -tagged cells is also objectionable on the grounds, first, that when the regression rate is slow, considerable extrapolation may be necessary, and, second, that use of the half-life concept depends on a strictly exponential decline which can only be true when random destruction is greatly in excess of loss due to age (Dornhorst, 1951).

It has been shown (Mollison and Veall, 1955) that in normal subjects survival curves plotted on semi-logarithmic paper (ignoring the "early loss" period) deviate from linearity little, up to 50 days after injection of labelled cells since the loss of ^{51}Cr is very slow; when the slope is steep, it is likely to be due to an increase in the rate of random destruction, and so the slope will even more nearly approximate an exponential. Using this empirical method the time at which 50% of the labelled cells are still in the circulation has been used as an expression of cell survival, and this is variously given as 25 to 32 days in the normal. The disadvantage in practice is that the length of time for which the survival must be followed in order to express results in this form is sometimes too prolonged (up to 30 days) when the survival is not grossly impaired, although in cases of special interest it may be desirable, since, as in measurement of any constant rate of change, the longer the rate of ^{51}Cr loss is measured the greater will be the accuracy of the measurement.

It has been shown, however (Wetherley-Mein, Epstein, Foster, and Grimes, 1958; Jones, Wetherley-Mein, Ingram, and Langmead, 1960), that if the values for ^{51}Cr activity are plotted on semilogarithmic paper for seven to 10 days, starting 48 hours after the injection of labelled cells (thus excluding the period of rapid early loss), and the best-fitting straight line drawn through the points, the percentage loss per day of ^{51}Cr can easily be calculated. By this method rates of loss greater than about 5% are abnormal. Although a figure of less than 5% per day over

such a short period does not necessarily mean that the survival is normal it does exclude any gross degree of haemolysis. As mentioned above, smaller degrees of impairment of survival would necessitate a longer period of study. The advantage of the technique is that it can readily be applied in routine clinical "work-outs" and can be combined with ^{59}Fe studies, i.e., plasma iron clearance, ^{59}Fe turnover and utilization, and surface counting (Wetherley-Mein, Hutt, Langmead, and Hill, 1956; Wetherley-Mein *et al.*, 1958). The method is also useful in before-and-after studies such as assessing the effects of treatment.

An ingenious method for testing the compatibility, which may be doubtful, of donor blood has been demonstrated. A small quantity of the donor cells is labelled with ^{51}Cr and a control sample of the possible recipient's with ^{32}P . The short-term survival of the donor's blood is then compared with that of the control in the recipient, and the presence of serologically unidentified antibody, if present, is detected (Mollison and Cutbush, 1955, 1958).

Surface Counting.— Directional scintillation counting over the liver and spleen when labelled cells are injected shows a slow and small rise in the normal presumably due to destruction of cells and/or accumulation of ^{51}Cr by these organs (Hughes Jones and Szur, 1957). In haemolytic anaemia, the quantity of ^{51}Cr in the spleen, and sometimes the liver, may be very large, and in some cases the removal of the spleen has been followed by good haematological remission (Hughes Jones and Szur, 1957; Jandl and Greenberg, 1957; Schloesser, Korst, Clatanoff, and Schilling, 1957). (Surface counting technique is further discussed in the section on iron metabolism.)

Platelet Survival.— Some information on the survival time of platelets is available. Platelets have been labelled with ^{35}S , and their survival and distribution followed in the rat (Cronkite, Bond, Robertson, and Paglia, 1957), in humans (Leeksa and Cohen, 1956), and in rats (Hjort and Paputchis, 1960), by labelling cells with di-isopropylfluorophosphate tagged with ^{32}P (DF ^{32}P). The half-life is two to four days; those investigations using platelets labelled *in vitro* are open to the criticism that the platelets may well be altered by the preparation.

Gastro-intestinal Bleeding.— Quantitative measurement of blood loss via the gastro-intestinal tract can be made by measuring the ^{51}Cr appearing in stools after an injected dose of ^{51}Cr -labelled red cells. ^{51}Cr is not normally excreted to any

appreciable extent in the gastro-intestinal tract so that, provided the blood volume is first measured, the fraction of the given dose appearing in the stools day by day can be expressed in terms of millilitres of blood lost (Owen, Bollman, and Grindlay, 1954). Counting is conveniently done on large faecal samples in a Geiger Muller tube ring counter (Hughes Jones, 1958) although, since this type of counter is relatively insensitive to ^{51}Cr , efficiency is somewhat reduced compared with well-type scintillation counting of emulsified aliquots of the faeces. For most clinical purposes, however, the Geiger Muller ring counter is good enough, and the unpleasantness of emulsifying faeces is avoided. Quantities in the order of 3 to 5 ml. blood loss per day can be detected.

Iron Metabolism.—Much new information on iron metabolism has become available since radioisotopes of iron have been introduced. Previously information on this topic was provided mainly by laborious balance studies and by interpretation of plasma iron concentration levels, which, though useful, are very limited in value. ^{59}Fe is the isotope in general use; it is a γ emitter, easy to count by conventional means, including external body counting, and it has a suitable half-life (46 days). A further isotope, ^{55}Fe , is available and has been used simultaneously with ^{59}Fe , but is considerably more difficult to detect (see below).

Absorption.—The method in common use is to give the tracer dose by mouth as ferrous salt in several milligrams of "carrier" (non-radioactive salt). It is said that ferrous salt is most readily absorbed (Moore, Minnich, and Roberts, 1944) and that achlorhydria does not affect absorption (Moore and Dubach, 1951; Pirzio-Biroli, Bothwell, and Finch, 1958), although recent claims have been made that the addition of ascorbic acid increases absorption even when ferrous salt is used (Bonnet, Hagedorn, and Owen, 1960). The amount absorbed is most frequently obtained by difference after counting the quantity of ^{59}Fe recovered in faeces, which must be collected for some days (Badenoch and Callender, 1954; Smith and Mallett, 1957). As usual, collection difficulties may be encountered. For clinical purposes a ring counter (Geiger Muller tubes) is suitable, and obviates the need for homogenizing the faeces.

A further method, which gives a direct measurement of the amount absorbed and is probably more accurate, uses ^{59}Fe and ^{55}Fe simultaneously. A dose of one is given by mouth, and of the other intravenously. After an interval (10–14 days) the percentage absorption is obtained from the relative amounts of the two isotopes in the red cells com-

pared with the dose given (Bothwell, Pirzio-Biroli, and Finch, 1958). Simple measurement of plasma concentration or iron turnover rate is too dependent on additional factors to reflect absorption using one isotope only. This technique has not yet been used extensively because of the technical difficulties of counting ^{55}Fe . This isotope emits very soft x rays only, and requires the use of the gasflow proportional counter or liquid scintillation counter. When suitably prepared the quantities of the two isotopes in a mixture can be separated by physical means (Dern, 1958), but preparation is laborious. For use in the proportional counter the iron is electroplated on to planchettes after digestion of the plasma; when liquid scintillators are used, the iron is first quantitatively electroplated on to wire and then dissolved as the perchlorate in a form suitable for solution in the phosphor-solvent system (Dern, 1960). Although the procedure is not simple it may well repay its use in future studies in intermediary iron metabolism.

