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THE MEDICAL APPLICATIONS OF RADIOACTIVE TRACERS

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More than a decade has passed since the first biological tracer application of artificial radioactivity by Hevesy in 1935, who used radiophosphorus to study the distribution of this element in the rat (1). Since that time, several hundred papers and reports have appeared which describe the use of radioactive tracers in the biological and medical sciences. These efforts have been devoted to the study of metabolism of organisms ranging in complexity from viruses to man and embracing the more important members of the plant and animal kingdoms. A large share of the earlier work with radioactive tracers was directed toward the exploration of the potentialities of this new and powerful research tool. These exercises of methodology, which have been described elsewhere in considerable detail (2), were the essential prelude to the effective application of the artificial radioactive elements to extending our knowledge of the nature of life processes.

World War II appeared at almost a midpoint in the brief chronology, to date, in this new field of research. Its influence had the usual disruptive effect which reduced to a considerable degree all work in fundamental investigation. The retardation of progress in this field during the interval of the war was counterbalanced by the most significant technological development of modern history--the release of atomic energy. The impact of this 20th century version of "Pandora's Box" upon the biological and medical applications of artificial radioactivity has produced several situations which most certainly will alter

the predictable pattern that this new field of research may assume in the immediate future. It is of interest to touch upon these briefly before describing in somewhat greater detail a number of the more representative uses of radioactive tracers in medicine.

The first, and probably the most significant of the factors, has been the discovery of the chain reacting uranium pile. This device can produce continuously a tremendous intensity of neutrons which in turn may be employed to prepare almost limitless quantities of a large number of radioactive isotopes. Included among the radioactive materials that can be produced by neutron irradiation from the pile, are the radioactive isotopes of a large proportion of the elements that are known to be essential to life, namely, hydrogen, carbon, sodium, phosphorus, sulfur, chlorine, potassium, calcium, iron, cobalt, copper, zinc, and iodine. Second, the release of nuclear energy is associated with the formation of incredible quantities of a large series of radio-elements called the fission products, as well as considerable amounts of several radioactive heavy elements, notably, neptunium and plutonium. The adequate protection of workers who come in contact with these materials in the peaceful applications of atomic energy has presented a number of new and unique problems in the field of industrial medicine. The grim significance of the atomic bomb and radioactive warfare does not require further emphasis--the unpleasant possibilities are all too clear. Third, the wartime developments in nuclear energy demanded a rapid extension of training of workers in all of the fields of applied nuclear physics, including those in the biological sciences and medicine. Fourth, this interval has seen a rapid and extensive development in many of the practical technics for the use of artificial radio-elements, which includes the ready availability of reliable devices for the detection and measurement of radioactivity, equipment for the protection of people

working in this field, special films and emulsions for radioautography, and many other technological advances too numerous to mention in this brief report. Over and above the factors enumerated, there is a widespread and intense public interest in the many aspects of atomic energy and, in particular, its applications to the advancement of knowledge in medical sciences. It can be readily appreciated that the availability of almost limitless supplies of radioactive tracers, the rapid increase in the number of scientific workers trained in this new field, the new medical problems presented by the toxicological factors arising from the release of atomic energy, and the widespread public interest that is manifested both by moral and material support, most certainly has a profound accelerating effect upon the future developments in this particular field of applied nuclear physics.

An attempt will be made in this report to review a number of the applications of radioactive tracers to medicine. It is hoped that the studies discussed in this paper may serve to bring together some of the representative investigations that have been done to date in this new field and, at the same time, to illustrate some of the potentialities these agents possess to further medical research in the future. Obviously, it is not possible to make a precise disassociation of its applications to medicine from its manifold uses in the related biological sciences. However, it is convenient at the present time to divide certain phases of this new field, which are predominate in the domain of medical research, into four major categories. First, investigations in the field of normal and abnormal human physiology and biochemistry. Work in this category has included, primarily, investigation of the mineral metabolism of a number of elements in human subjects, metabolism of the noble gases in man, and related studies. Second, the use of tracer technics for the search of either radio-elements or compounds will be accumulated in pathological

structures and thus act as radio-therapeutic agents. Third, the use of radioactive tracers as diagnostic aids in clinical medicine. Fourth, the employment of tracers as an aid to the investigation of toxicology of radioactive elements. This last division has been derived from the necessity of securing adequate information concerning the metabolic and biological properties of a large number of radioactive substances which arise from the release of nuclear energy. 3

Studies in Human Physiology and Biochemistry

A number of the earliest radioactive tracer studies were done with human subjects. These included a series of determinations of the rates of absorption of sodium, potassium, chlorine, bromine, and iodine following the oral ingestion of solutions containing radioactive isotopes of these five elements (3). The appearance and increase of radioactivity of the hand, which grasped a counter tube within a thick lead shield, was employed as the index of measurement of the absorption of these five radio-elements from the digestive tract. This procedure, which is commonly defined as the "in vivo" radioactive tracer technic, revealed that the sodium ion and the three halogen ions were rapidly absorbed at similar rates. Their first appearance in the hand was noted within a very few minutes after ingestion and an apparent equilibrium was established within two hours, which suggested that absorption from the digestive tract was completed at the end of this interval. Labelled potassium was observed not only to appear several minutes later in the hand than the other four elements, but an interval of nearly five hours was required before the steady increase of radioactivity in the hand leveled off. In another series of experiments, the excretion, and distribution of

sodium in body fluids, was made following the intravenous and intraduodenal administrations of radio-sodium in three leukemia patients (4). The excretion was observed to be almost exclusively by way of the kidney and took place at a rate of from 2% to 6% per day of the administered dose of radio-sodium. These early studies were largely exercises in methodology but they helped to demonstrate the possibilities of radioactive tracers as a new research tool.

