

# SURVEY OF CONTEMPORARY KNOWLEDGE OF BIO- GEOCHEMISTRY

## 1. ISOTOPIC PHENOMENA IN BIOGEOCHEMISTRY

MARTIN D. KAMEN

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## PREFACE

THE "SURVEY OF CONTEMPORARY KNOWLEDGE OF BIOGEOCHEMISTRY" owes its existence to the generosity of Robert Earll McConnell and George Monroe Moffett, and to the imagination and foresight of Albert Eide Parr, Director of the American Museum of Natural History. The purpose of the survey is to bring together, and publish as a series of monographs, the available information on all aspects of the interrelation of biology and geochemistry. The contact area between these two sciences, which have only recently begun to influence each other, is of direct concern in many of man's efforts to cope with his environment. This is obviously true in agriculture, in the utilization of aquatic resources, in human and animal nutrition, and in certain branches of medicine. It is equally true that aspects of biogeochemistry are involved in our understanding of the history of the earth and its inhabitants, in fact whenever questions arise as to the origin, potentialities, and fate of mankind. In order to consider adequately both the details of practical importance and the more general principles of theoretical significance, all contributors to the series are asked to cast their nets widely, and to report whatever of interest they may land. Much of the information that is available in theory is published in such scattered, miscellaneous, and inaccessible works as to

make it at present of very little utility. As such information is brought together, the interrelation of many previously disconnected facts becomes increasingly evident, and new generalizations emerge not previously available, because the facts known in theory were in practice never known by one and the same person.

The survey is intended as more than a synthetic summary. As much emphasis is to be placed on the lacunae in our present knowledge as on its established content. By defining the gaps carefully, a program for research is developing. Wherever well-defined problems can be investigated in the laboratory without prejudice to the development of the Survey, such studies are being made, but an enormous mass of uncertainty is bound to be disclosed without the prospect of any immediate investigation by those engaged in the project. It is, however, hoped that other workers will be attracted to the field by the inherent interest of the unsolved problems of biogeochemistry.

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*May 26, 1946*





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## INTRODUCTION

A CHEMICAL ELEMENT consists of atoms which are identical or which differ only in mass. In the latter case the element is said to be "complex," being comprised of a mixture of "isotopes."<sup>1</sup> Within the precision ordinarily obtained in atomic weight determinations, the composition of these isotopic mixtures remains invariant for any given element. In general, fluctuations in isotope ratios cannot be detected despite wide diversity in source materials and chemical purifications employed.<sup>2</sup> Conversely, attempts to separate isotopes in the laboratory reveal the extreme difficulty associated with the achievement of even very small separations.<sup>3</sup> Theoretically, it is possible to show that isotopic separations are to be expected in many kinds of processes, physical and biological, but that the separations are so minute as to require not only extraordinary care in chemical manipulation but also physical instrumentation exhibiting a high order of precision.

The discovery of the heavy hydrogen isotope (Urey, Brickwedde, and Murphy, 1932) has provided much of the incentive for a renewed effort to refine existing procedures and instruments. The resulting improvement in isotopic techniques suggests their possible employment in clarifying a

number of problems interesting to the biogeochemist.

Vernadsky (1938, 1944) has suggested that since organisms in the biosphere exhibit preferential assimilation of elements (mixtures of isotopes), they may also show differential utilization of isotopes of a single element. He has proposed isotopic differentiations as a possible basis for classification of biosphere components into "bioactive," "bio-inert," and "inert" categories. To establish a biogeochemical effect by study of isotopic separations, however, requires the ability to recognize operation of all unbiological factors active in producing such effects. Knowledge sufficient to provide a basis for such discrimination is now available from laboratory studies made possible through the development of adequate separation and assay procedures in the last 10 years. In this review, the material to be presented has been organized to include first a brief review of the existing methods for assay and separation of isotopes. This is followed by discussion of all the examples known (hydrogen, carbon, oxygen, potassium) for which strong evidence of isotopic differentiation exists. Fluctuations in isotope ratios brought about by radioactive disintegration will not be considered.

