Isotopic Tracers in Biochemistry and Physiology

JACOB SACKS PH.D., M.D.
Associate Professor of Chemistry
University of Arkansas, Fayetteville

FIRST EDITION 1953

McGraw-Hill Book Company, Inc.
NEW YORK TORONTO LONDON

ISOTOPIC TRACERS IN BIOCHEMISTRY AND PHYSIOLOGY

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52-12364

THE MAPLE PRESS COMPANY, YORK, PA.

PREFACE

The introduction of isotopic tracers into biochemistry and physiology has made possible a direct experimental approach to many types of problems which previously were in the province of the philosopher rather than the experimental scientist. The results obtained from experiments with the heavy stable and the radioactive isotopes of the elements of biological importance have brought about a revolution in the ways of thinking of investigators in these fields. The new technique has in turn given rise to its own specialized vocabulary, with which the student must become familiar for a clear understanding of the literature.

Many of the early studies with isotopic tracers were of a descriptive nature rather than an analytical one. With increasing familiarity with the potentialities of the technique, the emphasis has been increasingly on the use of isotopes to elucidate mechanisms. This is particularly the case in investigations on intermediary metabolism. As a result of this type of experiment, new concepts have arisen and become firmly established, displacing the speculations of an earlier day.

It cannot be too strongly emphasized that the proper use of isotopic tracers represents a specialized technique, and is not an end in itself. For this reason the organization of this book is in terms of subject matter rather than of isotope. It should also be pointed out that the isotopic tracer is not necessarily the only possible approach to the problem at hand, and that the data yielded by this type of experiment do not interpret themselves automatically. Properly designed experiments are still necessary, and the results obtained must be subjected to critical analysis in order to avoid reading into them more than is contained.

The purpose of this book is to discuss the principles involved in the use of isotopic tracers, to describe the major accomplishments that have resulted from their application to biochemistry and physiology,

and to point out some of the limitations inherent in their use. The object has been to select from the vast and rapidly growing literature those studies which represent major contributions, help to integrate results of other experiments, or serve to illustrate some of the problems and pitfalls that arise in the course of this type of experimentation. Many worth-while investigations have been omitted from consideration in order to preserve as much unity as possible in the presentation of such diverse material. The emphasis has been placed on mammalian physiology and biochemistry for the most part. References to studies on plant material and microorganisms have been made where these serve to emphasize or to clarify. A chapter on photosynthesis has been included because of the overwhelming importance of the process and the outstanding contributions that have resulted from the application of radioactive carbon to this problem.

The treatment has been in terms of the broad outlines of the methods used and the results obtained rather than of the details of the experimental procedure. For these details the original literature should be consulted. A brief account of the elements of nuclear physics and radioactivity and of the principles of measurement of both the heavy stable and the radioactive isotopes has been included, for the sake of completeness.

The author has had the benefit of critical examination of most of the chapters by specialists in the fields represented. The cooperation of Dr. Samuel B. Barker, Mr. William Bernstein, Dr. George B. Brown, Dr. Martin Gibbs, Dr. William H. Rubinson, Dr. Kurt Salomon, Dr. Sidney Weinhouse, Dr. Abraham White, and especially of Dr. Vaughn T. Bowen is acknowledged with deepest appreciation. For any errors of commission that have crept in, such as misinterpretations of results, and for any errors of omission of important contributions the author has sole responsibility.

Special thanks are also due to Dr. Wilma C. Sacks for many helpful comments and for invaluable aid in the tedious but all-important tasks of proofreading and of preparing and checking the index.