Radio-sodium has been employed to determine the volume of extra cellular fluid in normal human subjects (5). This was accomplished by simply administering a known quantity of radio-sodium as sodium chloride by intravenous injection and observing the equilibrium concentration in the blood plasma. The values obtained by this procedure were compared with similar determinations made at the same time with thiocyanate. The investigators point out that a correction of approximately 20% must be applied to the radio-sodium determinations, since one-fifth of the total exchangeable sodium in the body is presumably bound into the mineral structure of the skeleton. After applying this correction, and making allowance for the small fraction of radio-sodium excreted during the experiments, they obtained an average value of 21.1% of the total body weight for the extra cellular sodium space. The corresponding values obtained by the use of thiocyanate were 23.5% of the body weight. Although the results observed using these two procedures are very similar, it would appear that labelled sodium is to be preferred, since it is a normal constituent in the body.

Another use of labelled sodium has been in the study of the comparative physiology of the placental transfer in a number of different animals, including man (6), (7). A correlation was made of the variations of the rates

of sodium transfer per unit weight of placenta as a function of the morphological structure of this organ at different intervals of gestation. A summary of the transfer rates at the middle of nine-tenths of term for seven animal species is shown in Table I. The rate of transfer, expressed as milligram of sodium transferred per hour per gram of placenta, is characteristic for each of the four morphological types studied. The rate of transfer is apparently a function of the number of cellular layers between the maternal and fetal circulation. The sodium transfer rate per unit weight of placenta is also apparently influenced by the duration of gestation. The value increases steadily throughout pregnacy. This phenomena is characeristic of all the animal species studied with the exception of the pig, which showed a continued increase of transfer rate during the last tenth of pregnancy. It is suggested that this decrease in transfer rate, except for the pig, is related to the thinning of the chorionic and uterine cellular layers as term is approached.

An interesting observation concerning absorption from the vagina in normal human subjects and in post partum patients has been made with radio-sodium (8). A small but detectable absorption occurred through the vagina in normal subjects, which ranged from .05% to .1% of the administered dose at the end of two hours. At a similar time interval, from .88% to 9% of the labelled sodium was absorbed from the vagina in a group of post partum patients. The implications of these studies are obvious in suggesting that great caution be exercised in the introduction of toxic materials in the vagina when there has been trauma to this organ and the cervix.

The absorption of sodium through the intact skin has been apparently demonstrated (9). Labelled sodium, as sodium chloride, was incorporated into several different ointment bases which were then applied by thorough rubbing to

the inner surface of the upper arm. Absorption was determined by placing the hand of the opposite arm upon an adequately shielded counter tube and observing the presence of radioactivity at that point during the experiment. The presence of radio-sodium in the urine was also demonstrated, thus indicating that a detectable degree of absorption of sodium presumably took place through the intact skin. Unfortunately, no quantitative data were given, but it is clear that experiments of this character are worthy of far more attention than they have received up to the present time.

An investigation of the exchange of radio-potassium with the potassium of the body in man and laboratory animals has been made by several groups of workers (10), (11). These studies indicate that potassium, which is primarily an intracellular constituent of the tissues, attains an equilibrium in the body quite slowly as compared to sodium.

During the war, the radioactive isotopes of the noble gases and nitrogen were employed to attack the problem of decompression sickness (12). The "in vivo" technic was used during and following the inhalation of these radioactive gases with a large number of human subjects in a series of attempts to evaluate the various factors that may be responsible for occurrence of the "bends" at high altitudes. Counters, suitably shielded with lead, were placed over different portions of the body to measure the rates of change of content of these radioactive gases in the tissues following their inhalations by the subjects. The results of these very extensive and detailed studies indicate that the limiting factor of inert gas exchange in the body is apparently the blood-tissue perfusion rates of the different organs and not the diffusion and trans-cappillaric permeability to the labelled gas. The rate of release of an inert gas from the different tissues is, of course, influenced by its solubility in the particular

organ. But for a given amount of inert gas dissolved in a certain tissue, the primary factor governing its release will be the rate of blood flow passing into that tissue and not the diffusion coefficient of the gas.

The short lived radioactive carbon, C^{11} with a half life of 20.5 minutes, has been employed to investigate the metabolism of carbon monoxide in man (13). The labelled carbon monoxide was inhaled by normal human subjects and the presence of radioactive carbon dioxide was sought in an effort to ascertain if the inhaled carbon monoxide was oxidized in the body to form carbon dioxide. The results of these experiments indicated that less than .1% of the inhaled carbon monoxide that entered the blood stream was converted to carbon dioxide in the course of the experiments, which extended for an interval of approximately an hour with each subject. Longer time intervals could not be employed due to the relatively short half life of C^{11}. In addition, the rate of excretion of the radioactive carbon monoxide was followed while the subjects breathed pure oxygen and it was observed that the labelled carbon monoxide was excreted from the lungs at an apparently exponential rate with a half time of approximately sixty-six minutes. Later studies (14), in which the excretion of labelled carbon monoxide was followed with the subjects breathing air, indicated a half time of elimination of three hours. These differences in rates are presumably related to the effect of the variation of the partial pressure of oxygen in the lungs following the inhalation of pure oxygen as compared to air.