It is reasonable to suppose that a description of conditions under which appreciable isotopic differentiation has been achieved in the laboratory will suggest analogous situations obtaining in geochemical systems and serve as a reliable basis for the prediction of isotope effects of a biogeochemical nature.

<sup>1</sup> This term was proposed by F. Soddy from the Greek "*ισος*," same, and "*τοπος*," place (cf. Soddy, 1911).

<sup>2</sup> For a general discussion, cf. F. W. Aston (1933).

<sup>3</sup> A voluminous literature exists on this subject. Reference is made to the volumes by Aston (1933) and also by Hevesy and Paneth (1938).

## ISOTOPIC ASSAY METHODS

TWO MAIN TYPES OF METHOD are applicable to the study of isotopic ratios. One is based on the use of combined electric and magnetic fields to analyze isotopic components brought into a state of ionization by suitable means. The other type depends on measurement of density differences between isotopic samples in the liquid state.

Other methods are also available but are of no practical importance to the geochemist since they are extremely restricted and incapable of the high precision required. For example, in the case of the heavy hydrogen isotope, a microthermal conductivity method has been applied (Farkas and Farkas, 1933). The  $H^2$  content must not be lower than 1 per cent. The accuracy claimed is about 0.1 per cent. The method has the advantage that at the high temperature employed for the conducting element ( $2000^\circ C.$ ), contamination of the sample is minimized.

Historically, the methods of isotopic analy-

sis based on the isotope shift in line and band spectra are of interest. In hydrogen, modifications in the Rydberg constant brought about by change in nuclear mass result in a slight displacement of the characteristic lines in the Balmer series. This effect which is most marked for the two hydrogen isotopes because of the high mass ratio compared to other isotopic pairs has been used to demonstrate the existence of the heavy hydrogen isotope (Urey, Brickwedde, and Murphy, 1932). The change in oscillational, vibrational, and electronic energy levels in diatomic molecular spectra resulting from mass changes in the atomic force centers has been employed to establish the presence of the isotopes  $C^{18}$  (King and Birge, 1929),  $N^{15}$  (Naude, 1930),  $O^{17}$  (Giauque and Johnston, 1929), and  $O^{18}$  (Giauque and Johnston, 1929; Babcock, 1929) (general references; Jevons, 1932).

## MASS SPECTROGRAPHIC ASSAY

The principles of mass spectrography have been presented at some length in the literature of the last few decades. The reader is referred to Aston's "Mass spectra and isotopes" for a detailed presentation. It will suffice here to remind the reader that in the mass spectrograph<sup>1</sup> four essential operations must be performed:

1. The material to be assayed must be brought into the gaseous state at low pressure and positive ions produced from each isotope present in the ratio which obtains in the original sample.

2. These ions must be rendered monoenergetic by electrostatic means.

3. The ions from step 2 after suitable collimation are analyzed for ratio of charge to mass in a magnetic field.

4. The ions focused at a collecting slit in step 3 are collected and the currents so measured are related to original isotopic content of material assayed.

There are many ways in which these steps can be carried out. The multiplicity of mass spectrographs developed in recent years illustrates the many permutations possible (e.g., Dempster, 1918, 1938; Bainbridge and Jordan, 1936; Mattauch, 1936; Bleakney, 1936; Nier, 1937). In the precision measurement of isotope ratios, many precautions must be taken in the above procedures. Isotopic fractionation must be avoided in the preparation of samples for analysis. In step 1 this means avoiding a method such as volatilization of solid or liquid, since volatility is dependent on mass, the lightest isotope being the most volatile. An unduly high pressure in the ion source may also result in fractionation since neutralization of the positive ions takes place at different rates for the different isotopes (Sherwin, 1940). This is owing to the fact that neutralization at an

