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The Impact of Isotopic Tracers
on Physiological Concepts

JAMES S. ROBERTSON

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FOREWORD

The Brookhaven Lectures, held by and for the Brookhaven staff, are meant to provide an intellectual meeting ground for all scientists of the Laboratory. In this role they serve a double purpose: they are to acquaint the listeners with new developments and ideas not only in their own field, but also in other important fields of science, and to give them a heightened awareness of the aims and potentialities of Brookhaven National Laboratory.

Before describing some recent research or the novel design and possible uses of a machine or apparatus, the lecturers attempt to familiarize the audience with the background of the topic to be treated and to define unfamiliar terms as far as possible.

Of course we are fully conscious of the numerous hurdles and pitfalls which necessarily beset such a venture. In particular, the difference in outlook and method between physical and biological sciences presents formidable difficulties. However, if we wish to be aware of progress in other fields of science, we have to consider each obstacle as a challenge which can be met.

The lectures are found to yield some incidental rewards which heighten their spell: In order to organize his talk the lecturer has to look at his work with a new, wider perspective, which provides a satisfying contrast to the often very specialized point of view from which he usually approaches his theoretical or experimental research. Conversely, during the discussion period after his talk, he may derive valuable stimulation from searching questions or technical advice received from listeners with different scientific backgrounds. The audience, on the other hand, has an opportunity to see a colleague who may have long been a friend or acquaintance in a new and interesting light.

The lectures are being organized by a committee which consists of representatives of all departments of the Laboratory. A list of the lectures that have been given and of those which are now scheduled appears on the back of this report.

Gertrude Scharff-Goldhaber

The drawing on the cover is taken from a 5th Century B.C. relief on the Acropolis in Athens, the "Dreaming Athena," by an unknown sculptor.

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INTRODUCTION

Welcome to this 33rd Brookhaven Lecture. I am particularly pleased to introduce this evening's speaker, Dr. James Robertson. Although not one of the first Laboratory employees, Dr. Robertson has been here for a number of years and is well known to most of you.

Dr. Robertson was raised and received much of his formal training in Minnesota, but he actually is a Southerner by birth and spent the first years of his life in Virginia. He attended the University of Minnesota Medical School, where he obtained his M.D. in 1945. He interned with the Navy at the U.S. Naval Hospital at Annapolis and then spent some months on a Navy destroyer. I first encountered him in 1947, when we were both taking postgraduate work at the Donner Laboratory in Berkeley with Drs. John Lawrence, Hardin Jones, Cornelius Tobias, and the late Joseph Hamilton. I remember Jim well at the Donner Laboratory, operating a deuterium falling drop apparatus in one room and a small mass spectrometer in another room down the corridor. He certainly looked busy enough, but I was never too sure how much data he actually collected. These machines were homemade and crude, and there was an art to keeping them operative. As near as I could make out, when one became operative so he could start collecting data, he had to leave to start fixing the other one. He must have gotten some data, however, because he was awarded a Ph.D. in physiology by the University of California in 1949.

Dr. Robertson joined the Medical Staff here at Brookhaven in February 1950. He contributed greatly to the development of the Medical Department, particularly, initially, in the areas of setting up and standardizing procedures for use of isotopes, and in dosimetry. However, the Navy apparently was so impressed and pleased with his initial performance at Annapolis that he received another rather formal, rather insistent invitation to spend two more years in that Service. He left Brookhaven to complete this obligatory duty, and we spent two additional years together at the Naval Radiological Defense Laboratory in San Francisco. He returned to Brookhaven at the end of his military leave in February of 1955 and has been here since.

Dr. Robertson has always had a keen interest in mathematics and physics as well as in medicine and biology, and his knowledge of mathematics is almost unbecoming to a physician. Although his research interests and activities are broad, his principal scientific contributions have been in the field of tracer kinetics, both on the theoretical and the experimental sides. His studies have inevitably led him into the use of computers, and at present he is doing his best to computerize the Medical Department, with some appreciable measure of success. His scientific contributions in physiology, biophysics, and tracer theory have been quite substantial, and his reviews on tracer theory are widely regarded as standard and authoritative works in this field. I know of no one more qualified to speak on the subject that he has chosen, "The Impact of Isotopic Tracers on Physiological Concepts."

VICTOR P. BOND

The Impact of Isotopic Tracers on Physiological Concepts

Four score and seven years ago* isotopes hadn't been thought of. In trying to fit the occasion by citing something pertinent and important that happened 87 years ago (1877), the closest thing that I found was Edison's invention of the phonograph, which seems at best only remotely related to the present subject. George Hevesy, the originator of the use of isotopic tracers, wasn't born until 8 years later (1 August 1885).^{1, p. 11}

Now the use of isotopic tracers has become so extensive that it would be an impossible task to review in one evening their *applications* in physiology. Hundreds of articles appear every year, and in some areas of research the use of tracers has become so well established and so commonplace that frequently the use of a tracer in an investigation isn't even mentioned in the title of the report. If our attention is restricted to *concepts*, however, the material to be discussed becomes much more limited. Even so, it has been necessary to select a few outstanding examples and to omit much of perhaps equal importance. Some of the omitted topics have been covered in previous lectures in this series and others will be treated in future ones.