Radio-iodine has been employed for extensive studies of the metabolism of iodine in normal human subjects, as well as patients with different types of thyroid diseases. In the earlier experiments of radio-iodine (15), it was shown that the rate of excretion of the labelled iodine containing fourteen milligrams of iodine as sodium iodide, was similar for normal subjects and patients with endemic goiter and hyperthyroidism. Excretion was rapid and took

place almost entirely by way of the kidneys. Nearly 80% was eliminated during the first 48 hours. The rate of excretion of labelled iodine was observed to be perceptibly slower in patients with spontaneous myxoedema. Later, a series of studies was made of the uptake by the thyroid of radio-iodine following the oral administration of labelled iodide ion by placing a counter tube over the isthmus of the gland in situ (16 (17). This "in vivo" technic made it possible to observe quantitatively the accumulation of radio-iodine in the thyroid in normal subjects, as well as patients with different types of thyroid disorders. The uptake by the gland was followed for five days in the normal controls and patients with hyperthyroidism without goiter, hypothyroidism with goiter, and endemic goiter. Care was taken to insure that none of the normal subjects and patients had received any iodine containing drugs for at least two months before the radio-iodine studies. Two series of experiments were conducted. In the first group, the radio-iodine for each subject was diluted with 14 milligrams of iodine as sodium iodide, and in the second series, the radio-iodine given to each individual contained less than .1 micrograms of iodine. Thus it was possible to observe what may be defined as the exogenous and endogenous iodine metabolism of the thyroid in a variety of clinical states. Fortunately, it was possible in many instances to use the same subjects and patients in both series of experiments. Under these circumstances, the .1 microgram test dose was given first. When the test dose of 14 milligrams of labelled iodine was used, the uptake of the normal controls and the patients with endemic goiter reached a maximum level at 48 hours with no perceptible loss from the thyroid during the following three days of the experiment. The uptake averaged 3.5% and 10% respectively of the

administered radio-iodine. The accumulation of labelled iodine by the thyroids of the patients with hypothyroidism without goiter was observed to be less than .05%. The radio-iodine uptake by the thyroids of the patients with hyperthyroidism assumed a most unexpected pattern. The maximum accumulation by the gland was attained within three hours to reach an average value of 14% and then declined rapidly during the next 12 hours to approximately one third of the peak level of uptake. This effect was consistent for each of the ten patients studied. A similar type of uptake curve was observed in two patients with goiter and hypothyroidism. It is apparent that in these two very dissimilar clinical states, the thyroid cells have an abnormally high degree of affinity for iodine but the mechanism for the retention of recently deposited iodine has been altered. The negligible accumulation of the labelled iodine by the thyroids in the patients with hypothyroidism without goiter may be explained by the fact that the gland did not possess the ability to synthesize the thyroid hormone in appreciable quantities. When the studies were repeated with the test dose of radio-iodine containing less than .1 micrograms of iodine, the uptake patterns were quite different. The radio-iodine accumulation by the thyroids of the normal controls and patients with endemic goiter ranged from 20% to 25% at the end of 24 hours. The radio-iodine uptake of the patients with hyperthyroidism and hypothyroidism with goiter varied from 60% to 80% at the end of the same time interval. Moreover, in these two clinical thyroid states, the rates of uptake were very rapid. Approximately 50% of the administered radio-iodine was deposited in the gland within three hours. The rapid and marked loss of labelled iodine from the thyroids of these two types of goiter, which was observed with the 14 milligrams test

dose experiments, did not take place in this series of studies in which the total iodine administered to each subject was less than .1 micrograms. The thyroids of the patients with hypothyroidism without goiter accumulated an appreciable fraction of the .1 microgram test dose. The range of radio-iodine deposition in the thyroids of these patients varied from .2% to 2.5%. This observation suggests that the capacity of the thyroid tissue of these individuals to accumulate iodine was not completely lost, although the amount of hormone produced was apparently very small judging from the clinical degree of hypothyroidism noted in these individuals. Radio-iodine studies in normal human subjects and a large series of patients with hyperthyroidism have been done by other groups of investigators (18) (19). In these studies, the quantity of inert iodine added varied from 100 micrograms to 5 milligrams for each patient. The total thyroid uptake of the labelled iodine varied somewhat both with the degree of clinical severity of the disease, and with the amount of inert iodine carrier added.

The efficacy of whole blood transfusion in augmenting the red blood count volume, as well as the survival of the transfused cells, cannot be readily determined by ordinary procedures. Such methods have included red cell counts, hemoglobin and hematocrit determinations, circulatory red cell and plasma volume measurements, and agglutination methods for following the fate of transfused cells. The application of radio-iron to label erythrocytes for the purpose of determining red cell volumes and the post-transfusion survival of human red cells demonstrated the practical usefulness of this agent (20) (21) (22) (23) (24) (25).