JACOB SACKS

Fayetteville, Arkansas
January, 1953

CONTENTS

CHAPTER 1 GENERAL PRINCIPLES IN THE USE OF ISOTOPIC TRACERS

The concept of the use of the isotopic tracer derives from the pioneer investigations of George Hevesy with the naturally occurring radioactive isotopes of lead and bismuth. As early as 1920 [9] he began to use these isotopes in such diverse fields as the study of chemical exchange reactions in solution and of the self-diffusion of metals. These studies made use of the radium D and thorium B isotopes of lead. Three years later he initiated the use of these tracers in living matter, using thorium B to study the uptake and translocation of lead in plants, and a year after that he made the first isotopic tracer experiments in animals, using radium D and E as tracers in the study of the distribution of lead and bismuth, respectively, in animals. These experiments were the forerunners of many of the different types of tracer applications of isotopes which have contributed so much to our understanding of the dynamic processes in living matter.

Shortly after these experiments, Lacassagne [11] introduced into biology the technique of radioautography (p. 46), which was the means by which Becquerel discovered the existence of radioactivity. This technique has been of great utility in studying the localization of radioactive isotopes in the tissues. The experiments of Lacassagne were on the distribution of polonium in the tissues of the rabbit.

The possibilities of using the naturally radioactive elements as tracers were rather limited, on account of the small number of such isotopes available and the relative unimportance of these elements in normal body processes. The discovery of artificial radioactivity in the light elements by Joliot and Curie in 1934 was the prelude to a tremendous expansion of the scope of the isotopic tracer technique. The first tracer experiment with such a radioactive isotope was again made by Hevesy [4]. It consisted of a study of the distribution in the body of radioactive phosphorus injected in the form of sodium phosphate.

The development of the cyclotron made it possible to produce fairly large quantities of radioactive isotopes of many of the elements of biological importance. As a result, these isotopes began to be used in biological tracer experiments wherever cyclotrons were available. At that time, the Geiger-Müller counter, one of the most sensitive means for the measurement of radioactivity, was a hand-made instrument and many technical problems were involved in its use. The other types of instrument used for this purpose, while familiar to the nuclear physicist, were not generally known to biologists, so that the use of these isotopes as tracers was limited to a relatively few institutions.

All this was changed by the developments in the Second World War that culminated in the atomic bomb. After the war, when control of atomic energy was transferred from military to civilian hands and the Atomic Energy Commission was set up, the nuclear reactor at Oak Ridge was turned to the task of producing radioactive isotopes for research. The quantities that it could produce were such as to dwarf the capacities of the cyclotron, and some isotopes were made available in quantity which previously could be obtained in only the merest traces. The most important one in this category is C^{14}, the long-lived carbon isotope. Administrative machinery was set up to make these isotopes articles of commerce, which could be had by any laboratory that was properly equipped to utilize them in research projects. The direct cost of the radioactive isotopes produced by this means is so low that the isotope itself is generally the least expensive part of such research work. And, also as a result of the wartime developments, Geiger-Müller counters and the other paraphernalia for the detection and measurement of radioactivity have become articles of commerce.

HEAVY STABLE ISOTOPES

The discovery by Urey in 1932, of deuterium, the heavy stable isotope of hydrogen, and the finding that it could be concentrated relatively easily, by the electrolysis of water, opened the way to the use of this type of tracer and increased the possible scope of isotopic tracers in biology. Hevesy was among the first to make use of this type of tracer in experiments on the rate of elimination of heavy water from the body [10].

Urey then turned his attention to the problem of concentrating the heavy stable isotopes of some of the elements of biological importance and developed methods for obtaining the heavy isotopes of carbon, nitrogen, and sulfur in sufficiently high concentration to permit their use as tracers [20]. His colleague at Columbia University, Schoenheimer, had already made extensive use of deuterium in what may be

regarded as the first tracer experiments of an analytical nature, designed to elucidate mechanisms of body processes [18]. As soon as heavy nitrogen became available, Schoenheimer began to use it in experiments that have produced a revolution in the thinking of biochemists [17]. Heavy carbon and sulfur have been almost completely superseded by the radioactive isotopes of these elements, the production of which is feasible only in the nuclear reactor. Heavy nitrogen, N^{15}, remains as the only tracer of this element, because there is no radioactive isotope of nitrogen which has properties that will permit its use as a tracer in biology. Material is commercially available which contains N^{15} in almost a hundred times the natural abundance, namely, 0.38 per cent. Oxygen is the other element of biological importance for which no satisfactory radioactive isotope exists; the only available tracer is the heavy isotope, O^{18}, of which the normal abundance is 0.204 per cent. The concentration of this isotope has not been carried to so great a degree on a commercial scale as with heavy nitrogen or carbon.