To begin with a few definitions, it presumably would be safe to assume that this audience does not need a definition of the term "isotopic tracer." However, for the record, let us briefly say that a tracer is a labeled form of a substance that is, for practical purposes at least, identical in behavior with its unlabeled counterpart at the level of unit reaction. An isotopic tracer, of course, is one in which the label is an isotope, which may be stable or radioactive.

It may be more interesting to try to define what is meant by "physiology." A literal translation of the original ancient Greek roots would make it mean the study of physics (physics + knowledge). For ages the term "physiology" was used to mean *all* natural science and was applied impartially to the animal, vegetable, and mineral kingdoms.² The exclusion of inanimate matter did not occur before the 16th century. As the various disciplines developed, physiology became more restricted to the

study of living processes. When used without qualifying adjectives, as in general physiology or plant physiology, the term "physiology" now usually means the study of living processes in mammals.

For centuries the concept of a mysterious "vital force" was employed to explain the differences between living and nonliving matter. Life was regarded as a struggle between the vital forces and those of chemistry and physics, with the latter eventually overcoming the former and destroying the body at death. It was only about 100 years ago (1852) that Ludwig discredited the principle of vitalism and expounded upon the doctrine that living processes obey the same laws of chemistry and physics that all other matter does.³ An appealing modern definition of physiology is the attempt to explain living processes in terms of chemistry and physics. It should be mentioned, however, that the process of attrition is still going on, and about as fast as a process actually is explained in terms of chemistry and physics, it tends to be taken out of physiology and to become part of biochemistry or biophysics, so that what seems left for "pure physiology" are those areas that have not yet been so explained. In addition, the inroads of anatomy, pharmacology, psychology, hematology, nutrition, genetics, and other independent disciplines on the domain of physiology have been discussed by others to the point of questioning whether there remains a field of physiology *per se*.^{4,5} We shall ignore the fine points of the definition and consider all living processes as being within the field of physiology.

The balance of this lecture will be divided into three main parts: (1) an historical background intended to describe the state of thinking at the time of the discovery of radioisotopes; (2) the qualitative aspects of some basic discoveries made possible by and through the use of radioisotopes; and (3) analytic interpretation of the kinetic behavior of tracers – the quantitative aspects of tracer theory.

HISTORICAL BACKGROUND

The reason for inserting an historical background at this point is not only to indicate the place of the tracer method in physiology but also to introduce

*This lecture was delivered on Lincoln's Birthday.

the context of the interdependence of the sciences in general. When we say that much of physiology *has not* been explained in terms of chemistry and physics, this should not be construed to mean that we doubt that these areas will ultimately be explained – it is largely a matter of these problems being too difficult to attack at present, so that progress must await the development of appropriate physical and chemical methods.

This dependency of progress in physiology on the development of techniques in the more basic sciences is emphasized, because the discovery of radioactive isotopes and the development of the tracer method is just one of many possible examples characterizing the present era in which progress in one field has followed at first apparently unrelated discoveries in another field. Much of the recent progress in the biological sciences has come about through improvements in methods and in instrumentation that have been made by nonbiologists.

The dependence of one branch of science on the state of development of another is, of course, an old idea. It was suggested about 100 years ago by the French philosopher Comte, who predicted first a divergence, then a convergence of the sciences. He listed the sciences in such a way that each depends on the preceding science but is independent of those that follow.⁶ As improved by Spencer, the list runs: Mathematics, Physics, Chemistry, Biology, Psychology, and Sociology. For example, as has been discussed by I. e. Corbeiller,⁶ mathematics can be, and often is, presented without any reference to the physical world. Physics uses mathematics but avoids chemical reactions, and so on, until we reach sociology, the most complex, but which is still so much in the descriptive phase that its inclusion as an experimental science may be questioned. Tolman⁷ explains that although mathematics is the simplest of the sciences, “it is hard for you and me to understand, not because the mathematician talks about complicated things, but because he says such complicated things about them.” The sciences remain independent during their descriptive phases, but as they become experimental and predictive, progress becomes more dependent upon the state of the more basic fields. This sequence is both logical and historical. With this mutual dependency in mind, let us go back even further for a moment. As the Red Queen advised Alice, you should start at the beginning.