If radio-iron in a soluble form as, for example, ferric

ammonium citrate, is administered parenterally, almost all of the labelled iron is removed from the plasma and deposited in the body iron stores. The formation of erythrocytes is associated with the synthesis of hemoglobin which, in turn, will contain a significant proportion of the labelled iron atoms that were previously administered. Thus these red cells will be radioactive due to the presence of radio-iron in the recently synthesized hemoglobin of these newly developed erythrocytes. Since, presumably, hemoglobin does not escape from normal red cells and the iron in this hemoglobin does not exchange with the other iron atoms of the body, it is possible to label erythrocytes and detect their presence in the blood stream. When these cells are destroyed, the radio-iron will be released to the body iron stores and a portion of this labelled iron will appear in the hemoglobin of the erythrocytes being formed at that time. These investigators gave radio-iron to human subjects for the purpose of preparing labelled red cells. These radioactive erythrocytes were then employed initially to determine the values for the red cell volume of the blood. Later, they extended this labelling technic to investigate the post-transfusion survival of preserved human erythrocytes stored as whole blood, or in resuspension, after removal of the plasma. A "double labelling" technic was used which employed the fifty-seven day Fe^{59} and the five year Fe^{55}. The recipients pretransfusion red cell volume was determined by a small infusion of erythrocytes from a donor with labelled red cells who had previously received Fe^{59}. Transfusions of whole blood and re-suspended red cells were then give, the donor cells being labelled with Fe^{55}. Since the radiation characteristics of these two iron isotopes differ, it was possible to distinguish between the small amount of cells labelled with Fe^{59} that was given to determine the pretransfusion red cell

volume, from the erythrocytes administered in the experimental transfusion and which contained Fe^{55}. This procedure made it feasible to study the "in vivo" survival of transfused red cells under several different circumstances. The preservative action was studied of a number of different solutions added to both whole blood and to separated red cells. Whole blood, which was drawn into an acid citrate-dextrose solution, maintained satisfactory transfusion properties during a storage period of 21 days with a 70% viability of the transfused cells at the end of this interval. When the erythrocytes were separated from blood drawn into this solution and stored without dilutent, satisfactory transfusion properties were maintained after a storage period for the same period of time. An investigation of the optimal range of temperature during storage revealed that values between four degrees Centigrade and ten degrees Centigrade were the most satisfactory. Moreover, the rate of deterioration resulting from storage outside this optimal range was not retarded by subsequent storage at from four degrees Centigrade to six degrees Centigrade. In the course of these studies it was found that non-viable erythrocytes are rapidly removed from the blood streams. The majority disappear during the first two hours after the transfusion. Approximately 70% of the radio-iron released by the destruction in the body of these cells was quite rapidly incorporated into newly formed erythrocytes.

Section II

The Applications of Radioactive Tracers as Diagnostic Aids.

Two very interesting procedures have been developed which employed radioactive elements as tracers for the purpose of evaluating the character and degree of several pathological states. Both make use of the "in vivo" technic and appear to possess considerable practical value as well as being relatively simple to execute. The first of these diagnostic tests is the use of radio-sodium as a tracer in the study of peripheral vascular diseases (26). A solution of isotonic saline containing 100 microcuries of radio-sodium is injected into the vein of the ante cubital fossa and the rate of appearance of the labelled sodium in the extremity is measured by placing a shielded counter tube against the foot. The increase in counting rate is determined as more and more of the injected radio-sodium is carried to the extremity. This increase is the result of the interchange of labelled sodium between the blood and the extra cellular fluids of the foot. The radioactivity of the foot will rise until equilibrium is established, at which time the number of radio-sodium atoms leaving the blood vessels is equalled by the number returning to the circulation from the extravascular fluids in the foot. The manner in which the equilibrium is established depends upon the rate that the labelled atoms are delivered to the extremity and the relation between the volumes of blood and extravascular fluid in the tissue that the counter tube can "see". If frequent determinations are taken over a period of an hour or so and the results plotted, a smooth curve is obtained which becomes flat as equilibrium is approached. In normal subjects, the curve begins to

level off in thirty to forty minutes. The majority of patients with peripheral vascular disorders demonstrate equilibrium curves which are lower and level off more gradually. This procedure can be just as readily applied to measure equilibrium curves for other regions of the lower extremities, such as the calf, popliteal space, etc. Moreover, a comparison of the activities of the corresponding levels of the two legs is frequently useful for estimating the relative extent of the disease process. This method is not only of value to demonstrate the relative degree of increase of vascular function as the result of therapeutic procedures, but also is of great assistance in making the decision for the site of amputation when surgery becomes necessary. More than two hundred patients with nineteen different types of peripheral vascular disorders have been studied by this technic. The data obtained is stated to have provided information of sufficient reliability to permit a number of amputations at more distal levels than would have been done otherwise, using the more conventional criterion of circulatory efficiency. Another diagnostic procedure of possibly considerable practical value has been the measurement of radio-phosphorus taken up by breast tumors in situ (27). It was observed in a series of twenty-five patients with breast tumors, that following the intravenous administration of from 300 to 500 microcuries of radio-phosphorus, that there was an appreciable increase in radioactivity over the surfaces of the tumor in situ when the neoplasms were of malignant character. The radioactivity noted over the surface of benign tumors was not significantly different from the values observed at corresponding regions over the surface of the normal breast. The basis of this technic is the fact that malignant tissue, due to its

rapid rate of growth, will accumulate more of the labelled phosphorus which makes the presence of the tumor known by the increased degree of radioactivity in its immediate vicinity. A serious limitation is imposed upon the use of radio-phosphorus for this procedure because there are no gamma rays emitted and the beta particles have a range of about 1 centimeter in the tissue. For this reason, the method is restricted to the study of relatively superficial neoplasms when radio-phosphorus is used. The application of a radioactive element emitting gamma rays, and which will selectively accumulate in malignant tissue, would greatly extend the potential usefulness of this interesting technic.