BASIC CONCEPTS

The fundamental property that makes it possible to use isotopes as tracers is that all the isotopes of an element have identical chemical properties. This applies whether the isotopes are the naturally occurring stable ones or the radioactive ones that are produced by nuclear reactions. The distinctive physical property which serves to detect and measure the concentration of the isotope in question is independent of the chemical transformations that the element may undergo. The chemical properties of the element are determined entirely by the number and configuration of the extranuclear, or planetary, electrons. These are identical for all the isotopes of any given element. The number of these electrons is equal to the number of protons contained in the nucleus, which defines the *atomic number Z* of the element. The neutrons which go to complete the nucleus contribute mass, but no electrical charge. The number of protons and neutrons together gives the *mass number A* of the particular nuclear species, or *nuclide*. Nuclear species which contain the same number of protons (and therefore of extranuclear electrons) but different numbers of neutrons are *isotopes*. In the usual notation used by nuclear physicists in the United States, Z appears as a subscript to the left of the chemical symbol for the element and A as a superscript to the right of the symbol. Thus the two stable isotopes of carbon are written ${}_6C^{12}$ and ${}_6C^{13}$. In most references to tracer uses, Z is omitted, since the chemical symbol automatically includes this. There are no

special symbols or names assigned to the various isotopes except for hydrogen and the naturally radioactive elements. The lightweight stable isotopes of hydrogen is called *protium*, and the symbol H is retained for it. The heavy stable isotope, of mass number 2, is called *deuterium* and has the symbol D; the radioactive isotope, of mass number 3, is called *tritium* and given the symbol T. There are also nuclear species having the same mass number but different atomic number, for example, ${}_{18}A^{40}$ and ${}_{20}Ca^{40}$. Such pairs are called *isobars*.

EFFECT OF ISOTOPIC MASS ON REACTION RATE

The statement that the chemical properties of all the isotopes of any given element are identical requires some clarification. It is true that all isotopes will go through the same reactions, to form the same compounds, and that the isotopic composition of material from different natural sources is constant. However, there are differences in reaction rate, which depend on differences in isotopic mass, that may show up under nonequilibrium conditions. Ordinarily this difference in reaction rate is not great enough to be significant in a biological tracer experiment, but it may assume importance under certain conditions. In a reaction involving the breaking of a carbon-carbon or carbon-hydrogen bond, the rupture will take place more readily with the lighter isotope than with the heavier one. This is true whether the heavy isotope is stable or unstable, *i.e.*, radioactive. The activation energy, which is the energy that must be supplied to the system to permit the reaction to take place, is lower for the lighter isotope. This is because the internal energy of the bond is an inverse function of the effective mass of the atom. The principal component of the energy of a chemical bond at ordinary temperatures is the "zero-point" energy, that is, the residual vibrational energy of the bond at absolute zero temperature. This is equal to Planck's quantum constant h multiplied by the characteristic frequency of vibration ν. This frequency term is the one which varies inversely as the mass of the atom.