Shortly preceding Aristotle there was a brilliant philosopher named Empedocles,⁸ who was a disciple

QUALITY	ELEMENT	BODY HUMOR	TEMPERAMENT
HOT + DRY	→ FIRE	→ BLOOD	→ SANGUINE
COLD + MOIST	→ WATER	→ PHLEGM	→ PHLEGMATIC
DRY + COLD	→ EARTH	→ BLACK BILE	→ MELANCHOLIC
MOIST + HOT	→ AIR	→ YELLOW BILE	→ CHOLERIC

Figure 1. Empedocles to Hippocrates. The ancient Greek theory of the universe and some relationships among the “elements” and certain biological properties.

of the Pythagorean school. The Pythagoreans were fascinated by numbers, especially the number 4. On this basis, Empedocles decided that all the properties of matter were the result of various combinations of four principles: warm, cold, dry, and moist. The combinations shown in Figure 1 gave the four fundamental elements: earth, air, fire, and water. These elements were supposed to explain the workings of the universe. If Empedocles were working today, he would be a theoretical physicist.

With physics and chemistry in such a fix, one could hardly expect much of biology. The body was thought of as being rather like an orange insofar as the organization of its juices was concerned, except that there were some four varieties: blood, phlegm, black bile, and yellow bile, the predominance of one or the other being responsible for the temperament as indicated in the last column of Figure 1. The purpose of respiration was supposedly to cool the blood, and the role of food was as a lubricant for the motion of muscles and joints. Absurd as these ideas seem to us now, they held sway for centuries, and vestiges of them remain in our vocabulary today in the terms for temperament.

It wasn't until 1628 that Harvey described the circulation of the blood. Harvey's book on the motion of the heart and blood⁹ is one of the most important books in the history of physiology, and has been said to mark the beginning of physiology as an experimental science. This occurred at about the same time that Galileo was discovering the moons of Jupiter with his newly invented telescope (1610) and allegedly was dropping weights from the leaning tower of Pisa. Harvey's discovery is cited here not only because of its great physiological importance, but as the outstanding example of a discovery that was made by observation within the realm of biology, that is, without depending on help from other disciplines except for a certain amount of arithmetic. It may be pointed out that the microscope was not available to Harvey, and therefore the passage of the blood from arteries to veins could not be explained at that time. Isotopes,

Figure 2. Diagrams of the electrolyte composition of body fluids and sea water. Shown here primarily to emphasize the high K, low Na of cell fluid vs the low K, high Na of the other fluids. From Gamble.¹⁰

like the microscope, are among the useful tools that biology has acquired from physics.

With the discovery of oxygen, the role of respiration in the body began to be understood. The body became likened to a candle, and later to an internal combustion engine which requires fuel (food) and air to support a form of combustion, thus generating heat and being capable of achieving mechanical work. It was recognized that food was also needed for growth and that the body differed from a machine in that it could repair itself to a degree. Aside from growth and repair, however, the body constituents were regarded as being rather fixed, once they were formed. Not only were bones, teeth, and eyes regarded as being fixed

structures but, except for metabolites (that is, the various kinds of food in the form that reaches the cells), oxygen, CO₂, and other metabolic products, the constituents of cells were regarded as being quite permanent.

One of the biological facts that required explanation, for example, was the difference between the internal composition of cells and the composition of their surroundings, as shown in Figure 2.¹⁰ There is a relatively high intracellular potassium and a low extracellular potassium, and the question is, how is such a gradient maintained? The problem would be resolved if it could be shown that the potassium in the cell was trapped there and couldn't get out. This could happen if the cell

membrane were impermeable to potassium so that it couldn't pass in either direction. The experiment of suspending cells in a potassium-free medium and observing that potassium does leak out is open to the criticism that an unphysiological situation has been used and the results are therefore questionable. Before isotopes were available, some very competent workers had decided to resolve the question, and, as the result of a series of careful experiments, reached the conclusion that the red cell membrane was impermeable to sodium and potassium.¹¹ Of course we now know that the conclusion was incorrect. We shall return to this problem later – for the moment it has been mentioned only to illustrate the situation that existed.

THE DYNAMIC STATE OF BODY CONSTITUENTS

We are now ready to discuss the tracer method. The tracer concept itself is, of course, an ancient one and consists in essence of being able to label or tag some members of a group and, by observing the tag, learn something about the behavior of the group. The genius who first put a bell on a sheep as a means of helping to locate his flock may have originated the method.

Physiologists had long been familiar with the use of dyes as tracers for studies of the circulation.¹² The big advantage of an isotopic tracer is, of course, its chemical near-identity with the element being traced. Another advantage, especially with radioactive tracers, is that they can be introduced in quantities sufficiently small that the system under study is not disturbed.

George Hevesy started the use of radiotracers in 1923 when, after two years of unsuccessful effort to separate radium D from lead, he decided that it was practically impossible, and that radium D could be used as a tracer for lead.^{1, p. 14} Following a number of investigations with tracers in such chemical problems as the solubility of difficultly soluble compounds and self-diffusion in metals, Hevesy turned to biological studies. In 1924 he reported studies of the circulation of bismuth and lead in the body. These elements are, of course, not important as natural constituents of the body, but bismuth, at least, did have some then-current importance in certain therapeutic procedures.