<sup>1</sup> The term "mass spectrometer" is in common usage at present. This term has also been applied to an instrument developed by Smythe and Mattauch (1932), in which mass analysis is accomplished without the use of magnetic fields. For a discussion of the electrical momentum filter used, see Smythe (1926).

appreciable pressure of foreign gas molecules depends on the ion velocity and hence, at constant energy, on the mass. Therefore, high transient pressures must be avoided. In some cases it is possible to discriminate between true isotope currents and spurious effects by noting the dependence of ion current on pressure. In the mass spectrographic study of the

performed to bring the sample into the gaseous state no fractionation occurs.

Another source of error is introduced in the case where hydrogen or a source of hydrogen is present as a contaminant, since the possibility of hydride formation exists.<sup>1</sup> This will manifest itself by appearance of satellite peaks in the mass spectrogram. If the resolu-

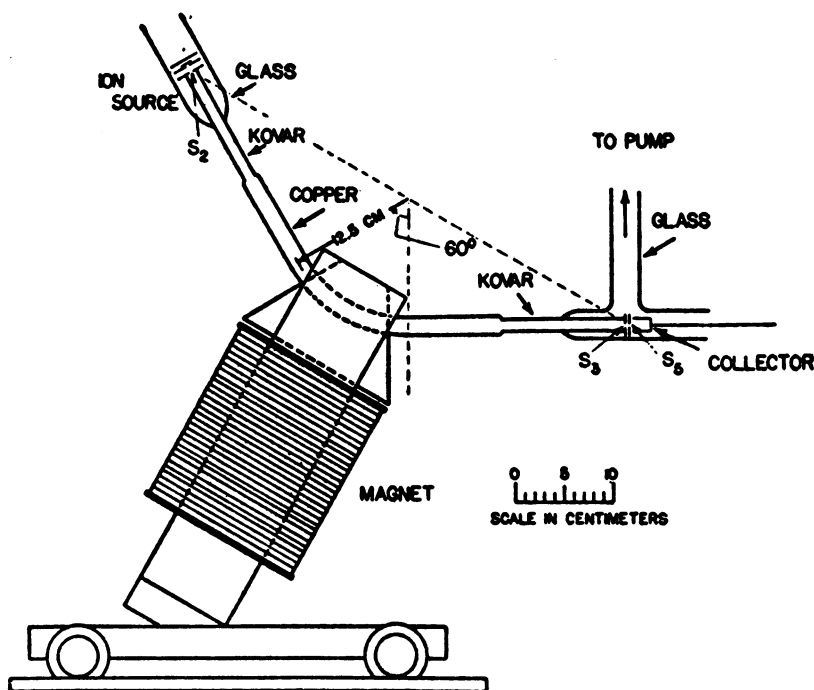


FIG. 1. Cross-sectional view of spectrometer (after Nier). Slits  $S_2$  and  $S_3$  and the center of curvature of the bent section of the copper tube lie in a straight line. Distance from  $S_2$  to  $S_3$  is 50.8 cm.;  $S_3 = 1.1 \times 6.5$  mm.,  $S_4 = 3 \times 14$  mm.  $S_4$  is in a plate held at potential 4.5 volts below ground (potential of analyzer). This plate suppresses secondary electrons formed by ions striking the  $S_4$  plate. The Kovar tube to the right of the magnet is perforated to facilitate pumping.

isotopes present in hydrogen gas samples, analysis is performed by varying the pressure, since, in a mixture of  $H_2$  and HD, the concentration of  $H_3^+$  ions is proportional to the pressure squared and that of  $HD^+$  ions to the pressure directly (Bleakney, 1932).

It is apparent that each element presents its own special problem in sampling. Although it is best to introduce the sample as a gas directly into the ion source, care must be taken that in the chemical procedure

tion is not adequate, overlapping sufficient to cause large errors in the isotope estimation will result. It is also possible that the presence of organic vapors affects volatilization of solid sources in the ion chamber.