Bigeleisen [1] has presented a theoretical discussion of the isotope effect on relative reaction velocities and has calculated the maximum ratios of the rate constants for many of the important tracer isotopes [2]. These maximum possible ratios are in general considerably higher than those likely to be encountered in experiment. For example, the theoretical maximum ratio for the rupture of a C^{12}-C^{12} bond in comparison with a C^{12}-C^{13} bond is given as 1.25, and for the C^{12}-C^{14} bond, 1.5. Yet in the decomposition of ordinary malonic acid to carbon dioxide and acetic acid, Bigeleisen and Friedman [3]

found a maximum ratio of 1.04 for C^{12}-C^{13} malonic acid, and Yankwich and Calvin [21] found a ratio of 1.14 for C^{12}-C^{14} malonic acid. The isotope effect decreases very rapidly with increasing mass number, so that for the radioactive isotopes of sodium, potassium, and phosphorus the theoretical maximum ratios are of the order of 1.02 to 1.05. These ratios are so close to unity as to be well below the limits of accuracy of experiments on biological systems and may therefore be neglected. However, in the case of the hydrogen isotopes, the difference in rate constants of the reaction is so great that the data obtained with deuterium, and especially with tritium, may not be valid for protium. The maximum ratio of the rate constants calculated by Bigeleisen for the protium-deuterium pair is 18, and for the protium-tritium pair, 60.

This caution applies only to those cases where the process being traced involves the transfer of the deuterium or tritium from one carbon to another or to oxygen. Where the tracer function is that of an auxiliary tracer for carbon, these strictures do not apply to those cases in which the deuterium or tritium atom can be expected to remain firmly bound to the same carbon in the course of the reaction. For example, in the experiments of Schoenheimer and Rittenberg [18] on the mobility of the depot fats, deuterium was useful as a tracer when it was introduced into those positions in the fatty acid molecule in which it would remain firmly bound if the carbon chain remained intact. Under these conditions there is no isotope effect, and the results obtained are valid as to rate as well as qualitatively.

SEPARATION OF HEAVY STABLE ISOTOPES

The effects of isotopic mass on the physical properties of a compound are appreciably greater than the effects on chemical properties, and some degree of separation can be accomplished by methods which depend on differences in physical properties. Most of these are based on the differences in rate of diffusion of molecules in the gaseous state. The best known example of this is the large-scale separation of U^{235} from U^{238} by diffusion of the hexafluorides through porous barriers. The degree of separation achieved by any such diffusion process is very small. The efficiency can be increased by combining what is essentially a diffusion process with a chemical exchange reaction, as was done by Urey [20] for a number of isotopes. For the concentration of N^{15}, the exchange reaction between ammonia and ammonium nitrate was utilized. The ammonia gas was bubbled through a long column of a concentrated solution of ammonium nitrate. Under these conditions, a certain concentration of the heavy isotope takes

place in the liquid phase. The equation for the process can be written

$$N^{15}H_3(\text{gas}) + N^{14}H_4NO_3(\text{aqueous}) \rightarrow N^{14}H_3(\text{gas}) + N^{15}H_4NO_3(\text{aqueous})$$

The calculated equilibrium constant for this reaction is 1.033, and the enrichment actually obtained was somewhat less than the theoretical. By converting most of the product of the first column to ammonia gas, and bubbling this through the remainder of the solution, a further concentration of the N^{15} in the solution was obtained, and by operating a third column with the product of the second, a high enrichment was obtained. Naturally, the total quantity of N^{15}-enriched material obtained was only a minute fraction of the starting material. Urey was able to concentrate C^{13} by a similar process, using HCN gas and a solution of NaCN. In this reaction, the equilibrium relations are such that the C^{13} is concentrated in the gas phase. No concentration of N^{15} takes place in this exchange reaction.

RADIATION EFFECTS IN TRACER EXPERIMENTS

Very early in the course of the use of radioactive isotopes as tracers, the point was raised that the radiation effect on the tissues might be so great as to produce profound alterations in cell physiology. If this were so, the results of tracer experiments with these isotopes would have no validity. Ionizing radiation, such as that given off by the radioactive isotopes, does have marked effects on living matter, and if the quantities of radioactive isotope needed were great enough to have such effects, the method would fail. However, it was soon seen that the quantities of isotope needed for most tracer experiments were so small that the radiation effects would be negligible. So well established has this thought become that there have been very few attempts to show by actual experiment that this is the case. Mullins [14] studied the effect of increasing amounts of radioactivity in the medium on the permeability of the cell membrane of *Nitella* and found that there was no effect below a concentration of 1 millicurie (mc) (p. 29) per liter. This concentration is far above the amount necessary in most tracer experiments.