Despite the relatively early availability and attractiveness of radioactive tracers, however, some of the most important work, insofar as the impact on physiological concepts is concerned, was

achieved with stable tracers, deuterium and nitrogen-15. Even at that time it was recognized that an isotope of carbon would be closer to being ideal, but none was then available.

In 1934 Rudolf Schoenheimer¹² and David Rittenberg teamed up and developed the idea of using deuterium (which had been discovered by Urey in 1932) as a label in organic compounds for studies of intermediary metabolism. Those of you who heard Dr. Bigeleisen's talk¹³ are aware of the hazard of an isotopic effect in these studies. Although, of course, the theory of isotopic effects hadn't been worked out, the possibility of an isotopic effect affecting the results of the early studies was not overlooked. The experiments were justified on the basis of the observation that the hydrogen in organic matter has the same isotopic composition as that of common water.

Among the early "surprises" was that, if animals (rats) were fed fats containing labeled deuterio-stearic acid, a large proportion of the label was rapidly deposited in the body fats. Not only did labeled fats appear in the fat depots, but, if the animals were given heavy water, deuterio-fatty acids appeared in the fat depots and reached a maximum in a strikingly short time. This occurred even in animals that were losing weight. Also, with reversion to the normal unlabeled diet and ordinary water, the isotopic label in the fats disappeared equally rapidly. This work not only showed a rapid interchange between components of the diet and of the tissues, but provided a basis for the study of many intermediate reactions. Interestingly enough, the only natural fatty acids which appeared not to be synthesized by the rat were the highly unsaturated acids known to be essential for health. This supported the idea that the reason these essential fatty acids have to be included in the diet, while others may be omitted, is that the body does not have the ability to synthesize the essential ones as it does the others.

Similarly, when N¹⁵ became available, Schoenheimer and his colleagues used it in studies of protein metabolism. Labeled amino acids fed to animals in nitrogen equilibrium were found to be rapidly and extensively incorporated into the tissue proteins.

The result of these investigations has been the development of a concept of metabolic "regeneration," meaning a continual release and uptake of chemical substances by tissues to and from a circulating metabolic "pool." This concept has been

summarized as *The Dynamic State of Body Constituents*, the title of a little book by Schoenheimer,¹² from which the preceding discussion was abstracted.

These experiments opened the gates to large areas of investigation that had previously been inaccessible. With the fact of the dynamic state of body constituents established, physiologists and biochemists were attracted to the possibilities of using tracers in investigating the problems of metabolic pathways and turnover rates.

Among the pioneer users of these new ideas was, again, George Hevesy.¹ After a series of important tracer studies in chemistry, this most remarkable man had turned to biological experiments. Hevesy seems to have tried to study everything. He initi-

ated studies of phosphorus metabolism in rats, including early papers on phosphorus exchange in teeth, the rate of rejuvenation of the skeleton, and retention of atoms of maternal origin in the adult white mouse. In the last-named study he concluded that it takes 11 generations before no phosphorus atoms from the original mother appear in the offspring. (Since the diminution is exponential, and therefore theoretically never reaches zero, this is evidently a statistical zero.)

With other isotopes Hevesy studied red blood cells, body water, potassium exchange, iron metabolism, nucleic acid formation, and many other biological systems or processes. Even now, anyone embarking on a new project concerning the use of isotopes in biology would be well advised to check first to see whether Hevesy has already done it.

Zilversmit et al.¹⁴ made a significant contribution to the quantitative aspects of the turnover concept by analyzing the specific activity relationships between metabolic products and their precursors. In Figure 3 the slope of the product curve is positive up to the time the curves cross, after which the slope is negative. It can be shown that in a direct precursor-product relationship the specific activity curves should cross at the maximum of the product curve. Equations based on these relationships make it possible to calculate the turnover rate of the product molecule regardless of the shape of the precursor curve. These relationships are modified when the precursor is not the immediate precursor of the product, and when a given product has multiple precursors. There have been many applications of the precursor-product relationship in the biochemistry of intermediate metabolism of fats, carbohydrates, and proteins.

An example at a more definitely physiological level is in the study of the formation of blood cells.¹⁵ One theory has it that a single type of cell, called the stem cell, gives rise to the multiplicity of cell types that are to be found in the bone marrow and in the blood. A great deal of work has been devoted to the problem of establishing the chain of cell types between a given blood cell type and the stem cell. Tritiated thymidine, a labeled compound developed at BNL by Hughes,¹⁶ is very useful in such studies. Using labeled thymidine, Bond et al.¹⁷ have found, for example, that in the chain leading to the formation of the red cell, the label appears in different cell types at different rates, as shown in Figure 4. These curves can be used as part of the evidence for the sequence in the chain

Figure 3. Specific activity-time relationships between product and precursor. From Zilversmit et al.¹⁴

Figure 4. Time course of tritiated thymidine labeling of hematopoietic series. From Bond et al.¹⁷

of cell types and can be used for measuring the time constants for the various cell types. Similarly, the lifetimes of various cell types can be measured. For the blood cells these lifetimes range from a few days for white cells to 120 days for red cells.