In general, the approved procedure involves introduction of the sample as a gas.

<sup>1</sup> Aston found that the existence of lead isotopes with masses 203, 205, 209, and 210 originally claimed on the basis of early mass spectrographic work was due mainly to hydride formation.

For ionization, a well-collimated beam of high-energy electrons is recommended.

In step 2, the type of energy filter employed may be an optical "lens" system or a direct electrostatic accelerator. Here again the literature contains numerous references to the focusing properties of various lens arrangements. Thus, in one case (Hughes and Rojansky, 1929) if the angle subtended by the ion path is  $\pi/\sqrt{2}$  radians (*ca.*  $127^\circ$ ), all ions with the same kinetic energy but diverging in direction by one degree from the axis of the beam are brought to a focus at the exit slit of the electrostatic filter. In general, it has been found satisfactory to employ direct acceleration in an electric field, carefully maintained constant. The voltage must be sufficiently high so that the initial spread in ion energies introduced by the thermal conditions in the ion source is minimized.

The type of magnetic analyzer is also optional. In general, a homogeneous field may be employed with focusing of the ion beam at an angle of arc  $180^\circ$ , or an inhomogeneous field with focusing at various angles such as  $127^\circ$ ,  $90^\circ$ , etc. In figure 1 there is reproduced an arrangement for the routine analysis of

isotope ratios employing the inhomogeneous field.

In detecting the ion currents, the original means employed was the photographic plate. It is preferable, however, to use electronic devices such as d-c amplifiers and electrometers since the photographic plate does not have a linear response in intensity of blackening as a function of ion beam intensity.<sup>1</sup> If the mass spectrograph is used with a single ion collector, the various ion beams are swept into the collector by varying the accelerating potential. Fluctuations in the ion production cannot be observed with such an arrangement. A surer method utilizes two chambers so arranged that each receives simultaneously a component beam. This null method has been employed advantageously by Strauss (1941), Sherwin and Dempster (1941), and Cook (1943).

The outstanding advantages inherent in the use of the mass spectrograph are:

1. The instrument is universally applicable.
2. High precision can be attained (*cf.* discussion on p. 114).
3. Only small amounts of sample (1–2 cc. of gas) are required.

## DENSITY METHODS

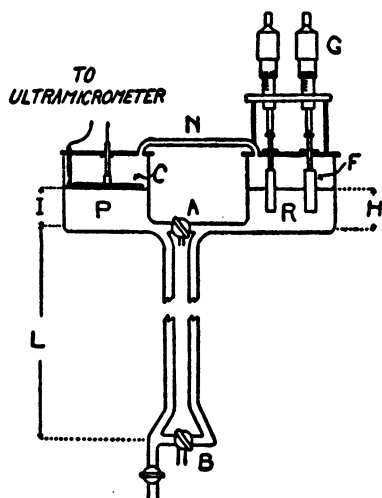


FIG. 2. Apparatus for determination of small density differences (after Wirth, Thompson, and Utterback). Significance of symbols is explained in text.

These methods find their major application in the study of fractionation in water and other hydrogen-containing liquids. The geochemical importance of water and its constituent elements, coupled with the elegance of several of the density methods developed for isotopic assay, justifies a somewhat detailed presentation of these procedures. The methods described in this section appear to be best suited for application in geochemistry.