A thorough investigation of the possible radiation effects in tracer experiments has been made by Skanse [19]. The isotope used was I^{131}, and the effect of various doses on the physiology of the thyroid was studied in chicks. He investigated the effects of the radiation on the capacity of the gland to collect iodine, to grow, and to respond to thiouracil and to thyrotropic hormone. Doses of 10 microcuries (μc) (p 29.), which are appreciably larger than those needed for most

tracer experiments in animals of the weight range of his chicks, did result in definite impairment of thyroid function in experiments which lasted over several days. In short-time experiments, in which only the capacity of the gland to collect iodine was determined, even 50 μc did not produce within 48 hours any radiation effect that could be demonstrated by statistical analysis of the data. These results show that, while ordinarily one can select a dose of radioactive isotope that is adequate for all tracer needs without being in danger of having radiation effects enter, one should be careful to use only the minimum quantity necessary, especially if the experiment is to be of long duration.

THE SCOPE OF TRACER EXPERIMENTATION

The tracer technique has made it possible to investigate a whole range of phenomena in biochemistry and physiology which otherwise would not have been subject to direct experimentation. In other instances, use of isotopic tracers has made possible a direct attack on problems which could have been studied only by relatively indirect means. The greatest successes of the technique have been in studying the dynamics of the steady state, in the analysis of the transport of ions across cell membranes, in the study of the pathways of intermediary metabolism and metabolic interconversions, and in the study of mineral metabolism, especially of the trace elements. Isotopic tracers have also introduced a new method of quantitative analysis, isotope dilution analysis, which is a very effective means of solving certain analytical problems. This does not by any means exhaust the list of general fields of usefulness of the technique.

The Dynamics of the Steady State. The steady state is represented by the condition in which the amount or concentration of the substance under study, or the rate at which a physiological activity takes place, remains constant during the period of observation. In biochemical terms this may be represented by a constant level of blood glucose or electrolytes, and in physiological terms, by a constant pulmonary ventilation or cardiac output. Before tracer isotopes were available, it was not possible in many cases to establish whether the steady state is maintained by a constant formation and breakdown of the substance in question, or represents a condition of immobility. This was particularly true of biochemical problems. One of the most important contributions of the tracer technique has been to demonstrate the dynamic nature of most steady states and to make it possible to study factors which affect the rates of these dynamic processes.

Transport of Ions across Cell Membranes. Formerly it was possible to

study permeability only in the special cases where the cells could be separated mechanically from the medium in which they were suspended, and with respect to substances which were not normal cell constituents, for only under such circumstances was it possible to determine the rates at which these substances crossed the cell membrane. In the mammalian organism, this meant, for all practical purposes, that the studies were limited to erythrocytes. Studies on permeability of these cells to ions were not particularly fruitful, because the introduction of an increased concentration of an ion into the medium in order to determine its effect on the rate of transport might of itself have an unknown and unmeasurable effect on the property being studied. These strictures do not apply to the isotopic tracer, for the added concentration of the ion being studied in the medium can be so small as to be imperceptible. Furthermore, the rates and mechanism of ion transport in all the fixed tissues of the body can now be studied under physiological conditions.

In a related field is the use of the isotopic tracer to study the size of certain compartments in the body: plasma volume, blood volume, the extracellular phase, etc. Some of the methods previously available in this type of investigation have been what might be called non-isotopic tracers: bromide or thiocyanate to determine the amount of extracellular phase, dye methods for blood volume. The substitution of the isotopic tracer in these instances has meant the replacement of a foreign substance by one which is a normal body constituent.

