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this brief review are but the opening wedges into an important field of research, which may be expected to grow rapidly in the near future, and which will make a lasting contribution to our knowledge of chemistry.

THE BIOLOGICAL USE OF THE STABLE MASS ISOTOPES

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The purpose of this article is to discuss briefly the uses, advantages, disadvantages, difficulties and limitations of the stable isotopes in biological research. The discovery of methods of concentrating the stable isotopes, for which major credit must be given to Prof. Harold Urey of Columbia University, has provided biologists with a new and important tool for investigating the course of chemical reactions in tissues. There are two types of isotopes which are used to trace chemical reactions of tissues, namely (1) the radioactive isotopes and (2) the stable mass isotopes. The radioactive isotopes are produced by bombardment of various elements with high speed particles with the result that an unstable element is produced which then disintegrates at a definite rate. On disintegration it emits radiation or electrons which can be measured electrically. The artificial radioactive elements are produced by high speed ionic bombardment in a Van de Graaf apparatus or a cyclotron. The radiation emitted on disintegration is measured with a Geiger-Muller counter which is activated by the ionization produced. Very minute quantities of radioactive materials can be detected with this apparatus, and the method has proved valuable in tracing the metabolic course of such elements as sodium, potassium, phosphorous, iron, and iodine. The radioactive method suffers from the disadvantages that the radioactive elements have a limited life with the result that there is a time limitation on the experimental work. Also, practically all substances contain slight traces of radioactive impurities, and cosmic radiation produces ionization in the measuring chamber, resulting in a "background" which invalidates measurement of weak radiation. The elements of importance to the organic and biological chemist, namely carbon, nitrogen and hydrogen, do not have suitable radioactive isotopes. However, carbon, nitrogen, and hydrogen do have stable mass isotopes which can be concentrated and accurately measured. The stable mass isotopes fill in the deficiency caused by the non-existence of suitable radioactive isotopes and in addition have certain advantages.

The stable mass isotopes are naturally occurring elements. In nature, carbon, nitrogen and hydrogen exist as mixtures of light and heavy elements. The natural abundance of deuterium, H^2 or D, the

heavy isotopes of hydrogen, is 0.02 per cent, of heavy nitrogen N^{15} 0.368 per cent, and carbon 1.10 per cent. Since the natural abundance ratio is found to be the same in elements from all biological sources, this is evidence that in processes of metabolism the heavy isotope behaves in the same manner as the lighter isotope. It is this property which makes the stable isotopes especially valuable. Professor Urey, Nobel prize winner in chemistry, and others have succeeded in producing isotopic mixtures in which the concentration of the heavy isotope has been increased many times. For biological work we use ammonium chloride furnished by Professor Urey, in which the percentage of N^{15} is 5.0 per cent, which is a 13 fold increase. Professor Nier of the physics department of the University of Minnesota is now producing methane with a C^{13} content of 6 per cent, a 6 fold concentration.

The physical chemist and physicist have furnished us with an isotopic mixture in which the heavy isotope is concentrated. This material is then used for synthesis of biological compounds. For example, in our investigations on the formation of creatine we have synthesized glycine, $CH_2(NH_2)COOH$, and sarcosine, CH_3NHCH_2-$COOH$, with the heavy isotopic mixture of nitrogen in the amino group and methyl amino groups, respectively. These synthetic procedures require an organic chemist who is familiar with special methods of synthesis in which precautions must be observed to prevent loss of valuable isotopic material.

With the synthesized biological compounds containing isotopic material, a physiological experiment is next performed. This experiment may consist of simply feeding the synthesized compound to a small animal. Other experiments consist of adding the compound to an enzyme system or perfusing it through isolated organs. Care must be taken to reduce dilution of the heavy element as much as possible and for this purpose small animals or organs must be used. The next step is the isolation and purification of compounds which may be metabolic products of the synthesized compounds. In our experiments we are isolating creatine, which is a possible metabolic product of sarcosine and glycine. The compound isolated must be in pure form and free from any contaminants which might contain the heavy elements. These isolation and purification procedures require careful semi-microchemical technique and in the future will be the limiting factors which decide what type of experiments are feasible when isotopes are used.