First, there is a procedure based on the method of communicating tubes described by Frivold (1920) in an article which contains references to earlier work. Wirth, Thompson, and Utterback (1935) have adapted this method to the study of isotopic variations in sea water. A diagram from their original

<sup>1</sup> Compare, however, Mattauch and Ewald (1944) for a recent development of the photographic detector.



variation in density of the liquid brought about by a small pressure or temperature change can be determined in this way. Thus, a difference of  $0.0002^{\circ}$  C. can be readily measured. This corresponds to a density change of no more than  $0.1\gamma$ . The apparatus used by Schoenheimer and Rittenberg (1935) (cf. fig. 3) is cited to illustrate an arrangement and procedure employed in determining deuterium (heavy hydrogen) content of water samples. The glass float (*a*) is constructed so that it maintains its equilibrium position at  $0^{\circ}$  C. under a pressure of one atmosphere. A reduction in pressure (the

temperature being kept constant) causes the float to sink since the water is more compressible. A linear relation exists between the density of the liquid and the pressure for which the float moves neither up nor down. The motion of the float is observed through a microscope fitted with two cross hairs in the eyepiece. The rate of movement down across the field of the eyepiece is noted as the pressure is reduced. Likewise, the velocity upward is measured for a pressure increase. From the points found in this way, a graph is constructed, and the pressure for no movement is read off where the line intersects the ordinate for zero velocity. The float is calibrated with a solution of heavy water for which the density has been found to  $1\gamma$  unit or better by the pycnometer method. A

change in pressure of 1 cm. Hg, easily determined in this manner, corresponds to a change in density of approximately  $0.37\gamma$ . A change of  $1\gamma$  unit corresponds to a change in deuterium content of 0.001 per cent. Obviously, scrupulous cleanliness is required. In practice, the water is distilled *in vacuo* from b into c, the latter being immersed in a Dewar flask filled with a bath of ice and distilled water. The pressure adjustments are made with the stopcocks 1, 2, 3, 4. The range given for the method by the authors extends to  $28\gamma$ . The volume of sample required is approximately 2.5 cc. This is no limitation in practice since, owing to the sensitivity obtainable, dilution can be made of most samples to the required volume.<sup>1</sup>

### OTHER METHODS

In addition to the above methods, the difference in refractive index of light and heavy water has been exploited as an assay method (Lewis and Luten, 1933; Crist, Murphy, and Urey, 1933). Furthermore, by measuring simultaneously the specific gravity and refractive index of a given sample it is possible to assay for both  $H^2$  and  $O^{18}$  content (Lewis and Luten, 1933). The standard pycnometer and drop weight methods, while adequate for tracer studies with enriched isotopic mixtures, are not capable of the precision required for most studies in geochemical isotope effects.

A special case of isotopic assay occurs when the isotopes are radioactive. The rapid development of atomic transmutation technique has extended this possibility to all the elements of the periodic system. More general application of radioactive isotopes to studies of geochemical phenomena is possible, therefore, than in the early days of radioactivity when radioactive isotopes existed for only the heavy elements. The techniques employed in radioactive assay are described elsewhere and do not form part of the subject matter of this review.<sup>2</sup>

### REMARKS ON THE PRECISION REQUIRED IN ISOTOPIC ASSAY

The assay of the oxygen isotopic content of water offers a good example for discussion of precision requirements. If the density ratio between  $H^1O^{16}H^1$  and  $H^1O^{18}H^1$  is assumed to be the ratio of the atomic weights, then

$$\frac{d_{H_2O^{18}}}{d_{H_2O^{16}}} = \frac{20}{18} = 1.111.$$

Thus, addition of one part in 500  $O^{18}$  to  $H_2O^{16}$  (as in normal water oxygen) would mean an increase in density relative to pure  $H^1O^{16}H^1$  of  $0.111/500 \dots$  or  $222\gamma$  units. A

further increase of  $1\gamma$  unit in density representing a total difference of  $223\gamma$  units would correspond to a change in the isotope ratio from 1.000/500 to 1.004/500. As will be seen in the discussion of isotope separation, such an effect is just about what is to be expected in naturally occurring physicochemical processes. In the laboratory, it is possible to

<sup>1</sup> Gilfillan and Polanyi (1933) have described a micro-float method that requires only a few cubic milliliters of liquid for assay.