Intermediary Metabolism and Metabolic Interconversions. When a normal metabolite is introduced into the body, its carbon atoms cannot be distinguished from those already present, and the metabolic fate of that particular material cannot be followed. However, when a sugar, fatty acid, or amino acid containing an isotopic tracer is introduced into the body, the metabolic fate of the labeled atoms can be followed after their entry into the general metabolic pool. In this way it is possible, in theory at least, to integrate the observations on the whole animal with those obtained by the study of such systems as tissue slices, homogenates, and isolated enzymes. The utility of the labeled metabolite or metabolic intermediate extends much beyond this, for the enzyme chemists have found that the isotopic tracer is necessary in many cases to determine the mechanism of the process, even in cell-free extracts.

Within this general field should be included the use of the tracer to establish the nature of the specific precursor in synthetic processes. In a number of instances, it has been possible by this means to determine the parts played by certain small molecules in the synthesis of more complex ones, not only with respect to whether the small mole-

cule is used, but to identify the individual carbon atoms of the large one which it furnishes.

Mineral Metabolism. The use of isotopic tracers in this field divides into two major aspects: the study of those elements which are present in the body in fairly large quantity, and the study of the trace elements. It is possible to study absorption, distribution, and excretion patterns by the use of amounts of material which are so small that they are comparable to the daily dietary intake and, therefore, impose no unphysiological condition that might lead to an alteration in the normal metabolic pathways. This is particularly important in studies on the trace elements, which may be present in the tissues in such small quantities as to tax the most sensitive chemical methods for their determination. Measurements of radioactivity may be more sensitive than microchemical methods by two or three orders of magnitude.

This is one of the fields in which radioautography is particularly useful. This procedure makes it possible to study the localization of the trace element within the tissue or organ.

Isotope Dilution Analysis. This highly specialized technique is useful where the quantity of material available for analysis is very small, or where it is not possible to separate quantitatively the material being studied from other substances present. In essence it consists of adding to the material to be analyzed a known amount of the substance to be determined, of known isotopic composition, *i.e.*, either a radioactive isotope of known specific activity or a heavy stable isotope in known concentration. The material is then recovered from the mixture, and even if only a fraction of the quantity added is recovered in the pure state, the degree of dilution of the isotopic marker permits calculation of the amount initially present. The details of the method, and some applications, will be discussed in Chapter 6.

PRACTICAL CONSIDERATIONS IN TRACER EXPERIMENTATION

A number of factors that enter into the design and carrying out of a tracer experiment do not enter into consideration when ordinary techniques are used. These arise from both the special character of the isotopic tracers themselves and the processes by which the radioactive isotopes are produced. Among these are the possibility of a chemical exchange reaction taking place instead of a metabolic one, the necessity of ensuring radiochemical purity of the isotopic material used, the necessity of limiting the total amount of tracer substance to one which is within the physiological range, and the requirement that the metabolic fate of the whole molecule being traced be the same as that of the part carrying the isotopic marker. In many cases this

last requirement is one that cannot be met, by the very nature of the experiment. In such cases it is essential to design the experiment so that it is possible to recognize when separation of the isotopic tracer from the remainder of the molecule has taken place.

Chemical Exchange Reactions. The very first tracer experiments carried out by Hevesy dealt with the question of interchange of atoms between two chemical combinations. Hevesy found that there is rapid and complete interchange between two ionized salts of lead, as was to be expected, but no exchange between an ionized salt such as lead nitrate and a compound in which lead is attached to carbon, such as lead tetraphenyl. Obviously, if such chemical exchanges did take place with the isotopic tracers, it would not be possible to utilize them for the study of metabolic interchanges. It therefore becomes necessary to establish whether such an exchange does take place. The general procedure for this is the one Hevesy used: one compound, usually the inorganic one, is prepared containing the radioactive isotope. A solution of this material is mixed with a solution of the other compound, unlabeled, of course. The two compounds are then separated quantitatively, and measurements of radioactivity are made on both. For example, sodium phosphate containing P^{32} is mixed with ordinary glycerophosphate, and the orthophosphate is precipitated by magnesia mixture. The glycerophosphate is then isolated from the filtrate. All the radioactivity is found in the magnesia mixture precipitate, and none in the glycerophosphate isolated. This example and a number of others are cited by Hevesy [9]. In every case of an organic phosphorus compound that has been examined in this way, it has been found that no unexpected exchange reaction takes place between the two compounds. There are, however, cases of other isotopes in which the exchange reaction does take place.