While the fractional absorption of a given dose of iron is dependent on the state of the iron stores and/or erythropoietic activity at the time (Bothwell *et al.*, 1958), it is also dependent on the size of the dose, the absolute absorption increasing with the quantity given (Smith and Mallett, 1957). Thus comparative studies should always be made with a standard dose (Bonnet *et al.*, 1960). Iron absorption is decreased in steatorrhoea (Badenoch and Callender, 1954) and sometimes decreased in post-gastrectomy states (Chodos, Ross, Apt, Pollycove, and Halkett, 1957). Decreased absorption in this case may be due to unavailability of dietary iron. However, in many post-gastrectomy iron deficiency anaemias (and probably in most other cases which appear to be unresponsive to oral iron therapy), the iron absorption is increased, the anaemia being due to occult bleeding (Smith and Mallett, 1957). Recently, interest has been shown in the problem of availability for absorption of dietary iron, and methods of labelling the iron in foodstuffs during their growth, e.g., in vegetables and eggs, are being developed (Halkett, Chodos, and Ross, 1959).

Plasma Turnover.—The curve of disappearance (or "clearance") of an injected tracer of ^{59}Fe is exponential (though not always simple). The normal range of half-clearance is 70–140 minutes (Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence, 1950) and will depend on the size of the plasma iron pool, the exchangeable stores, and utilization rate of the bone marrow and other tissues (Huff *et al.*, 1950). Thus, in both pol-

cythaemia vera and iron-deficiency anaemia the disappearance rate will be high, in the first place because the marrow utilization is increased, and in the second place because although marrow utilization may be low, the plasma pool is low and the turnover rate, therefore, still high. The pathways of disappearance are not all clearly defined; it is probable that the iron stores cannot be looked upon as a single compartment dynamically, and although such iron depots as the myoglobin do not appear to be part of the pool (West, Hahn, Clark, and Chappelle, 1952) in the normal state, it is clear that iron deficiency affects not only the haemoglobin pool, which is probably the largest single fraction of the total iron (Huff and Judd, 1956), but other iron-dependent functions as well. In chronic severe iron deficiency the skin and nails at least suffer, and it appears that depletion of iron in these is delayed until the total circulatory iron is considerably diminished. In abnormal states, then, some compartments of the pool may be avid for iron when normally equilibration with them is very slow. Such considerations make assessment of bone marrow reactivity, in terms of iron utilization, difficult and inaccurate (see below).

The disappearance rate is increased in haemolytic anaemia and in pernicious anaemia as well as iron deficiency and polycythaemia; it is decreased in bone marrow aplasia and most acute anaemias (Fig. 1). Considerations already discussed make it clear that plasma iron turnover would give a better idea of utilization than half-clearance; this can be calculated from the half-clearance and the quantity of the total plasma pool, which can be calculated from the plasma volume and plasma iron concentration (Huff *et al.*, 1950). This is usually expressed as plasma iron turnover per day, but since the disappearance rate is very variable over a short period (Bothwell and Mallett, 1955) the results may be considerably in error.

Erythropoiesis.—The curve of appearance of iron in red blood cells after an intravenous injection of ^{59}Fe is an inverse exponential approaching equilibrium (80–90% of the injected iron) 10 to 16 days after injection. This will be achieved more or less rapidly according to how far the actual turnover of the marrow deviates from the normal, but the value attained will be affected also if the rate of return of ^{59}Fe to the pool due to destruction of red blood cells is significant. Maximum utilization will always be achieved rapidly in iron deficiency in spite of obviously lowered haemopoietic activity, since the rate and degree of utilization will be

FIG. 1.—Disappearance curves after intravenous injections of ^{59}Fe . Normal range for 50% disappearance (shown on graph) is 70 to 140 minutes.

affected by the size of the plasma and storage pool. Haemolytic anaemias often show a lowered apparent utilization (in spite of obviously increased bone marrow reactivity), presumably due to the random nature of the haemolytic process. Typical utilization curves are shown in Fig. 2. The curve marked "polycythaemia" would also serve as an illustration of Fe deficiency, and that marked "renal failure" would serve for any marrow hypoplasia and for increased haemolysis. Relating the fraction of the dose appearing in the red cell mass at equilibrium to the plasma turnover rate gives a somewhat better index (Huff *et al.*, 1950), but this introduces the errors in iron turnover estimation previously discussed. Further indices, all empirical, have been suggested, such as relating the rate of appearance of labelled red cells to maximum utilization of ^{59}Fe (Joske, McAlister, and Pranker, 1956), but none gives an entirely satisfactory measure of erythropoietic activity. One of the principal difficulties lies in the inability to determine the fraction of iron which is utilized for production of red blood cells; a large quantity of the body iron is not concerned with haemoglobin, and the behaviour of this portion, especially

FIG. 2.— ^{59}Fe utilization curves. Quantity of radioactivity appearing in red cells after dose of ^{59}Fe . Normal utilization (70–90% of administered dose) achieved in seven to 10 days. Full utilization would not appear to be achieved if considerable haemolysis were taking place. "Polycythaemia" represents typical response in high erythropoietic rates, "renal failure" typical of low rates. Fe deficiency, in spite of low erythropoietic rate, would show rapid and complete utilization because of smallness of the iron pool.

in disease, is virtually unknown. Other factors may become of major importance in disease states; one is that reticulocytes take up iron directly from the plasma (Belcher and Courtenay, 1959).

Combined ^{51}Cr and ^{59}Fe Investigations.—Simultaneous measurement of marrow reactivity and red cell survival is comparatively simple with conventional well type scintillation crystals (Weinstein and Beutler, 1955), and several workers have investigated common anaemias in this way. Uraemic anaemia is shown to be due to lowered marrow reactivity, together with a progressive shortening of cell survival time (Joske *et al.*, 1956); in leukaemia the anaemia may be due to decreased marrow reactivity and/or a shortened cell survival (Wetherley-Mein *et al.*, 1958); in pernicious anaemia the effective erythropoiesis is reduced in spite of a high plasma iron turnover, and the red cell survival is reduced (Finch, Coleman, Motulsky, Donohue, and Reiff, 1956). For clinical purposes a technique which will give semi-quantitative data on marrow reactivity and cell survival in seven to 10 days has been worked out (Wetherley-Mein *et al.*, 1958; Bailey and Pranker, 1958).

Surface Counting Technique.— ^{59}Fe emits γ rays of sufficient energy for external detection. A directional scintillation counter placed over an organ will measure changes in the concentration of ^{59}Fe within it, but the measurement will not be quantitative, due to the size and shape of the organ relative to the counter, the proximity of other organs in which the concentration may be changing, the general scatter of γ rays by the tissue, and the concentration of the isotope in the blood perfusing the organ (Huff, Elmlinger, Garcia, Oda, Cockrell, and Lawrence, 1951). The sacrum is chosen as a suitable site for marrow concentration measurement, and the heart represents blood concentration; the other sites commonly measured are liver and spleen. Considerable qualitative information is available from following the counts over these organs with time. The counts over each organ are crudely corrected for blood activity represented by counts over the heart (Wetherley-Mein *et al.*, 1956) and a curve representing activity for each organ is plotted against time. In normals maximum activity is present in the marrow at about 24 hours; thereafter the marrow activity falls and the blood level, previously having dropped, rises once more as the ^{59}Fe moves into the circulation tagged to red blood cells. The pattern of events in abnormal states, such as marrow aplasia, leukaemia, and myelofibrosis, have been clearly shown (Wetherley-Mein *et al.*, 1958), and their clinical significance discussed.