<sup>2</sup> Consult G. T. Seaborg (1940) for a general review of this topic.

demonstrate certain chemical processes for which equilibrium is reached with isotopic fractionation involving changes ten-fold higher than the above. Smythe (1934) gives the figure 0.994/500 for the isotopic  $O^{18}$  content in ordinary water. If the accuracy is taken to be  $\pm .005$  for the numerator in this ratio, as appears reasonable for precision spectrometry, then these measurements with the mass spectrograph correspond to an uncertainty of 0.5 to  $1\gamma$  unit in the density values. It appears quite certain that this un-

certainty can be decreased to about  $0.1\gamma$  unit which is precision comparable with the results from use of the submerged float method.

The errors introduced by purification procedures may well limit isotopic assay to precision of this order. Generalizations on the nature of fluctuations introduced by chemical methods employed in preparing isotope samples are not possible since the reliability of such procedures depends on the specific element studied.

## SEPARATION OF ISOTOPES BY PHYSICOCHEMICAL SYSTEMS

AN EXTENSIVE LITERATURE has been accumulated in the last two decades on this topic. A detailed review of the many varied procedures available is not indicated here.<sup>1</sup> From the geochemical standpoint, a brief summary of the laboratory experience in this field should be sufficient to indicate under

what conditions geochemical isotope effects may be noted in the absence of biological systems. The processes which exhibit isotope separation may be classified in the following groups: (1) diffusion, (2) distillation, and (3) chemical exchange reactions.

### DIFFUSION METHODS

Diffusion of gases or liquids leading to separation effects occurs under a variety of conditions. Three types of diffusion may be distinguished. In the first, the gas mixture diffuses at low pressure through a porous material. Since the velocity of diffusion varies inversely with the square root of the mass, lighter molecules will diffuse more rapidly. If provision is made to prevent accumulation of heavy component at the diffusion interface, a separation of isotopic constituents can be effected.<sup>2</sup> At atmospheric pressure where this condition is not fulfilled no measurable isotope separation is noted (Aston, 1933). A single diffusion process of this type for a monatomic gas such as neon will give a change in density of 0.3 per cent in the heavy residue if diffusion at low pressure is continued until the residual volume is 1/500 of the initial volume.

Cyclic diffusion of this type has been exploited in a spectacular fashion by Hertz and his coworkers (Hertz, 1932; Harmsen, Hertz, and Schütze, 1934). One type of apparatus uses 48 diffusion units and 24 mercury diffusion pumps. Another type is based on diffusion of gas through streaming mercury vapor (Hertz, 1934). With such apparatus it has been possible to prepare in a day several cubic centimeters of neon gas containing almost pure Ne<sup>20</sup> or Ne<sup>22</sup>. Similar results can be obtained with other gases.

The second type of diffusion process occurs under the influence of a thermal gradient. Enskog (1911) and Chapman (1916) have

shown independently that a gas mixture will evidence a separation of mass constituents if allowed to diffuse freely in a vessel with ends maintained at different temperatures, excess of heavy constituent being found at the cold end. Clusius and Dickel (1938) have exploited this principle in a brilliant research. They use a vertical column with a central heated wire. As an alternative to the wire, a central cylinder heated either electrically or by circulation of hot fluid may also be employed. Thermal diffusion results in a concentration of heavy isotope at the cold outer wall. Convection is set up under the gravity gradient, the heavy isotope flowing downward, the light isotope upward. This counter-current flow results in a concentration of heavy isotope at the bottom of the tube and light isotope at the top. Isotope concentration continues until limited by back diffusion. Biologically important isotopes, such as H<sup>2</sup>, C<sup>13</sup>, N<sup>15</sup>, etc., can be prepared in laboratory amounts by this method. The extension of the method to separation of components in liquid solutions has also been reported. An idea of the efficacy of the method may be obtained from results given by Clusius and Dickel using a 36-meter tube with central wire 600° C. hotter than the wall. Using HCl, a twenty-four hour run produced 8 cc. of vapor containing 99.4 per cent Cl<sup>37</sup>. (Normal HCl contains 25 per cent Cl<sup>37</sup>.)