Several cases require special mention. One is the exchange between the hydroxyapatite of bone and the dentin and enamel of the teeth, with such ions as calcium and phosphate. These have been studied extensively by Hodge and his coworkers, both *in vitro* and *in vivo* (Chap. 14). Since these reactions are essentially those of ionized compounds, it is not surprising to find that the exchanges do take place. This does not altogether vitiate tracer studies on the metabolism of bone and teeth with these isotopes, but it does make it necessary to use extreme caution both in the design of the tracer experiment and in the interpretation of the experimental data.

A second case is that involving the reaction between iodide and such compounds as diiodotyrosine and thyroxin, in which iodine is present in an aromatic ring. It is well known to organic chemists that in acid solution it is possible for reactions to take place in which an iodine

atom may be removed from the ring or introduced into it in another position. Miller *et al.* [13] investigated this particular reaction with radioactive iodide and diiodotyrosine and found that the reaction takes place in acid solution to an appreciable extent under relatively mild conditions, but does not appear to take place at an alkaline pH. Hence in tracer experiments with radioactive iodine and the thyroid, it is imperative to use alkaline hydrolysis of the protein material and to limit the exposure of the material to acid media, in order to avoid the possibility of this exchange reaction.

Another type of possible exchange reaction that needs to be taken into consideration is in the use of C^{14}. In most cases involving this isotope, measurements of radioactivity are made after conversion of the carbon to $BaCO_3$. The claim has been made that considerable loss of C^{14} can take place by exchange with atmospheric CO_2 in the presence of moisture. There is some disagreement in the literature as to the extent to which this exchange reaction takes place, but proper technique in handling the precipitates of $BaCO_3$ will avoid the possibility. This includes the avoidance of exposure to extraneous CO_2 during the filtration of the precipitate, drying *in vacuo*, and measurement of radioactivity in a dry CO_2-free atmosphere. These are the precautions necessary, in any event, to obtain valid data.

The situations in which exchange reactions are of the greatest importance are those in which deuterium is used as an auxiliary tracer for carbon. Certain hydrogen atoms in organic compounds are stably bound and, therefore, may be labeled with deuterium as a tracer, while others are labile, subject to exchange, and therefore not suited for tracer applications in metabolic studies. The hydrogen atoms of hydroxyl and carboxyl groups are loosely bound and readily exchangeable. The hydrogen atoms attached to a carbon atom alpha to a carbonyl group are also loosely bound, and so exchangeable, by virtue of the enolization that such compounds undergo. As an example of this, the reaction of deuterioacetone with water may be given:

$$CD_3{\cdot}CO{\cdot}CD_3 \rightleftharpoons CD_2 = \underset{\displaystyle OD}{C}{\cdot}CD_3 + H_2O \rightarrow HOD + CD_2 = \underset{\displaystyle OH}{C}{\cdot}CD_3 \rightleftharpoons CD_2HCO{\cdot}CD_3$$

The process of enolization would be repeated until ultimately complete replacement of the deuterium by hydrogen would be possible. This restriction against the tracer use of deuterium alpha to a carbonyl group applies also to any deuterium atoms so placed in the molecule that they may be converted into positions alpha to a carbonyl in metabolism.

Radiochemical Purity. The biochemist or physiologist using radio-