Typical curves are shown in Fig. 3. The high figures for concentration in the liver and spleen are not generally associated with extramedullary haemopoiesis, although this of course does occur in these conditions. It is presumed that large non-erythrocytogenic sources of Fe uptake are involved which are not normally present.

Comment.—The development of the techniques discussed above has led to an improved concept of the dynamics of erythropoietic turnover. The static view of the relation of marrow activity, total red blood cell (and haemoglobin) pool, and survival of red blood cells, had given rise to the chaotic "classifications" of anaemia which still survive in many textbooks. Most abnormal states can now be interpreted in terms of the interaction of these variables, and a more rational approach to disturbed mechanisms in blood diseases not yet worked out is to be expected.

B_{12} Absorption.— ^{58}Co -labelled B_{12} is now almost universally used, the vitamin being produced biosynthetically using *Streptomyces griseus* (Radiochemical Centre, Amersham). ^{58}Co

FIG. 3.— ^{59}Fe surface counting graphs showing distribution in organs of injected ^{59}Fe with time. The "organ content" curves are crudely corrected for contained blood activity by subtracting the "heart" count, assuming the "heart" to represent blood. Marrow activity from count over sacrum, giving a crude measure of bone marrow reactivity. Similar procedures are used to estimate sequestration of red cells in spleen in, e.g., hypersplenism, using ^{51}Cr as red cell label.

has the advantage of a shorter half-life (71 days) over ^{60}Co (5.3 years) which was used in earlier work. The vitamin tends to be destroyed by auto-irradiation, but freeze-drying prevents this. Supplies from the Radiochemical Centre are therefore in this form. When the stock quantity is dissolved for dispensing as tracer doses, however, degradation must be expected, so that small quantities only should be obtained and used as quickly as possible.

The fraction of B_{12} which is absorbed varies with the dose given, and a standard quantity (0.5–1.0 $\mu\text{g.}$) should be administered. Reasonably accurate measurement can be made using less than 1.0 $\mu\text{c.}$ The tracer is given after the patient has fasted overnight.

Three main methods for measuring absorption are used: (1) measurement of faecal excretion of ^{58}Co , and therefore, by difference, the fraction absorbed (Heinle, Welch, Scharf, Meacham, and Prusoff, 1952); (2) excretion in the urine after "saturation" by a parenteral dose of unlabelled B_{12} (Schilling, 1953, 1955; Ellenbogen, Williams, Rabiner, and Lichtman, 1955); and (3) by measurement of hepatic uptake (Glass, Boyd, Gellin, and Stephanson, 1954; Glass, Boyd, and Gellin, 1955a; Glass, Pack, and Mersheimer, 1955b; Booth and Mollin, 1956).

In the first method faeces are collected and counted until consecutive stools contain less than 1% of the administered dose. This is conveniently done in the Geiger Muller ring counter previously mentioned (Veall and Vetter, 1952). The difficulties are the usual ones where collection of faeces over several days is required. The urine excretion method requires that a "flushing" or "saturation" dose of B_{12} be given parenterally at the same time as the oral tracer dose. Urine is then collected over the next 24 hours and an aliquot measured in a well-type scintillation counter. The flushing dose ensures that a measurable proportion of the absorbed labelled vitamin is excreted, but haematologically it may "queer the pitch" for further investigation in cases of doubt. Urine collection troubles may of course be experienced. Concentration of the ^{58}Co in the urine can be achieved quite simply (Buchholz, 1958) by adsorption on charcoal, thus improving sensitivity, and allowing a reduction in dose. There is some evidence that parenteral flushing itself depresses B_{12} absorption (Baker and Mollin, 1955a; Callender and Evans, 1955), and renal excretory failure may give anomalous results. Measuring hepatic uptake by surface counting with a directional scintillation counter is simple, but quantitative measurements are not possible due to

FIG. 4.—Comparison of surface activity over liver and gut in normal patients and patients with pernicious anaemia. Liver activity is the average of counts at two sites over the liver, and gut activity average of four fixed sites over the abdomen. Sodium iodide directional scintillator used: no collimator.

variations in anatomy, except perhaps by direct comparison with a subsequent intravenous dose. In pernicious anaemia, however, the activity over the liver, after time has been allowed for the gut activity to fall by excretion, is practically undetectable whereas in normal absorption a significant count is present for many months (Glass *et al.*, 1955b). Clinically the method may be useful as an out-patient screening test, or in cases of faecal or urinary incontinence, which occasionally occurs in subacute combined degeneration of the cord. Typical curves for a normal subject and a patient with pernicious anaemia are shown in Fig. 4.

Plasma activity levels are usually not accurate enough for diagnosis using B_{12} at the specific activity usually obtainable, and in the doses normally used, but some studies have been done (Booth and Mollin, 1956) and more information on turnover and utilization could be obtained using material of a high specific activity.

Experiments have shown that B_{12} absorption is greatly reduced or absent in pernicious anaemia, that absorption is restored to normal provided intrinsic factor is given with B_{12} , and that the

fraction absorbed is, over a short range, proportional to the amount of intrinsic factor given. Thus assay of intrinsic factor is possible (Baker and Mollin, 1955b; Callender, Turnbull, and Wakisaka, 1954) in patients without endogenous intrinsic factor. Hepatic turnover of B_{12} is very slow (Glass *et al.*, 1955b) as would be expected from the delayed appearance of megaloblastic anaemia following total gastrectomy and of haematological relapse when B_{12} therapy is stopped. The renal excretion of B_{12} appears to be more rapid than its uptake by the liver, which, even when depleted, appears to be slow.

In total gastrectomy absorption is similar to that in pernicious anaemia, and is likewise restored by intrinsic factor (Halsted, Gasster, and Drenick, 1954; Heinle *et al.*, 1952). In some normal fasting patients it appears that intrinsic factor may not be secreted in sufficient quantities to ensure absorption of a tracer dose; these are distinguished from abnormals by stimulation of gastric secretion by carbachol (Baker and Mollin, 1955a) whereupon absorption is restored. Absorption failure in steatorrhoea due to various causes may not be complete, as in pernicious anaemia, but neither is it restored by the addition of intrinsic factor. Fig. 5 gives typical data for normals and patients with pernicious anaemia, after total gastrectomy, and with idiopathic steatorrhoea. Some of the examples shown include the response to intrinsic factor or carbachol.

Authoradiography.—Sections or smears of tissue are suitably fixed and stained and then overlaid with a prepared film of emulsion backed with gelatin (Pelc, 1956) or coated with a fluid emulsion (Bélanger and Leblond, 1946). β particles produce blackening of grain, and the resolution depends on the characteristics of the film, the length of exposure, development, and the β -particle range. Quite accurate quantitative results can be obtained and the technique has considerable histochemical potentialities (Lajtha and Oliver, 1959). For these results, however, considerable attention to technique is necessary. Generally speaking, the dose of an isotope required in order to obtain reasonable labelling is quite high, and few studies *in vivo* except on the thyroid, have been done. Some work on blood *in vitro* (Ellis, Lajtha, and Oliver, 1955; Boyd, Casarett, Altman, Noonan, and Salomon, 1948) is accumulating, and a very large literature on animals, in particular with the leucocyte series (Perry, Craddock, Paul, and Lawrence, 1959; Everett, Rheinhardt, and Yoffey, 1960; Perry, Craddock, Ventzke, Crepaldi, and Lawrence, 1959; Yoffey, Hanks, and Kelly, 1958).