Having isolated the possible metabolic compound in pure form, it is then analyzed for the heavy elements. If the heavy element is carbon it is burned and the CO_2 liberated by combustion is collected in alkali. If the heavy element is nitrogen the isolated compound is digested by the Kjeldahl process and the nitrogen converted to ammonium sulphate. The next step is the collection of gaseous carbon dioxide or nitrogen free from contaminants, such as air nitrogen. This collection is performed in an evacuated system

from which all gases have been removed and the pressure reduced to 10^{-4} mm. of Hg or less. If CO_2 is collected the CO_2 is released from carbonate by adding to the carbonate in the evacuated system of a non-volatile acid such as H_2SO_4. When nitrogen gas is collected, potassium hypobromite, KOBr, is added to the ammonium sulphate in the evacuated system. The liberated gases are pumped into small bulbs and analyzed to determine the content of N_{15} or C_{13}. The analysis is made with a mass spectrometer. The mass spectrometer is an instrument developed in the past few years, the first instrument of the type now used having been made by Walker Bleakney at Minnesota in 1932. At the present time two of these instruments are being used at Minnesota, one by Professor Alfred Nier in the physics department and the other by Professor Ivan Taylor in the School of Chemistry. The mass spectrometer consists of an evacuated tube about three feet in length with a bend in the center and the two ends forming an obtuse angle. The bend near the center of the tube is located between the two pole pieces of a powerful magnet. The gas is admitted to one end of the tube through a small opening and is bombarded by a stream of electrons. The gas molecules by this bombardment are converted to gaseous positive ions. A strong electrical field accelerates the ions along the axis of the tube and when the ions reach the magnetic field they are spread out into a spectrum of ionic beams. Each beam corresponds to a definite mass. The deflection and dispersion depends on the strength of the magnetic field and the accelerating voltage. An ion collector is placed at the end of the tube to which the ions are directed, and the various ionic beams which correspond to the various isotopic masses can be directed into the collector. The magnitude of the ionic current is a relative measure of the abundance of the various isotopes. The relative abundance of isotopes can be measured by this instrument with a high degree of accuracy.

When it is found that an isotopic element can be transferred from one compound to another in a complex process this is evidence that the second compound is a metabolic product of the first. There is one disturbing factor in all isotopic biological research whether of the radioactive type or that of the stable mass isotopes. This is the phenomenon of exchange. Thus it is possible for a hydrogen to move from one compound to another if the hydrogen is in a group which can ionize as, for example, an amino group or a carboxyl group. In work with deuterium only stably bound deuterium can be used as a tracer element. There are enzymes in the tissues capable of exchanging an amino group of one amino acid for an oxy group of another amino acid. This exchange only occurs in the presence of tissue enzymes. Hence loss of an amino group is evidence only that an amino acid has been deaminated and one cannot conclude that the compound has been completely broken down. These processes make interpretation of results a cautious matter and emphasize the need of adequate control experiments.

The apparatus involved in the biological use of isotopes is expensive and complex. Several university departments must have facilities and men engaged in a particular highly specialized field of research. The physicist or physical chemist must supply the isotopic material and make mass spectrometer abundance analyses. Organic chemists are required for syntheses of isotopic compounds. Careful analytical chemists are needed for semimicrochemical analytical procedures and physiologists are needed for the animal experimentation. The initial expenditure for apparatus and isotopic chemicals is considerable but once the projects are under way the operation becomes a routine procedure presenting only usual analytical and physiological difficulties.

The stable mass isotopes as their name implies are indefinitely stable. There is no need to finish the problem in a time period before disintegration is complete as is the case with the radioactive isotopes. Synthesized compounds can be made in relatively large quantities and used for years at one's leisure. As a result of experiences with the stable mass isotopes we would conclude that they will prove of unusual value in solving problems of intermediary metabolism and chemical reaction mechanisms.

CHARACTERISTICS OF SPECTROGRAPHIC PLATES IN THE ULTRA VIOLET REGION BETWEEN λ2300–2000Å*

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Introduction

In the use of spectrographic methods for quantitative studies in the ultraviolet region, it is necessary to determine the characteristics of emulsions sensitized for a *certain* spectral region. To date, only a limited number of studies have been made on this topic. Harrison and Leighton[1] presented evidence to show that it was possible to increase the sensitivity of spectroscopic plates to the extreme ultraviolet region by coating them with fluorescent materials.

A report of a detailed study of photographic emulsions in the region between wavelengths 2500–2000 Å has been published by

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[1] Harrison, G. R. and Leighton, P. A. Journal of Optical Society of America, *20*, 313, 1930. Phys. Rev. *38*, 899, 1931.