The third diffusion method is based on the equilibrium reached by a heterogeneous gas or fluid mixture in a gravitational field. Here heavier particles tend to concentrate in the direction of the field. Lindemann and Aston (1919) have calculated the variation in neon isotopic content at various levels in the at-

<sup>1</sup> Consult Urey (1939) for a general critical discussion.

<sup>2</sup> A detailed treatment of this process is given by Rayleigh (1896).



mosphere. They find that if  $h_0$  represents the height above which mixing by convection ceases, then the relative isotope density ( $K/K_0$ ) at a height  $\Delta h$  greater than  $h_0$  is given by

$$e^{-1.075 \times 10^{-2} \Delta h}$$

At 100,000 feet the relative  $\text{Ne}^{22}$  content ( $\text{Ne}^{20}$  being the other component) should drop from 10 per cent to approximately 8.15 per cent, the atomic weight for neon at such a height thus becoming 20.163. Normal neon has an atomic weight of 20.20. At 200,000 feet, the atomic weight is calculated to be 20.12.

The influence of gravitational field is best investigated using peripheral fields developed in the high-speed ultra centrifuge. Mulliken (1922, 1923) has given the theory of isotope separation both by thermal diffusion and centrifugation. He finds that maximal separation is obtained if the two fractions condensed at the periphery and at the center are allowed to evaporate slowly. Beams (1938) and his coworkers have developed this method using the air or hydrogen-driven ultra centrifuge. A separation factor of 1.1 for the

chlorine isotopes has been obtained. This corresponds to an increase of 10 per cent in  $\text{Cl}^{37}$  content of the peripheral sample.

From the foregoing discussion, it can be concluded there is little likelihood any diffusion process other than that obtaining under influence of gravity is important in a geochemical sense. As will be discussed later, examples of isotope fractionation in the ocean water due to gravity diffusion have been found. It does not appear that any appreciable isotope effects can be expected as a result of gaseous diffusion in heterogeneous systems or under a thermal gradient unless a cyclic process is set up. The omnipresent mixing due to convection will, in general, nullify what small effects are produced. Diastrophic cracking of rock strata resulting in fissures through which diffusion of liquids or gases could flow from and into pockets representing a kind of crude free diffusion apparatus might give local separations, but it is difficult to see how such effects could approach even the minute separations noted under much better controlled laboratory conditions.

## DISTILLATION OR EVAPORATION METHODS

Two types of distillation are now known which lead to isotope fractionation. In one type, a monatomic solid with isotopic constituents is allowed to evaporate or sublime at a temperature sufficiently low so that the heat capacity is considerably less than the classical value  $6R$ , where  $R$  is Avogadro's number. On the basis of the Debye theory of the solid state, a monatomic solid which sublimates at such a temperature can be expected to exhibit isotopic fractionation. The method has been applied to hydrogen (Urey, Brickwedde, and Murphy, 1932a) and neon (Keesom, Van Dijk, and Haantjes, 1934). In the former case, it can be shown that at the triple point of hydrogen the ratio of vapor pressures of  $\text{H}_2$  and  $\text{HD}$  should be 2.42. A simple distillation of about 4 kilograms hydrogen down to a few cubic centimeters results in a change of isotopic ratio of 30. For neon a difference of 4 per cent exists in the vapor pressures of  $\text{Ne}^{20}$  and  $\text{Ne}^{22}$  at the boiling point.