FIG. 5.—Urine excretion of ^{58}Co after oral dose of ^{58}Co -labelled B_{12} (Schilling) in various groups. In some the test has been repeated with the addition of carbachol or intrinsic factor, and the results compared.

Many of the recent applications involve tritiated thymidine which is incorporated into nuclear protein. Tritium, due to its very short β particle range, gives very high resolution, and since a high tracer dose is allowable, it may be assumed that some results *in vivo* will soon be forthcoming.

Chemical Pathology

Body Fluids and Electrolytes.—Considerable information on the body composition of electrolytes and fluids is now available in normal and diseased states. Balance studies had previously been made on turnover rates, and measurement of fluid compartments using antipyrine (total body water), inulin (E.C.F.), and dyes (plasma volume) had been well established. The former are tedious, almost impossible except in a metabolic ward, and cumulative errors of measurement of input and output are very easily made; no information on the size of the pool is available. The latter are classical indicator dilution techniques, and depend on specific properties of limitation to certain fluid compartments. In order to distinguish them chemically they must of necessity be foreign to the compartment measured, and may not necessarily be distributed normally. Nevertheless, the techniques are well established, and in the case of antipyrine space, estimation of the antipyrine (chemically rather difficult) is facilitated by the use of ^{131}I -labelled 4-iodo-antipyrine (Talso, Lahr, Spafford, Ferenzi, and Jackson, 1955).

Since suitable isotopes have been available, it has been possible to measure the total pools of the common electrolytes, within certain important limitations.

It is clear that the body represents a more or less complicated system of "compartments" with which equilibrium may be attained to differing degrees. Various assumptions must be made about the dilution curve of the isotope with time. It is assumed that distribution is uniform throughout the pool at the time when the measured sample is taken; the assumption is made on the basis that the dilution curve has levelled out. This takes about 12 hours in the case of sodium (Miller, Munro, and Wilson, 1957), but the levelling out is usually only relative, the steep fall of the curve being succeeded by a shallower, slower one. The point at which the sample is taken will represent an average, for that time, of the whole body, but not necessarily equilibrium for each compartment. Thus, the figure obtained is approximate and represents a fraction only of the total sodium being measured. It is therefore necessary to adopt the same procedure from case to case in order to compare results, although in certain circumstances where there is a gross change in the size (and possibly other factors, such as permeability) of the compartments compared one with another, the assumptions made for normals may not be valid in disease. For instance, in oedema, ascites, and in cardiac failure, the time for flattening of the

dilution curve is about twice as long (O'Meara, Birkenfeld, Gotch, and Edelman, 1957).

It is customary to express the calculated figure for sodium or potassium content as "total exchangeable" sodium (T.E.S.) or potassium (T.E.K.) due to the above considerations. From carcass analysis T.E.S. represents about 70% of the total sodium in the body, much of the rest being relatively inaccessible in the bones (Forbes and Lewis, 1956) although over a long period equilibrium is reached with most of it. Total exchangeable potassium represents about 85% of total potassium as compared with estimation by measurement of the naturally-occurring ^{40}K in the body using whole-body scintillation counters (Rundo and Sagild, 1955). Equilibrium of potassium is much slower than of sodium, partly because, unlike sodium, most of the potassium is in cells, and partly because the low plasma concentration of potassium renders equilibrium almost entirely dependent on rate of blood flow (Black, Davies, and Emery, 1955).

Sodium space, T.E.S., and T.E.K. are measured with ^{24}Na (half-life 15.0 hours) and ^{42}K (half-life 12.4 hours). Other Na isotopes have unsuitably long or short half-lives; there are no other suitable K isotopes. The techniques for separate counting of ^{24}Na (Miller *et al.*, 1957) and ^{42}K (Corsa, Olney, Steenburg, Ball, and Moore, 1950) are well established.

Exchangeable Chloride, E.C.F., and Total Body Water.—No suitable radioisotope of chlorine exists (^{38}Cl , half-life 37 minutes, ^{36}Cl , half-life 3×10^5 years) so that ^{82}Br (half-life 36 hours) is used. Certain tissues show different concentrations of chloride and bromide, but the error is probably not great (Staffurth and Birchall, 1960; Reid, Forbes, Bondurant, and Etheridge, 1956). Excretion rates of chloride and bromide by the kidney differ considerably, so that plasma samples are counted rather than urine. Total bromide is rarely particularly informative; ^{82}Br space is more commonly used as a measure of E.C.F. Comparison of E.C.F. estimates using large, chemically inert molecules (inulin, mannitol, sucrose) and ^{82}Br ("bromide" space) shows considerable difference, the ^{82}Br space giving a figure of about 17% of body weight, and that of the large molecule methods 15% (Elkinton and Danowski, 1955). Various reasons are possible: the large molecules may not properly equilibrate with the E.C.F.; bromide space includes some intracellular bromide, the amount being governed by the Donnan effect. (It is usual to apply a correction factor for this, and for ^{82}Br in the red cells (Ljunggren, 1957; Manery, 1954).) In addition,

^{82}Br space probably measures (and over-measures) fluid contents of the gut.

Pragmatically, however, although the concept of E.C.F. is explicit, the relation of the derived estimate to whatever is the absolute value is not clear, nor is it particularly important. It is better to accept the values given by the particular method used, remembering that the methods are not strictly comparable one with another. ^{82}Br space is a function of E.C.F. and varies in the expected way with changes in E.C.F. Its main advantage over other methods lies in the speed of (apparent) equilibration and the simplicity of measurement.

Total body water measurements using tritiated water are simple, equilibrium is reached in a few hours, and measurement is made on cumulative urine excretion, thus obviating the need for the repeated venepunctures of the antipyrine method. The values obtained are somewhat higher than for antipyrine (Prentice, Siri, Berlin, Hyde, Parsons, Joiner, and Lawrence, 1952), presumably due to the fact that ^3H measures exchangeable hydrogen in, e.g., hydroxyl groups. Certainly after initial rapid equilibration, there is a slower, more prolonged disappearance rate, and the concentration of tritium rises slightly in the liver.

The counting of ^3H , previously difficult, is simpler since the advent of liquid scintillation counting (see above). ^3H in urine presents no difficulties provided the urine is first decolorized by adsorption with charcoal. Plasma ^3H may be counted after previous separation of plasma water from its solutes by distillation or by precipitation.

Total body water in adults is about 55 to 60% of body weight, according to the amount of fat. Body water apparently bears a constant relation to "lean body mass" and this has given rise to a considerable literature in which various absolute quantities, e.g., red cell mass (Muldowney, 1957), are related to lean body mass, which is in turn derived by an approximation from the total body water. Unless specific information on lean or fat mass is required, it seems logical to refer other quantities to total body water and leave it at that. Certainly total body water is a much better index than body weight, height, or surface area, all widely used indices. Other quantities, such as cardiac output, oxygen consumption, are more reasonably quoted in terms of total body water, and, since ^3H measurement is simple and rapid, in future this might be done.