PHOTOSYNTHESIS

The methods used in studying complicated metabolic pathways are perhaps best illustrated by the work of Calvin et al. at the University of California in the now classic study of the path of carbon in photosynthesis.^{18,19} Photosynthesis is, of course, the process by which green plants use sunlight as a source of energy in building carbohydrates $(\text{CH}_2\text{O})_x$ from CO_2 and water. Free oxygen is also released in this process. The over-all reaction is usually represented as

In the apparatus used in some of the studies at the University of California, incandescent lights of 1000 to 10,000 foot candles served as a source of artificial sunlight. Green algae (*Chlorella* and *Scenedesmus*) contained in a flask between the lights could be exposed to light or to darkness at the will of the investigators. Provision was also made for the rapid introduction and mixing of CO_2 labeled

with carbon-14. Another important feature of the apparatus was that the contents could very quickly be dumped into boiling absolute ethanol, which stopped all metabolic reactions. With this apparatus the investigators searched for the first products of photosynthesis. Working with increasingly shorter exposures to light and labeled CO_2 , they continued to find many labeled compounds. Finally, to make a long story short, they had to reduce the overlap of the light and the CO_2 exposures to zero, that is, to expose to CO_2 after the light was off. The light absorption and the CO_2 utilization are separate processes. The light produces an energy state in the plant so that it can pick up CO_2 in the dark. There is evidence that the initial energy conversion reaction is associated with the separation of the water molecule into a reducing agent and an oxidizing agent. After CO_2 exposures of 90 seconds, some 15 labeled compounds could be identified by the technique of making autoradiographs from paper chromatographs. Figure 5 shows typical autoradiochromatographs. When the exposure was reduced to 5 seconds, there were only 4 or 5 compounds and 65% of the fixed C^{14} was in 2- and 3-phosphoglyceric acid, which were apparently the first products to be labeled.

Diphosphates of fructose and glucose are also among the compounds labeled very early, and these combine to form sucrose in at least one of the proposed mechanisms. Sucrose is the first free sugar

Figure 5. Autoradiographs of paper chromatographs of C^{14} -labeled products of photosynthesis in green algae. Courtesy of A.A. Benson.

to appear. As illustrated in Figure 6, the CO_2 carbon enters a cycle of successive compounds connected through a series of reactions. The only precursor necessarily common to carbohydrates, fats, and amino acids is 3-phosphoglyceric acid. Some plants, such as the sugar beet, incorporate most of their incoming carbon into carbohydrate, but with tracers it has been shown that fats and amino acids can be produced without intervention of hexose intermediates.

Similar CO_2 fixing cycles occur in mammalian systems, but of course only in green plants is light the source of energy. Incidentally, it may be mentioned that Ruben,²⁰ using oxygen-18 as the label, showed that the oxygen that is evolved in photosynthesis originates entirely from water, not from CO_2 .

In respiration, there is a similar cycle that runs in the opposite direction in the sense that carbohydrates are oxidized to CO_2 with an associated release of energy. This is known as the Krebs, or tricarboxylic acid, cycle. The Krebs cycle may also be entered through protein metabolism.

Isotopic studies have made it possible to establish many important relationships among carbohydrates, fatty acids, and proteins. For example, it was shown that while there is some conversion of carbon-14-labeled glucose to fatty acids in other tissues, the liver is 10 times as active in this proc-

ess.²¹ One of the major metabolic defects in diabetes is the failure of the ability of the liver to utilize glucose for fatty acid synthesis. It has also been shown that the short-chain fatty acids can be incorporated into glucose, a process that an earlier textbook described as a "figment of the imagination." Insulin controls the conversion in both directions between carbohydrates and fats. It is also possible that insulin exerts much of its control through its effect on protein synthesis. Some of these findings are not only of physiological interest, but have clinical implications that may affect the treatment of diabetes.²² When insulin is given to a patient, his blood glucose concentration falls. Without isotopic tracers, it would be very difficult or impossible to determine whether this fall is due to a decreased glucose production rate or to an increased utilization rate, but with tracers each process can be studied independently. Actually both processes may be important, with utilization being the better established. Insulin controls the rate of entry of glucose into muscle and fat cells, and also affects the output of glucose by conversion from glycogen.²³