The Use of Radioactive Tracers as a Prelude to Therapeutic Applications of the Radio-elements.

The therapeutic applications of the artificial radio-elements are based upon the fact that certain radioactive materials will selectively localize in pathological structures, thus subjecting such tissues to a greater degree of irradiation than to the surrounding normal area. It is of interest to review some of the tracer studies which have led to the actual therapeutic utilization of artificial radio-activity and to describe experiments now in progress which may have significant therapeutic potentialities in the future.

Radio-phosphorus was shown, in the very earliest tracer studies, to accumulate to a considerable degree in the skeleton and the more metabolically active soft tissues, notably, liver, stomach, and small intestines; heart, spleen, lymph nodes, and kidneys (28)(29)(30). Moreover, it was observed that malignant tissue demonstrated to a marked

degree the selective accumulation of labelled phosphorus following its administration as di-sodium phosphate. Significant information has been obtained from studies of this character with regard to the accumulation and retention of labelled phosphorus in normal and tumor tissue. Apparently the degree of uptake by the different tissues is related to three factors: first, the total phosphorus content of the tissue; second, its rate of turnover in the tissue; and third, the laying down of new tissue. The relatively large accumulation of administered radio-phosphorus in the bone, which is composed chiefly of calcium and phosphorus, is an example of the first factor. An illustration of the second factor is the effect of the rate of turnover of phosphorus in tissue upon the uptake of a single dose of labelled phosphorus. An example of this phenomena is the marked difference observed under these conditions of the accumulation of radio-phosphorus in the liver and in the brain. The total content of phosphorus in these two tissues is similar, but the uptake of labelled phosphorus varies considerably. The relatively low uptake in brain tissue indicates a much slower rate of phosphorus metabolism than such tissues as liver, kidney, spleen, small intestine, etc. Since in adult animals, the total phosphorus content of such tissues remains constant with relatively narrow limits, the presence of a high proportion of labelled phosphorus in an organ, such as the liver, suggests that the rate of turnover of phosphorus atoms between the cells of the liver and the blood stream is considerably more rapid than in the brain tissue. In other words, it would appear that an atom of phosphorus, whether it be radio-active or stable, remains within a cortical neuron for a much longer interval of time than in a hepatic cell. Many investigators have

demonstrated that the labelled phosphorus taken into the different tissues is bound in firm organic combination. Therefore, the varying rates of phosphorus turnover apparently are a manifestation of the different speeds with which the cells of the body build up and break down the complex organic phosphorus compounds that make up their internal structure. The third factor which influences the degree of uptake of phosphorus is the rate of growth and development of new tissue. This is illustrated by the relatively high degree of accumulation of radio-phosphorus that takes place in tumor tissues in animals, and has also been demonstrated in studies with human subjects with neoplasms (31) (32).

A comparison of the phosphorus metabolism in normal and leukemic mice demonstrated a marked increase of labelled phosphorus uptake by the leukemic lymph nodes, spleens, and livers (33) (34) (35). The total phosphorus content of the normal and leukemic tissues did not differ appreciably. No significant variation in the uptake of radio-phosphorus by the skeleton was noted between the normal and leukemic mice. It was suggested that any variations in phosphorus uptake in the marrow of the leukemic animals were masked by the relatively large quantities of radio-phosphorus laid down in the bone in both groups of animals. The greater accumulation of radio-phosphorus in the leukemic organs, although the total phosphorus content was similar to that of the normal tissues, was interpreted as an indication of the rapid laying down of new tissues as well as of an increased metabolic activity of the pre-existing leukemic cells. A comparison was also made of the distribution of radio-phosphorus in the acid soluble, phospholipid, and nucleoprotein fraction of the tissues of normal and leukemic animals. The nucleoprotein fraction from

the leukemic tissues contained from three to ten times the concentration of radio-phosphorus that was observed in the corresponding organs of the normal animals and this effect persisted for intervals ranging up to more than one week. These results were interpreted as indicating that nucleoproteins were synthesized from the assimilated labelled inorganic phosphorus at a more rapid rate by the leukemic cells than by the normal tissues.

The role of phosphorus in metabolism of normal and malignant tissues has been explored by studying the distribution of labelled phosphorus in the separated cytoplasms and nuclei of cells (36) (37). These studies made it possible to observe the relative uptake of administered inorganic radio-phosphorus in the nuclei and cytoplasms of malignant cells in animal tumors, such as lymphoma, sarcoma 180, carcinoma 256, as well as in normal tissues. These experiments revealed not only the nuclei of the malignant cells accumulate more of the labelled phosphorus than those of the normal cells, but also the relative proportion of the radio-phosphorus in the nuclei, as compared to that in the cytoplasm, was much greater in the neoplastic than in the normal cells. This metabolic pattern of phosphorus in malignant cells was not restricted to neoplastic tissues but was found as well in rapidly multiplying normal cells. A comparison of uptake of labelled phosphorus in the nuclei of malignant cells and of rapidly regenerating liver cells in animals, from whom a large part of the liver had been previously removed, revealed that the radio-phosphorus uptake by the nuclei by these two very different types of cells was essentially the same. The investigators concluded from these observations that the variations in phosphorus metabolism of nuclei are

dependent, at least in part, upon the rate of cell multiplication and are not a peculiarity of malignancy per se. It has been estimated from the observed rate of radio-phosphorus uptake by the nuclei of the lymphoma that an average of 52 hours was required for the formation of a new lymphoma nucleus. This value is in close agreement with the observed rate of the growth of the tumor. Moreover, it has been calculated from these data that in a lymphoma nucleus approximately 3×10^4 molecules of tetranucleotide are synthesized per second.