Intracellular fluid space cannot be derived directly, but is obtained by difference between total body water and bromide space. The total exchangeable sodium and potassium likewise have greater significance when related to body water

and bromide space. The average values for T.E.S. and T.E.K. in normals are: for sodium, males 43 mEq./kg. body weight, and females 40 mEq./kg. (Moore, 1946; Moore, McMurrey, Parker, and Magnus, 1956), and for potassium, males 45 mEq./kg. body weight, and females 35 mEq./kg. (Aikawa, Harrell, and Eisenberg, 1952; Blainey, Cooke, Quinton, and Scott, 1954; Corsa *et al.*, 1950). The ranges quoted are large; this is presumably due to the variation in proportion of fat to lean both in normals of the same sex and between male and female. Reference of these values to total body water would almost certainly give a narrower range for normal values.

Since for most investigations it is desirable to measure all the parameters mentioned above, a number of techniques have been developed for simultaneous measurement of T.E.S., T.E.K., and bromide space. Total body water presents no problem since the half-life of ^3H is 12.3 years and it is necessary merely to wait for the decay of the other isotopes, all of them with short half-lives, before counting the tritium in samples or to separate the water by some method. Separation of the others, since their half-lives are similar, must be done by chemical means (James, Brooks, Edelman, Olney, and Moore, 1954; Wittig and Raff, 1951; Munro, Renschler, and Wilson, 1958; Arons, Vanderlinde, and Solomon, 1954), by physical means (Tait and Williams, 1952; Robinson, Arons, and Solomon, 1955; Flear, Cawley, Quinton, and Cooke, 1958; Munro *et al.*, 1958), or some convenient combination of both. A comparison of both techniques is given by Munro *et al.* (1958).

Metabolic Turnover.—Information on turnover rates of sodium and chlorine is obtainable because suitable isotopes (^{22}Na , half-life 2.6 years; ^{36}Cl , half-life 4.4×10^5 years) are available. The metabolic half-life of these elements is about 10 days. Since ^{36}Cl is a β emitter, measurement of turnover must be made by urine and faecal collection (Ray, Burch, and Threefoot, 1952), but by using a suitable whole body counter (Veall *et al.*, 1955a) ^{22}Na turnover can be measured by direct estimation of the amount of the isotope remaining in the body. Thus measuring excretion is unnecessary; given suitable isotopes it may be possible to adapt this method to other metabolic processes.

The advent of more sensitive whole body counters (Anderson, Schuch, Perrings, and Langham, 1955; Langham, 1958) has made possible measurement of body content of the naturally occurring isotope ^{40}K , the quantity of which is very closely related to lean body mass. Changes

in lean body mass, and indirectly overall protein metabolism in the human body in disease states, should therefore be measurable in the future. Fat/lean proportions of pigs, produced commercially for different purposes in the bacon and pork products industries, are already measured in this way.

Plasma Proteins.—These are labelled in two ways: the proteins are autogenously labelled by giving ^{14}C -labelled precursors (Campbell, Cuthbertson, Matthews, and McFarlane, 1956; Abdou and Tarver, 1951; Volwiler, Goldsworthy, MacMartin, Wood, MacKay, and Fremont-Smith, 1955), or by labelling *in vitro* protein preparations for injection (McFarlane, 1956).

The plasma proteins are collectively labelled by the former method, so that differentiation is difficult; turnover and half-life studies are affected by re-utilization of amino-acids in protein anabolism (Armstrong, Kukral, Hershman, McLeod, and Wolter, 1954; Masouredis and Beeckmans, 1955; Volwiler *et al.*, 1955). In general, doses of ^{14}C or ^{35}S are comparatively large and not very suitable for human studies.

Labelling *in vitro* is open to criticism on the grounds that the protein may be altered in preparation and may therefore not behave in the same way as the protein it is meant to tag (Berson, Yalow, Shreiber, and Post, 1953; Volwiler *et al.*, 1955); it has been shown that molecules heavily laden with ^{131}I do not have normal physical characteristics. Improved methods of preparation (McFarlane, 1956) have reduced the number of iodine atoms per molecule and the evidence is that ^{131}I -labelled proteins behave in a similar way to autogenously produced ^{14}C -labelled ones. This can be assumed to be true of ^{131}I -labelled human serum albumin when carefully prepared since good quality serum albumin is readily available, but probably not of the more complex globulins and fibrinogen.

Calculation of distribution, pool size, and turnover using ^{131}I -H.S.A. depends on assumptions that the compartments through which the albumin is distributed are defined, and that the system is in equilibrium over the period of measurement (Campbell *et al.*, 1956). In practice, the thyroid is blocked to iodine uptake by previous administration of Lugol's iodine, and, after intravenous administration of the dose of ^{131}I -H.S.A., blood samples are taken over several days and plasma activity measured. Urine is collected continuously and the excreted ^{131}I measured. (In cases of urinary protein loss, e.g., the nephrotic syndrome, urinary protein is calculated separately from the free iodine in the urine.)

Values for the plasma pool, plasma metabolic turnover rate, total body pool, and total body turnover rate can be obtained. Mere information on half-life, without regard to the size of the pool, is misleading (Bauman, Rothschild, Yalow, and Berson, 1955), and some earlier work should be treated with reserve. Nevertheless, the normal range of plasma ^{131}I -H.S.A. half-life appears to be somewhere between five and nine days, and that of the total albumin pool about 16 to 21 days. In a few cases where there appears to be a defect of albumin production, the clumsily named "idiopathic anabolic hypoproteinaemia," the metabolic half-life is as long as 35 days (Gordon, Bartter, and Waldmann, 1959), while in some cases of accelerated protein loss (nephrotic syndrome, exudative enteropathy, "hypercatabolic" hypoproteinaemia) it is as short as two to four days (Gordon, 1959; Gordon *et al.*, 1959; Bland, Fields, and Goldman, 1955; Gitlin, Janeway, and Farr, 1956). Chronic liver disease and possibly starvation result in a reduced albumin pool and a slow rate of production of protein has been demonstrated (Bauer, Bland, Fields, and Getchell, 1954). Other failures of protein synthesis have been shown in congenital afibrinogenaemia and agammaglobulinaemia (Gitlin and Borges, 1953). Changes in distribution of the pool have been examined in ascites and oedema (Berson and Yalow, 1954) and the multicompartment systems involved treated mathematically.

Excessive loss of protein can occur in the gut, but except in diarrhoea, due, for example, to ulcerative colitis (Steinfeld, Davidson, and Gordon, 1957), such loss is not easily measured because of complete or partial reabsorption of the ^{131}I after protein destruction within the gut. Recent investigation using ^{131}I -labelled polyvinylpyrrolidone (P.V.P.) (Gordon, 1958), which has a molecular structure and size analogous to H.S.A. but is not destroyed in the gut, has shown that some hypoproteinaemic conditions exist due to an abnormal loss of protein in the stomach or small intestine (Gordon *et al.*, 1959; Gordon, 1959). Some cases described are characterized by abnormally low total plasma proteins, high E.S.R., and high plasma cholesterol, and some have a condition similar to chronic hypertrophic gastritis. More investigation of gut conditions of this sort is required; ^{131}I -P.V.P. might also help in settling the question of whether active excretion of protein into the lower gut can occur in, e.g., steatorrhoea.