Many pathways besides that of carbon have been worked out with radioisotopes. The course of iodine in the body, as illustrated in Figure 7, is another good example. As is well known, iodine is concentrated by the thyroid gland. However, as

Figure 6. Carbon compound synthesis from carbon dioxide in plants. From Bassham and Calvin.¹⁸

Figure 7. The iodine cycle. From Berson.¹²

indicated in Figure 7, the thyroid has to compete with gastric and salivary iodide secretion and with iodide excretion by the kidneys. These other processes can affect the results of thyroid iodine uptake studies. The steps in the synthesis of the thyroid hormone have largely been elucidated through the use of radioiodine.²⁴ The importance of being able to use a minute amount of a tracer was brought out in a study of the transport of the thyroid hormone in the blood.²⁵ With small amounts of tracer, most of the labeled hormone appears in the globulin fraction of blood plasma, but when larger amounts are used, much of the activity appears in the albumin fraction.

ANALYSIS OF COMPARTMENTED SYSTEMS

A large class of substances, such as water and the electrolytes, exist in the body in the same chemical form on both sides of various anatomical barriers, such as the walls of blood vessels, the membranes of cells, and the membranes of cell nuclei. Even within the cell there are other structures that are defined by definite boundaries; for example, little bodies called mitochondria. Within fairly recent times there was still some question as to whether mitochondria were artifacts, that is, a result of staining methods used in preparing cells for study, and not natural, living constituents of the cell. Now these structures are recognized as being very important in the energy-producing reactions in the cell.²⁶

In all these cases, the item of interest is often simply the rate of passage or transport of substances back and forth across the boundary. To determine these rates it is sometimes sufficient to rely on observations of the behavior of a tracer, without requiring chemical isolation and identification of the substance being traced. This is important, because in work with mammals, and in particular with human beings, it frequently is not possible, or perhaps not permissible, to obtain a sample for chemical analysis. With human beings as the subjects, often the only samples that can be obtained are blood, breath, and excreta. Much as we would like to have them, we can't grab samples of bone, muscle, liver, etc., for analysis whenever we please. Some information about the amount and location of a gamma-emitting radioisotopic tracer, however, can be obtained by external counting methods, particularly with scintillation counters. The design of such counters ranges from

those useful for rather precise geometric localization of activity to whole-body counters that are used to assess the total amount of a tracer in the body. In these studies the interpretation of the behavior of the tracer is heavily dependent on mathematical analysis of the data. This kind of analysis was originated by Sheppard and Householder²⁷ and has subsequently become a very useful procedure.²⁸⁻³⁰ In order to discuss this type of analysis, it is necessary to define a few terms and to point out the assumptions involved.

The system being studied is regarded as consisting of a number of subdivisions called *compartments*. It is assumed that in what is called the steady state, the unlabeled composition of each compartment remains constant, so that the rates of flow in and out are equal. It is also assumed that within a given compartment mixing is perfect; that is, the material of interest is homogeneously distributed throughout the compartment, and that any material entering the compartment is instantaneously mixed with the material present. It is realized that these assumptions are oversimplifications of the real situation, but they are necessary as a starting point for the mathematical analysis and are adequate for many purposes. Modifications may be made later as they are needed.³¹

It should be mentioned that a compartment does not have to be defined in terms of anatomical boundaries. A compartment may be a chemical state or a functional subdivision. It may even have a shifting location. For example, all the cells in the DNA synthesis stage of mitosis at one time may be regarded as a compartment for some purposes.

Another extremely important assumption concerns the behavioral identity of the tracer with the material being traced. It is assumed that, at the level of the unit being considered, the labeled material behaves just like its unlabeled counterpart with respect to participation in chemical reactions and with respect to reaction kinetics. When the unit that is labeled is a cell or a large molecule, isotopic effects are negligible. In some reactions at the atomic level, however, the isotopic effect may have to be considered.¹³ There is no question, for example, that heavy water (D_2O) behaves differently in the body than does H_2O , because it has repeatedly been shown that D_2O is toxic to mice and rats.³² These animals die when the heavy water concentration in their body exceeds about 30%. It may be noted, however, that some plants can be essentially 100% deuterated,

and this opens up the interesting speculation that it may be possible to breed mammals with a progressive tolerance for D_2O in succeeding generations. An isotopic effect can even be shown in some carbon reactions.

In most physiological studies, however, it may be assumed that the isotope effect is negligible. Formally, this assumption is expressed by using the same turnover rate constant, k , for both the labeled and the unlabeled material:

$$\frac{\rho}{S} = k = \frac{\rho x}{Sx} = \frac{\rho^*}{Q},$$

where S = the amount of the substance being traced within a given compartment, ρ = the rate of flow of the substance, x = specific activity of the tracer in S , ρ^* = rate of flow of the tracer, and Q = total amount of the tracer in S .