The bones of animals contain large quantities of both calcium and phosphorus. Unlike phosphorus, the calcium content of the soft tissue is relatively small. Early tracer studies with radio-calcium (38), showed that it was almost exclusively stored in the bones and that only small traces were deposited in the soft tissues. This selective localization of radio-calcium suggested that it might be of therapeutic value in the treatment of malignant bone tumors. The very long half life of calcium (180 days) and the small amounts that are available have precluded its trial as a therapeutic agent. It was predicted and subsequently demonstrated that the metabolism of strontium, which is in the same group in the periodic table of elements, is very similar to that of calcium (39). The radioactive properties of the radio-strontium isotope employed are far superior to the characteristics of radio-calcium in that it has a half life of 55 days and, furthermore, it can be prepared in large amounts. Later studies with patients with several types of malignant bone tumors showed that the accumulation of radio-strontium in the neoplasm was comparable to the concentration in the adjacent uninvolved bone and occasionally was considerably greater (40). The very low concentration in the soft tissues

of the animal experiments was also noted in the human experiments. However, the degree of selective localization in the tumor tissue, as reported in these experiments, does not appear sufficiently great to produce an adequate degree of destructive action upon the neoplasms without producing irreversible damage to the bone marrow from the large amounts of radio-strontium that are accumulated in normal bone. It would appear that further study of the selective accumulation of radio-strontium in bone tumors is warranted in view of the results of the experiments noted above.

The tracer studies with radio-iodine, described earlier in this paper, demonstrated that the thyroids of patients with thyrotoxicosis accumulate and retain a large proportion of this administered radio-element, particularly when the labelled iodine is not diluted with large quantities of non-radioactive iodine. In addition to the various experiments described earlier, another series of studies was made with radio-iodine in man which show further the desirable potentialities of radio-iodine in the treatment of hyperthyroidism. These studies comprised an investigation of the histological distribution of radio-iodine in thyroid tissue by means of the radioautographic technic (41) (42). Thin sections of radioactive thyroid tissue were placed against photographic films. After a suitable interval of exposure, the films were removed and developed and the sections stained. The histological structure of each section and its corresponding radioautograph were compared under a microscope. The areas of darkening represented the regions in the sections in which the greatest accumulation of radio-iodine had taken place. Characteristic patterns of distribution were noted for normal and pathological tissue of different types. With both the normal and pathological specimens,

it was consistently noted that the greatest accumulation of radio-iodine took place in the areas where there was evidence of increased metabolic activity of the gland. This was particularly striking in sections obtained from thyrotoxic goiter. The therapeutic significance of this finding is that the area of hyperplasia, to which presumably the excessive elaboration of the thyroid hormone is mainly referable, would be subjected to the greatest degree of irradiation from the beta rays of the accumulated radio-iodine. The selective irradiation of these hyperplastic areas can thus be expected to produce a relatively greater depression of the hyperplastic portion of the gland as compared to more normally functioning regions. Thus the therapeutic action of radio-iodine in thyrotoxicosis is presumably based on the high degree of accumulation of this radio-element by the gland as a whole and the additional factor of selective localization that apparently takes place within the thyroid tissue itself.

The first radioactive tracer studies to be done in patients with carcinoma of the thyroid were quite discouraging since the uptake of the labelled iodine was observed to be extremely small (2) (16). However only four patients were studied in these experiments. Moreover, a test dose of radio-iodine containing 14 milligrams of iodine was employed, which may have flooded the malignant tissue to such a degree with stable iodine that whatever uptake that might have taken place with the .1 microgram test dose was reduced to the vanishing point. The first positive evidence of a significant degree of deposition of radio-iodine by a thyroid carcinoma was observed in a patient who had one distant metastasis which possessed a considerable degree of differentiation (43) (44). The tracer

dose of radio-iodine employed apparently contained very little non-radioactive iodine and the metastasis accumulated approximately 6% of the administered radio-iodine. It is of interest to note that following the administration of 10 millicuries of a mixture of the 13 hour and the 8 day radio-iodine (I^{130} and I^{131}), the metastasis lost its capacity to take up radio-iodine several weeks later. After several months had elapsed following the administration of the therapeutic quantity of radio-iodine, the metastasis had regained its capacity to accumulate and retain this radio-element. This work was confirmed in another series of experiments in which five patients with thyroid carcinoma were observed to accumulate significant quantities of radio-iodine in the malignant lesions (45) (46). One of these patients is of particular interest since there had been a total thyroidectomy fifteen years earlier, but subsequently a severe thyrotoxicosis developed from the metastasis which apparently contained large amounts of functional thyroid tissue. The tracer studies done with this patient indicated that approximately 50% of the labelled iodine was accumulated by the metastasis which was estimated to weigh at least 300 grams. Biopsy was obtained from a metastasis from one of the ribs and radioautographs prepared. These revealed a considerable degree of deposition of radio-iodine in the viable portions of the tumor tissue. The tumor itself apparently was quite well differentiated. This inference was based upon the fact that the metastasis was capable of synthesizing the thyroid hormone in excessive amounts and the pathological evaluation of the biopsy specimen which was stated to be a metastatic adeno-carcinoma of the differentiated small follicle type. Therapy with radio-iodine produced a definite and prolonged clinical improvement with a remission