Fat Absorption.—Triolein and oleic acid can be iodinated without great difficulty in the laboratory and a suitable preparation for absorption tests

obtained. That obtainable from the Radiochemical Centre, Amersham, is iodinated to completion and unlabelled triolein added to give the required specific activity. It is thus expensive, but for ordinary work is not necessary. As a diagnostic test a tracer dose of iodinated triolein is given by mouth and the fraction absorbed obtained by difference after counting the activity excreted in the faeces (Lubran and Pearson, 1958), or blood samples obtained and the plasma activity measured (Baylin, Sanders, Isley, Shingleton, Hymans, Johnston, and Ruffin, 1955; Beres, Wenger, and Kirsner, 1957; McKenna, Bourne, and Matzko, 1957). The former method requires faecal collection with its attendant difficulties, and contamination with urine cannot be allowed due to excretion of iodine after endogenous splitting of fat.

The results, however, agree fairly well with those of the more laborious chemically determined faecal fat excretion and measurement is simpler. Table I shows the figures for ^{131}I excretion after

TABLE I
COMPARISON OF DAILY FAECAL FAT EXCRETION (CONVENTIONAL METHOD OF ESTIMATION) WITH CUMULATIVE EXCRETION OF ^{131}I AFTER ADMINISTRATION OF ^{131}I -TRIOLEIN

	Faecal Fat in g./Day (Normal up to 7 g.)	A.D. ^{131}I in Faeces (%)
Normal	3.0	0.4
.. ..	5.0	4.6
Crohn's disease ..	72.0	76.0
Jejunal resection ..	13.4	12.0
Ulcerative colitis* ..	10.9	1.3

*Note anomalous result for "ulcerative colitis."

giving ^{131}I triolein, compared with the simultaneous chemical measurement of fat excretion in a small group of patients. It will be seen that one result is anomalous and is not easily explained. More data on the correlation of these two tests in different fat-absorption states should be available soon (Vaughan Jones, 1960). There appears to be little difference whether the dose is taken alone, with a meal, or with a quantity of fat; the easiest way is to enclose it in a gelatin capsule, since some correction is otherwise necessary for the portion of the dose left after drinking an emulsion. It is also less unpleasant to take. In normals not more than about 5% of the administered dose is recovered in the faeces.

Blood levels of ^{131}I are often equivocal. The normal levels of activity are not very high and there is evidence that ^{131}I is rapidly split off (Beres *et al.*, 1957), presumably due to the instability of the iodinated bond, since ^{14}C -labelled triolein disappears less quickly. There is evidence, too (van

Handel and Zilversmit, 1958), that ^{131}I -triolein may be split during absorption, based on comparison of ^{131}I - and ^{14}C -triolein absorption, and thus interpretation of plasma concentration curves is very difficult. The value of plasma levels is that a peak figure is reached in a few hours, and the test may therefore be used as a screen to exclude normals.

In pancreatic disease and steatorrhoea due to other causes, e.g., idiopathic, post-gastrectomy, absorption of ^{131}I -triolein is reduced. In pancreatic failure (when not due to fibrocystic disease) the absorption of ^{131}I -oleic acid is normal, however, and this is in contrast to other causes of steatorrhoea, in which the ^{131}I -oleic acid absorption is impaired along with the ^{131}I -triolein (Turner, Parker, Coffey, and Duffy, 1956; Shingleton, Isley, Floyd, Sanders, Baylin, Postlethwait, and Ruffin, 1957). In fibrocystic disease of the pancreas, the ^{131}I -oleic acid absorption may be impaired (Reemtsma, Malm, and Barker, 1957) which accords with the more recently held view that the condition is one involving the gut and probably other systems as well as the pancreas. Table II illustrates the differences in absorption in three typical cases.

TABLE II
COMPARISON OF DAILY FAECAL FAT EXCRETION, AND ^{131}I FROM ^{131}I -TRIOLEIN AND ^{131}I -OLEIC ACIDS

	Faecal Fat Excretion (g./Day)	Faecal ^{131}I Excretion after ^{131}I -Triolein (% A.D.)	Faecal ^{131}I Excretion after ^{131}I -Oleic Acid (% A.D.)
Normal	4.2	2.0	1.3
Pancreatectomy	35.2	95.8	5.7
Idiopathic steatorrhoea	21.8	37.3	28.1

Recently ^{82}Br -triolein has been used and, once more, labelling in the laboratory is quite simple (Lubran and Corsini, 1959). Results seem to be comparable to those using the ^{131}I label, and since its half-life is short (36 hours) and its γ remission more energetic, it may have advantages from the point of view of repeatability. Since ^{131}I and ^{82}Br are readily separable physically, simultaneous tests of absorption, e.g., oleic acid and triolein, should be possible with much saving in time.

The effect on absorption of, e.g., pancreatin, bile salts, is not very clear at present, due mainly to the laborious fat balances previously necessary, and ^{82}Br -labelled fats and fatty acids could well be used in such investigations.

Thyroid Metabolism

Investigations of thyroid function are numerous and a voluminous literature has accumulated.

Radioactive iodine is taken up by the thyroid gland, incorporated into thyroid hormone, and secreted by the gland. The iodine on injection or ingestion is distributed rapidly throughout a large fluid space, and its disappearance from this space is a function of renal excretion (G.F.R.) and the avidity of the thyroid for iodine. An inverse relationship therefore exists between the amount taken up by the thyroid and the amount excreted in the urine; in general, but not always, a high uptake and low urine excretion over a given period mean that the gland is overactive and therefore that the patient is thyrotoxic. Similarly the appearance in the blood of a high concentration of radioactive protein-bound iodine over a given period is indicative of a high iodine turnover rate in the gland, in general indicating hyperthyroidism. Measurement of urine excretion of radioactive iodine, of the uptake of iodine by the thyroid, or of the concentration of protein-bound radioactive iodine might

FIG. 6.—General curves for ^{131}I neck uptake and PB^{131}I with time, in various conditions. Line at 24 hours indicates point at which most routine readings of these are taken. Note that at 24 hours the neck uptake in a nodular goitre may have fallen into the normal range. PB^{131}I will usually be abnormally high, however.

each be expected to provide an indication of the functional state; each does, but individually they present considerable difficulties and results may be equivocal. Fig. 6 shows the general pattern of uptake with time of the thyroid gland in various conditions together with the corresponding values for $PB^{131}I$. It should be noted that the patterns for the "hot" nodule and the toxic diffuse goitre are different (for reasons discussed later) and that in particular the 24-hour thyroid uptake level in the former may be indistinguishable from the normal.

Measurement of urine excretion is simple, but the best information is provided by splitting up a collection period over 24 to 48 hours, and this presents difficulties; it can never be certain that all urine is collected. In addition, renal or cardiac failure may impair renal excretion, and although some correction may be made for this (Fraser, Hobson, Arnott, and Emery, 1953) results are sometimes difficult to interpret. By itself, correlation with the clinical state of the patient is not, in our hands, very good (Keating, Power, Berkson, and Haines, 1947; Skanse, 1949).