With these assumptions, the differential equation describing the behavior of the tracer with some starting value, x_0 , in the compartment and with no tracer in the inflow is just like the one that describes radioactive decay, with the turnover rate constant being analogous to the decay constant. Of course, the solution equation, $x = Ae^{-\lambda t}$, is analogous also, and the curve that describes the disappearance of the tracer from a single compartment is the familiar exponential decay curve. Thus by determining the rate constant, λ , for the curve that describes disappearance of the tracer, one obtains the turnover rate constant for the unlabeled substance in the compartment. To get the absolute value of the turnover rate, it is necessary to know the amount of S . Alternatively, if ρ is known, S can be calculated. The convenient equality between the turnover rate constant and the exponent in the solution equation also holds for multicompartment systems having "one-way traffic." In chains of compartments with no feedback, it can be shown that the solution equation will have several exponential terms and each exponent will correspond to one of the turnover rate constants, again in analogy with the decay of mixtures of radioactive isotopes or chains of parent-daughter isotopes.

When there is feedback, however, as in the two-compartment closed system, the relationships between the rate constants of the system and the slopes of the curves change. In the two-compartment closed system with the label confined to the first compartment at time zero, the solution equations

Figure 8. Redistribution of labeled substances in a three-compartment closed system in the steady state. From the initial conditions indicated in the left diagram, the labeled material is redistributed among the three compartments according to the curves in the middle until a final equilibrium condition indicated on the right is reached. In this system, analysis of any one of the disappearance curves provides all the information needed to calculate the relative sizes of the compartments and the rates of flow between them. In higher-order multicompartment systems, it is necessary to sample several compartments in order to obtain enough information for a complete solution.

$$x_1 = Ae^{-\lambda t} + B \quad \text{and} \quad x_2 = B(1 - e^{-\lambda t})$$

have only one exponential term, the second having degenerated to a constant, the equilibrium value. The exponential constant, λ , is the same for both curves, and λ , instead of being either of the two turnover rate constants, ρ/S_1 or ρ/S_2 , can be shown to be their sum, $\rho/S_1 + \rho/S_2$. Similarly, in the case of the three-compartment closed system, the solution equations have only two exponential terms and the λ 's are the roots of a quadratic equation in which the coefficients are various groupings of the turnover rate constants. The variety of curves that result from the introduction of a tracer into a three-compartment closed system is shown in Figure 8, which illustrates the changes in distribution between time zero and equilibrium.

In going through the equations this way the parameters of the system have been used to predict the behavior of the tracer, as described by the solution equations. The real problem, however, is how to proceed when given the solution equation,

for example, by fitting a curve to experimental data, and what is required is to deduce the characteristics of the system; in other words, to work the reverse problem. This can be done as follows. If the solution values for x_1 , x_2 , etc., are substituted back into the original differential equations, a matrix consisting of the product of the rate constants and the coefficients from the solution equation is obtained. Also, if the solution equations are differentiated, derivatives of x_1 , x_2 , etc., are obtained that can be equated to the original derivatives, but in this case the matrix consists of products of the λ 's and the coefficients. Therefore

$$|k||C| = |C| - \lambda|$$

and with matrix algebra one can solve for the k 's by using the inverse of $|C|$ as a post-multiplier:

$$|k| = |C|^{-1} |C| - \lambda|$$

Thus in principle it is possible to solve for the rate constants of the system from the constants (the coefficients and exponents) in the equations that describe the behavior of the tracer. For the two- and three-compartment systems this is simple enough to be achieved by desk calculations, but for more complicated systems the equations are too unwieldy and some help is needed. One approach is to use an electrical analog computer. The equations for the discharge of capacitors through resistors are analogous to those for compartmented systems. Thus a model of the real system can be set up on an analog computer and electrical values can be suitably chosen to obtain curves that fit the data.^{33,34}

Two things should be pointed out at this time. First, the use of an analog computer in this way is not just a fancy curve-fitting method; it is a way of by-passing all the intermediate steps involved in writing and solving differential equations, and provides a means of jumping directly from the data to a model of the system. Second, any model so obtained may or may not be unique for several reasons. In the three-compartment system, for example, the same data can be fitted by one model, assuming that the tracer is originally in one compartment, and by a different model if it is assumed that a different compartment is involved. In this case other information has to be used to make the decision. Another cause of ambiguity is that the data are of a statistical nature, so that any fit is a compromise.

With an analog computer it is difficult to decide which of several possible combinations of values is the best one to use. For this purpose it is often more satisfactory to use a digital computer and a least-squares program for achieving the curve-fitting. We are using an IBM-7094 program that was borrowed from Mones Berman.³⁵ By an iterative method, this program selects a set of values that gives a least-squares fit between a set of data and a preselected configuration of compartments. The digital computer, on the other hand, is less flexible than an analog computer at the stage of searching for the model, or configuration, to use. Thus a good working procedure is to use an analog computer to search for the model and then to switch to the digital computer for obtaining the best fits and for routine fitting. This program can also be used to give a fit simply in terms of exponential components and is being used by at least one other group in the Laboratory for this purpose; there is a bit of two-way traffic in the matter of cooperation between the departments of the Laboratory.