of the signs and symptoms of thyrotoxicosis. Concurrently, with the clinical improvement of the patient, the quantity of radio-iodine accumulated by the metastasis in subsequent tracer studies was found to be sharply reduced. It is quite likely that highly de-differentiated thyroid tissue may have a very limited capacity to accumulate radio-iodine but an appraisal of this situation most obviously awaits more extensive studies on a larger group of patients suffering from this type of neoplastic disease.

It has been demonstrated that element 85, recently named astatine, is accumulated and retained by the thyroid in animals and man to almost the same degree that has been observed with radio-iodine (47) (2). This element does not exist in a stable form in nature but can be prepared by the alpha particle transmutation of bismuth in the cyclotron. The only isotope known to date of this element is radioactive. This isotope has a half life of 7.5 hours and decays by the emission of six million electron volt alpha particles. The combination of short half life and alpha particle radioactivity might conceivably make this substance more valuable in the therapy of hyperthyroidism. The basis for the suggestion is predicated upon the possibility that astatine may show the same degree of selective localization within the hyperplastic area of thyroid tissue that has been observed by radio-iodine. Should this be the case with astatine, such hyperplastic areas would be subjected to very intense and highly localized radiation due to the short range of alpha particles in the tissue and their property of producing a very dense ionization.

It has been known for many years that colloidal substances, such as India ink, are deposited and retained for a long period in the

liver and spleen following intravenous administration. An interesting series of studies has been made of the deposition in the liver and spleen of radioactive finely divided chromic phosphate (48). Chromic phosphate was prepared from radioactive phosphorus and ground into a fine powder in which the particle size was approximately one micron in diameter. Suspensions of this material were administered by intravenous injection to mice and dogs and the uptake and retention of various organs of the body followed for intervals of time ranging up to a year. Over 90% of the administered suspension was found in the liver and spleen and the remainder being distributed more or less uniformly throughout the other tissues. The per gram concentration of this radioactive suspension was twenty to one hundred times greater in these two organs than in any of the other structures. It is of interest to note in passing that the concentration in the lymph nodes and bone marrow was at the same low level that was encountered in all other tissues except for the liver and spleen. By using larger quantities of radioactivity, it is obvious that the liver and spleen could be subjected to selective irradiation. A similar type of selective accumulation has also been observed with radioactive dyes (49) (50) (51). In addition to the accumulation in liver and spleen, an increased deposition was noted in both inflamatory lesions and experimental animal tumors. More recently, further investigations have been made in this general direction, employing colloids of radio-zirconium and radio-yttrium (50). In these studies, hydrated colloids of zirconium oxide and yttrium hydroxide were employed. By the addition of lactate to the zirconium and citrate to yttrium in varying proportions, it was found possible to prepare colloids of which

the estimated particle size could be made to range from approximately 1 micron down to .01 microns. With both substances; when the colloidal particle size was in the range of 1 micron, approximately 90% was deposited in the liver and spleen and thus showed no significant variation from the results cited above employing the suspension of finely powdered chromic phospate. However, in the administration of radio-zirconium and radio-yttrium colloids in which the particle size was of the order of .01 microns, a marked difference of behavior in the animal body was noted. In the first place, the very rapid disappearance of the injected material from the blood stream that occured with the coarser colloids and the chromic phosphate, did not take place. Secondly, the distribution in the tissues was quite different in that the accumulation in the liver and spleen was approximately 45% and a similar quantity was taken up by the skeleton. Further investigation showed that most of the active material present in the skeleton was deposited in the bone marrow. This accumulation by the marrow was from seven to ten times greater per gram wet weight than the deposition in the mineral structure of the bone itself. The uptake in the other tissues of the body following the intravenous administration of these finely divided colloids was very low and comparable to the results noted from the chromic phosphate and the coarser colloids of radio-zirconium and radio-yttrium.

An investigation of the accumulation of colloidal manganese dioxide has been made following the intravenous administration of this material to human patients (53) (54). The material was dispersed in a colloidal sol using gelatine as the supporting colloid. The particle size was not stated, but from the description of the procedure employed,

it would appear that the dimension were probably in the range of .1 to 1 micron in diameter. By use of the "in vivo" counting technic, it was possible to detect the presence of the accumulated manganese by the emitted gamma rays. It was demonstrated that there was an appreciably greater amount of radioactivity over the region of the liver than in any other area of the body. The data presented are not sufficiently precise to permit a quantitative evaluation of the accumulation in the liver as compared to the other structures of the body. In all of these experiments with radioactive colloids, it is obvious that the controlling factor is not primarily the radio-element, but rather the size and nature of the colloid employed. A large number of the 96 elements can be incorporated into various types of relatively stable colloids. Thus, should radioactive colloids show promise as therapeutic agents, there is a wide choice of different radioactive isotopes which may be used with regards to half life, as well as the character and energy of radiations.