Measurement of thyroid uptake depends on surface counting, and the techniques employed are almost as numerous as the laboratories employing them. A "directional" scintillation counter placed at a standard distance to "view" the thyroid, and comparison of the count thus obtained with a standard, is widely used, but differences in anatomy of the gland and the scattering effect of the tissues introduce errors. More satisfactory geometry, minimizing variations in anatomy, is achieved using a combination of counters (Geiger Muller tubes) which "see" a cylindrical section of neck (Freedberg, Chamovitz, Ureles, and VanDilla, 1950). Comparison is then made with a standard. The use of a standard is avoided by comparing counts over the neck with those from non-thyroid tissue, e.g., of the thigh, expressing these as a ratio (Pochin, 1950; Foote and MacLagan, 1951). This method is particularly useful for repeated counts over a short period after administration of radio-iodine.

Uptake figures are usually expressed as the percentage uptake at a given time (Keating, Haines, Power, and Williams, 1950) after administration of a dose (24 hours is a convenient time after an oral dose) or as a curve of uptake rate over a few hours after an oral or (better) intravenous dose. Due to the wide variation in methods used, results from a given laboratory can only be fairly compared within the range of values obtained in that laboratory. Uptake curves show wide variation with respect to time and concen-

tration and give little information in absolute terms. This is due to several factors. First, the mass of the gland is virtually unmeasurable: although attempts have been made to do this by multiplane surface counting, the effort is hardly worth while. Second, the gland frequently does not function equally throughout its anatomical mass (Sinclair, Abbatt, Farran, Harriss, and Lamerton, 1956) even in the apparently "diffuse" goitres of Graves's disease; and third, since the amount of iodine in the gland at any time is a function of uptake, release (as hormone) and storage (as thyroglobulin), the "uptake" figure is not simple and may be distorted very considerably. For example, a small functional quantity of gland may be working to capacity to maintain either a normal or abnormal output of thyroid hormone, with little or no storage, while a large functional quantity may be producing exactly the same results with a much lower turnover rate per unit of gland, and with considerable capacity for storage. The reasons for these differences are obscure; although the former tendency is exemplified by the "hot nodule," even the apparently homogeneously active gland often shows areas of varying behaviour.

The value of thyroid uptake in the diagnosis of thyroid disease is undoubtedly high. Fig. 7 shows the distribution of thyroid uptake in a number of consecutive routine diagnostic tests, a clinical assessment having been made of each patient previously by the same observer. There is a considerable region of doubt, however, and a number of clinically doubtful cases remain

Fig. 7.—Distribution of patients in their clinical gradings, according to their ^{131}I neck uptakes. Note considerable overlap of grades.

unelucidated. It is convenient usually to give ^{131}I by mouth and measure the uptake at 24 hours, but the more recent introduction of ^{132}I with a half-life of 2.3 hours has made possible assessment of thyroid function with a much smaller radiation dose. Its short half-life precludes urine and P.B.I. measurement and allows only plotting the rate of uptake over a few hours, so it is probably better to inject the ^{132}I intravenously. The ^{132}I is eluted, when necessary, directly from a ^{132}Te column (supplied by the Radiochemical Centre, Amersham). Neck-thigh ratio measurements (Halnan, 1958) are very suitable, providing useful screening tests which will protect the majority from the greater radiation of a ^{131}I tracer dose. Very small doses allow repetition of the test when necessary, and thyroid assessment in infants and children may be considered feasible.

Information on the hormone output of the thyroid depends on measurement of the quantity of ^{131}I appearing in the protein-bound fraction of iodine in the plasma. In practice this is measured by counting the total quantity of radioactivity in the protein precipitated from a sample of plasma taken, say, 24 hours after a tracer dose. The ^{131}I is in fact bound to the α_1 globulin fraction and to a lesser extent to the albumin (Horst and Rösler, 1953). The concentration of ^{131}I will depend on the rate of release of hormone by the gland, the rate of utilization, the amount of available protein for binding, and the total pool of thyroid hormone.

In pregnancy the available binding protein fraction rises, and so does the bound thyroid hormone, presumably passively. This is the probable cause of the goitre and high ^{131}I uptake in pregnancy. The rate of utilization of hormone does not increase, however. Conversely, in the nephrotic syndrome the binding protein falls, but the fraction of free hormone is normal. Thus it appears that the protein-bound iodine is not consistently proportionate to the total hormonal pool, and therefore the PB^{131}I is not always simply related to the total hormone turnover.

The PB^{131}I is frequently used as a measure of thyroid function, either by itself or as a "conversion" ratio (Clark, Moe, and Adams, 1949) with the unbound plasma ^{131}I representing free iodine. This depends on the fact that as the PB^{131}I rises the free ^{131}I falls; therefore the ratio rises in thyrotoxicosis and falls in myxoedema. Cardiac or renal failure, or iodine-deficient goitre, will, however, give anomalous results.

Fig. 8 shows the distribution of PB^{131}I results in a number of consecutive routine diagnostic tests, a clinical assessment having been made of each patient previously by the same

FIG. 8.—Distribution of patients in their clinical gradings, according to their PB^{131}I values. Note considerable overlap of grades.

observer. As with the figure showing thyroid uptake results (Fig. 7), the overlap is rather large. Many of the clinically "doubtful" patients remain doubtful.

Probably the best results are obtained by measuring both the thyroid uptake and the PB^{131}I in each patient. In this laboratory differentiation (in patients previously assessed clinically by the same observer) was obtained in 90% of nearly 450 consecutive routine uptakes (Fig. 9). It will be seen that the great majority of those diagnosed as thyrotoxic give values for thyroid uptake of more than 60% of the administered dose and for PB^{131}I of above 0.3% per litre. (It must be stressed that these values are probably unique to this laboratory, and that the calibration will be different for different laboratories.) It will also be seen that

FIG. 9.—Uptakes of patients (clinically graded) plotted against their $PB^{131}I$ readings. A very large majority (90%) can be discriminated, normals lying below the 60% uptake level and to the left of 0.3% $PB^{131}I$ level, thyrotoxicos lying above and to the right of these levels. Myxoedema patients lie to the left of 0.3% $PB^{131}I$ level and below 20% uptake level. Points to the left of 0.3% $PB^{131}I$ line are plotted on the uptake axis only. Points scattered widely from these limits are often explicable (see text); those labelled "N" are nodular goitres, "H" are Hashimoto's disease; "I" and "T" have recently had exogenous iodine and thyroid respectively. Some of the points lying above the 60% uptake level, but to the left of 0.3% $PB^{131}I$ level, are brought into the normal range by thyroid suppression tests.

some of those giving apparently anomalous results are due to explicable causes. For instance, in some of the cases of Hashimoto's disease, the uptake is very low while the $PB^{131}I$ is abnormally high; the 24-hour uptake figure may be low in comparison with the $PB^{131}I$ figure in nodular thyrotoxicosis (although the peak may be much higher shortly after the dose); some results are invalidated by previous unsuspected administration of iodine or thyroid (two of these latter patients were thyroid "addicts").

A few of the results show very high thyroid uptakes but with $PB^{131}I$ figures which are in the normal range. This behaviour is sometimes observed in cases which later develop a clearly thyrotoxic picture, but sometimes the simple non-toxic goitre gives these results. On the assumption that in simple goitre the high uptake is due to a relative iodine deficiency, a standard test (Burrell and Fraser, 1957) has been applied which discriminates between the two conditions. A small quantity of potassium iodide is given daily for a week, followed after an interval by another uptake test. In simple goitre the uptake is reduced compared with that of

the first test, the iodine deficiency being made up by the iodide; in thyrotoxicosis the high uptake continues. A further discriminatory test is also available; the high uptake of the simple goitre is suppressed by a short course of tri-iodothyronine (a few days), while that of the hyperthyroid gland is not (Werner and Spooner, 1955). This test is also easily performed and has the advantages of a shorter time scale. Some of the anomalous results shown in Fig. 11 were clarified in this way.