Earlier in this lecture I promised to return to the question of the permeability of the red blood cell to sodium and potassium. If a tracer quantity of potassium-42 is added to a medium in which red blood cells are suspended, it is easily shown that the tracer enters the cells.³⁶ Similarly, once the cells are labeled, if they are resuspended in a tracer-free medium, the tracer leaves the cells, which shows that the membrane is permeable to potassium in both directions. From the tracer data the rate of exchange of unlabeled potassium between red cells and plasma can be calculated by using the two-compartment model discussed above. Thus a tracer experiment simple enough to be used for teaching generates data about a process that only a few years ago was inaccessible to investigation. By an extension of this technique, Tosteson³⁷ was able to show that the flux of potassium both into and out of the red blood cells of sickle cell anemia patients was faster than normal under conditions such that the cells were losing potassium. Modern explanations of the high intracellular potassium content are in terms of active transport and discrimination on the basis of differences in the sizes of the hydrated ions of sodium and potassium.

Another group of studies involves the analysis of the rates of disappearance of labeled materials injected into the circulation. In Figure 9 the inverse

of the concentration, or the apparent volume of dilution, is plotted for several substances.¹ It may be seen that Na, Br, and Cl level off with apparent volume of dilution of the order of 20 to 30% of the body water volume. This is consistent with the volume of the extracellular fluid. Potassium, however, levels off at 150%. This is possible because potassium has a higher intracellular concentration. The curve for phosphorus doesn't level off because phosphorus is metabolized and continues to leave indefinitely.

Studies of this kind may be used as measures of the circulation rates for various organs because the blood perfusion rate is the rate-limiting process. The true transcapillary exchange rates are usually much faster and cannot readily be obtained from blood samples only.

It should be pointed out that tracers sometimes create questions rather than providing answers. For example, surprising as it may sound, there is still a controversy in the literature concerning the mechanism of one of the most fundamental of biological processes, the flow of the solvent in osmosis. Figure 10 is an attempt to describe the major opposing views. In the upper left diagram the arrows indicate that diffusion of solvent in the two directions across a membrane is equal. In the diffusion theory (middle left) the presence of a solute reduces the diffusion rate of the solvent, giving a net flow from pure solvent into solution. The hydro-

Figure 10. Comparison of the conflicting theories on the mechanism of solvent transfer in osmosis. Explanation in text.

static pressure needed to restore the diffusion rate is a measure of the osmotic pressure³⁸ (lower left). Mauro,³⁹ however, has data indicating that a hydrostatic pressure produces a much greater flow rate per unit of pressure than does diffusion, as measured by the use of H_2O^{18} labeled water (upper right). In an alternative theory (middle right) the presence of the solute creates a momentum deficiency on the solution side of a pore in the membrane (Onsager, cited in ref. 39), giving rise to a hydraulic pressure, with bulk flow from solvent into solution. An applied hydrostatic pressure (lower right) stops this flow by opposing the generated pressure, again leaving diffusion as the remaining component of motion.

Nims has undertaken to resolve the situation through consideration of the balance between electrochemical forces and external forces, concluding that in the osmotic situation the observed flow of the tracer bears little relation to the flow of the substances concerned.⁴⁰ There is evidently still much to be done, both experimentally and theoretically, before these issues are finally decided.

CONCLUSION

Many additional examples of the applications of the uses of isotopes could be cited, but that is not

Figure 9. Apparent volumes of dilution of various labeled substances introduced into the circulation. The apparent volume of dilution indicates the distribution of the unlabeled material and the rate of interfusion of the label with its unlabeled counterpart. From Hevesy.¹

the purpose of this lecture. What I have tried to do is to show that the tracer has made it possible to study such things as the pathways of metabolized substances in the body and the kinetics of steady state systems. These studies have helped change the idea of the body as being composed of fixed constituents to one in which most of the tissues are subject to constant renewal, and have greatly modified our ideas about many living processes and the relationships among body constituents. At the same time I have indicated that this kind of research is one that has resulted from, and still depends heavily upon, ideas and methods from the more basic disciplines. Perhaps I have also indirectly helped to explain why the Medical Department needs pulse-height analyzers, whole-body counters, and computer time.

Finally, to symbolize the progress that has been made in bringing the physical sciences and mathematics into physiology and medicine, I quote a slightly ungrammatical limerick by Lusted:⁴¹

There was a young Doc from Atlantic
Whose data was all automatic
To make a house call
Computer and all
He needed a bag most gigantic!

Maybe in the future computers can be miniaturized so that this won't be such a problem.

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