The Toxicological Application of Radioactive Tracers to Medicine

The recent advances in nuclear physics have culminated in the successful release of atomic energy. The phenomena of fission of uranium and plutonium, which is the only known artificial procedure to date that can unlock nuclear energy on a large scale, is associated with production of 34 elements which are radioactive. There are nearly 200 radioactive isotopes of these elements which are defined as the fission products, and they possess half lives ranging from seconds to millions of years (55). Concurrently, with the release of nuclear energy, there is the production of these fission products as well as neptunium and plutonium, which are also radioactive. The wartime developments in this field have resulted

in the production of fission products in the range of hundred of megacuries and, at the same time, kilogram quantities of plutonium. The creation of such immense quantities of radioactive substances presented a serious toxicological problem to the health division of the Plutonium Project, for it was their responsibility to prevent any of the workers from being poisoned by these agents. One of the first steps to avoid medical hazards of a large number of these most unpleasant substances, was the determination of their fate in the body. This was achieved by a long series of animal studies which were conducted at various laboratories of the Atomic Energy projects. The assimilation, distribution, retention, and excretion of the major members of the fission products series (56), as well as neptunium and plutonium (57), were done using laboratory animals. In addition to investigating the metabolic properties of this group of substances, similar studies were made of the behavior of thorium, protoactinium, uranium, americium and curium (58) (59) (60) (61). Since these elements are also radioactive, they have been of concern to the Atomic Energy program. Space does not permit a detailed recapitulation of the immense amount of work done in this field, however, it is possible to summarize some of the high points of a number of investigations. Only five of the fission products studied are absorbed from the digestive tract to a significant degree, notably, strontium, barium, tellurium, iodine, and cesium. Xenon is rapidly absorbed through the lungs following inhalation and is as rapidly eliminated from the lungs. Strontium and barium are deposited and retained to a high degree by the skeleton. Iodine, as indicated earlier, is accumulated and retained by the thyroid. Tellurium shows some accumulation in the kidneys and blood, with a rather rapid rate

of release from these tissues. Cesium is distributed quite uniformly throughout all the tissues with the greatest accumulation occurring in the muscle and it is quite promptly excreted. The pattern of distribution of strontium, barium, tellurium, iodine, and cesium, following absorption from the digestive tract, is indistinguishable from a metabolism after parenteral administration. Ruthenium, which is a fission product, is not absorbed from the digestive tract and is quite rapidly eliminated following parenteral administration. No very striking degree of selective localization of this radio-element was noted in any of the tissues. The remainder of the fission products studied, which include yttrium, lanthanum, cerium, praseodymium, element 61, zirconium, and columbium, are not absorbed from the digestive tract and show to an appreciable degree a high level of accumulation and prolonged retention in the skeleton. Thorium, actinium, protoactinium, plutonium, americium, and curium likewise are not absorbed from the digestive tract and demonstrate a high uptake and marked retention in the bone. Radioautographic investigation of the histological distribution of a number of the fission products in heavy elements that are stored in the skeleton presented several unexpected findings (62) (63). As might be expected, strontium is distributed primarily in the mineral structure of the skeleton, since its clinical and biological properties are closely related to those of calcium. The other members of the fission product and heavy element series, which include yttrium, zirconium, cerium, thorium, plutonium, and americium, that were studied by this technic, show a very different type of histological distribution in the bone structure. These substances have been found to be deposited to quite a limited degree in the mineral structure of the bone in contrast to

strontium, but rather, they were selectively accumulated in the region of the periosteum, endosteum, and the superficial surfaces of the trabeculae. Evidence has been accumulated to suggest these substances are laid down in the osteoid matrix. Some accumulation of a very spotty character occurs in the cortex with cerium and americium. Apparently these substances are deposited in the region of the small blood vessels of the diaphysis.

This series of fission products and heavy elements differ from strontium, as well as calcium, in another very interesting respect. Various agents, such as age, prolonged lactation, dietary deficiency, ammonium chloride, the parathyroid hormone, rickets, and scurvy, which are known to produce significant disturbances of calcium metabolism in the skeleton and have been shown to alter the pattern of strontium metabolism in the bone, have little or no effect upon the deposition, retention, and localization of these other fission products that accumulate in this organ (62) (64). The failure of the procedures noted above to influence the metabolism in bone of certain fission products and the heavy elements has been observed with yttrium, zirconium, cerium, and plutonium. It is probable that most of the other members of these two groups of elements, which show such a high affinity for the skeleton, will behave in a comparable manner. It can be appreciated from this rather unique behavior that many of the fission products and all of the heavy elements studied present a potential hazard if they gain entry into the body. This situation was considerably aggravated by the fact that a large proportion of the material

stored in the skeleton is laid down immediately adjacent to the very radio-sensitve bone marrow.

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TABLE I

The variation of sodium transfer rates for the four principle morphological types of placenta expressed as milligrams of sodium transferred per hour per gram of placenta at the middle of the 9/10th of pregnancy.

Animal	Transfer Rate	Morphological Type	Cell Layer
Man	4.5	Hemochorial	3
Rat	8.3	Hemochorial	3
Rabbit	6.8	Hemochorial	3
Guinea pig	6.1	Hemochorial	3
Cat	.69	Endotheliałchorial	4
Goat	.41	Syndesmochorial	5
Pig	.026	Epitheliochorial	6

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