Recent work (Hamolsky, Golodetz, and Freedberg, 1959; Robbins, 1959) has demonstrated that when ^{131}I -tri-iodothyronine is incubated and shaken with blood, it is divided between the cells and plasma in a ratio which increases in thyrotoxicosis and decreases in hypothyroidism. In a large group of experiments a clear distinction is shown, although not very adequately related to the clinical state of the patients used. The explanation is presumably that the remaining unbound plasma protein takes up ^{131}I -tri-iodothyronine preferentially, and that the excess is bound on to red blood cells. When the available protein is reduced (because a large part is already bound to the patients' hormone) the red blood cell binding is

FIG. 10.—Typical examples of surface scans over (1) “hot nodule,” (2) “silent nodule” (thyroid carcinoma), and (3) a normal gland. The isocount lines show 10% decrements from the point of highest count.

large and vice versa. Further evaluation is necessary; the system appears to be sensitive to CO_2 (and therefore pH ?), but this method may well offer a useful screening test which is simple and avoids the giving of radioactivity to the patients. Its usefulness in infants and children is obvious.

Hashimoto's disease and other forms of thyroiditis in general show lowish or low uptakes, although a high uptake is occasionally seen in Hashimoto's disease. Characteristically ^{131}I is expelled from a Hashimoto goitre if a small quantity (400 mg.) of potassium perchlorate is given by mouth shortly after the ^{131}I dose. This contrasts with the behaviour of a simple goitre in which the ^{131}I uptake continues unimpaired (Morgans and Trotter, 1957). The reason is obscure, but presumably some abnormality of organic binding of the iodine must be involved. Typical behaviour in Hashimoto's compared with that in simple goitre is shown in Fig. 10. The PB^{131}I tends to be high compared with uptake in Hashimoto's disease; this is possibly related to a high turnover rate in a small remaining quantity of functioning gland. The sensitivity of the ^{131}I tests to pick out these cases is not very good; where there is doubt thyroglobulin precipitin tests should be done.

Congenital and familial abnormalities of thyroid function have been shown but usually are very rare. Various metabolic blocks have been demonstrated (McGirr and Hutchison, 1953; Stanbury and Querido, 1956) including iodine deficiency

goitre due, presumably, to the absence of an enzyme which normally de-iodinates the mono-iodotyrosine freed when thyroglobulin is broken down (Stanbury, Kassenaar, Meijer, and Terpstra, 1955).

Thyroid carcinoma tissue is characterized by a low or absent uptake, but the rest of the gland in which the carcinoma arises may behave normally or may even be overactive. Surface scanning may reveal a “silent” nodule if it is big enough compared with the adjacent normal gland, and it is wise to consider any silent area or nodule as a possible neoplasm. However, small neoplastic areas are not likely to be revealed by scanning, since resolution of the distribution of activity is not good enough. Further difficulties arise due to the fact that, although the gland may appear homogeneous anatomically, it very often is not so functionally.

Treatment of thyroid carcinoma with ^{131}I depends on the ability of carcinoma tissue to concentrate radioactive iodine; it is almost always negligible when normally functioning thyroid tissue is present. Ability to concentrate is (not surprisingly) related to the degree of differentiation histologically, and generally speaking the results are disappointing. “Silent” nodules may also be due to degenerative cysts and some appear to consist of normal quiescent gland tissue.

“Active” nodules may cover a range of functional behaviour from the normal to extremely

FIG. 11.— ^{131}I neck counts from a patient with a simple goitre and with Hashimoto's disease after an oral dose of KClO_4 . The ^{131}I is given by mouth one hour earlier, and the readings after the KClO_4 compared with that immediately before, which is taken as 100%.

high concentration. Such "hot" nodules may secrete sufficient hormone to produce thyrotoxicosis; on the other hand, a similar appearance on the scan can be given by a localized mass of thyroid which by rapid turnover and low thyroglobulin storage is maintaining normal hormonal output, the rest of the gland contributing little. Such nodular behaviour, by no means uncommon, often presents a difficult diagnostic problem, as the quantitative difference between the nodule producing the disease and that maintaining normal function is not easily measured.

The difference in uptake and PB^{131}I curves between the diffuse goitre and the nodule is shown in Fig. 6. Typical scans of a normal gland, a hot nodule, and a large thyroid carcinoma are shown in Fig. 11.

General Considerations

Use of Radioisotopes in Diagnosis.—In view of the concern felt by a public which is rapidly becoming aware of the danger of ionizing radiations, it is probably better not to carry out radioisotope studies unless there is a clear case for them. In the general run of investigation or diagnosis it is quite often reasonable to dispense

with an isotope "test" when an equally informative conventional test is available. For instance, when documentary evidence is needed "for the record" in a clear case of thyrotoxicosis, it seems proper to estimate the B.M.R. rather than ^{131}I uptake, since the more sensitive differentiation possible with the latter is not needed. Similarly, most of the electrolyte imbalance states arising in disease can be inferred from clinical observation, and the plasma electrolyte concentration figures, so that measurement of total exchangeable sodium or potassium is not necessary. This attitude is justified further by the undoubted truth that the general level of ionizing radiation received by the public during its "patient" life is increasing, and a tracer study should be thought of in the same terms as a radiological procedure when considering the question, Is this justified? Finally, there is at the moment little legislation regarding the use of, and disposal of, radioactive isotopes, and an attitude of caution in their use is less likely to make any subsequent statutory control unduly restrictive. For all these reasons the aim should be to increase the sensitivity of the counting procedure so as to decrease the tracer dose, but retaining the same degree of accuracy.

Record of Tracer and Treatment Doses.

It appears essential to have a clear and permanent record in the patient's notes of all tracer and treatment doses given which should be entered at the time the dose is given, preferably in some prominent place which is a fixture. This is necessary for several reasons. First, it provides a complete record of the accumulation of doses that the patient may receive during his hospital life; this could be considerable over many years. Second, such a record, easily referred to, will prevent the possibility (not so very unlikely!) of a second tracer dose being given for a second investigation while the first is still in progress; and third, a clear record is available if the patient comes to the post-mortem room, so that the pathologist and his staff can take the necessary precautions, and not be exposed to radiation of which they might otherwise be unaware. This is not usually a risk with tracer doses, but is of very considerable importance should the patient die soon after a treatment dose. Tracer dose levels can be of importance when they are concentrated in a particular organ, such as ^{131}I in the thyroid, when that tissue is manipulated during histological preparation.

In this hospital a gummed ticket, suitably coloured red, is stuck into the inside cover of the patient's folder, when he has his first tracer dose, and the particulars of the dose and all subsequent

doses are entered on the ticket as they are given. The further arrangement is made that should any patient die who has a red ticket in his folder, the cadaver is automatically given a prominent distinguishing mark before leaving the ward. This warns post-mortem room staff of possible radiation risks which can be confirmed or denied by reference to the date and dose record displayed on the red ticket.

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