In the other method, slow evaporation at reduced pressure is used. When a liquid evaporates *in vacuo* or under low pressure, the number of light atoms escaping from the surface in unit time is greater than the number of heavier atoms in inverse proportion to the square roots of the masses. The pressure above the surface must be kept low so that no atoms escaping can return to the liquid. Brönsted and Hevesy (1920) first used this method of "ideal" evaporation to effect partial separation of mercury isotopes. The rate at which evaporation should take place is determined by the rate of diffusion of mercury atoms to the surface. This can be fixed approximately from the known rate of diffusion of lead in mercury. Since radioactive mercury is now available as "tracer," it is also possible to determine this diffusion rate directly. The value calculated from the work with lead (Gróh and Hevesy, 1920) shows that the limiting evaporation rate is  $5 \times 10^{-3}$  c.cm per  $\text{cm}^2$  per second. When several kilo-

grams of mercury are evaporated to a volume about 1/100,000 of the original volume in this fashion, a change in density of about  $5 \times 10^{-4}$  units (500 $\gamma$ ) occurs. Harkins and Mortimer (1928, cf. Mulliken, 1922a) have published extended studies on the separation of mercury isotopes by this process. The method has also been applied with some success to the isotopes of potassium (Hevesy, 1927) and chlorine (Brönsted and Hevesy, 1921).

In connection with the first method involving fractionation at the boiling point, the restriction of the Debye theory to low temperatures does not seem to apply to molecules such as H<sub>2</sub>O and D<sub>2</sub>O. Here, at temperatures so high that the heat capacity approaches 6R, differences in vapor pressure are observed. Thus, the vapor pressures at 20° C. for light and heavy water (H<sub>2</sub>O and D<sub>2</sub>O) are

17.5 and 15.2 mm. Hg, respectively (Lewis and MacDonald, 1933). Similarly for the molecules H<sub>2</sub>O<sup>16</sup> and H<sub>2</sub>O<sup>18</sup> the ratio of vapor pressures at 23° C. is 1.008 (Urey, 1939, p. 53). Such differences have been utilized with the help of appropriate fractionating equipment to produce good separations of the hydrogen and oxygen isotopes (Huffman and Urey, 1937).

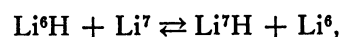
With distillation as well as diffusion little isotopic fractionation can be expected on a large scale geochemically unless cyclic conditions are involved. Of the two processes for which data are available, only the slow evaporation process seems of geochemical significance.<sup>1</sup> Here again, there is some evidence that this process occurs in the biosphere (cf. discussion of the hydrogen and oxygen isotopes on pp. 122–126).

## CHEMICAL SEPARATIONS

Binding energy of atoms in molecules is influenced to some extent by the masses of the atoms. At ordinary temperatures most of the mass effect is given by the difference in "zero-point" energy, that is, the energy residual in the vibrational oscillations for diatomic and polyatomic molecules at the absolute zero of temperature. The heavier atoms exhibit the lower zero-point energy since the vibration frequency for the oscillation depends on an inverse function of the mass. Thus, a difference in activation energy of the various isotopic constituents, while small in magnitude, can be expected to influence the rate at which the various isotopes enter into chemical reactions.

Also, it can be shown by statistical theory that the calculated equilibrium constants for

"exchange" reactions can deviate markedly from unity. Thus, in the reaction



the equilibrium constant at 0° C. is 1.028. Urey and his coworkers have investigated a large number of "exchange" systems and have confirmed experimentally the equilibrium constants calculated from spectroscopic data (Urey and Greiff, 1935). A few of the values calculated for some typical exchange reactions are given in table 1.

<sup>1</sup> Tucholski (1934) finds that when 25 liters of distilled water are evaporated for three years to a final volume of 600 cc., the concentration of D<sub>2</sub>O is increased to 1.65 per cent. Rapid boiling gives no significant increase in density.

TABLE 1  
(After Urey and Greiff)

Reaction	Equilibrium Constants		
	273.1° K	298.1° K	600° K
$\frac{1}{2}\text{SO}_2^{16} + \text{H}_2\text{O}^{18} \rightleftharpoons \frac{1}{2}\text{SO}_2^{18} + \text{H}_2\text{O}^{16}$	1.034	1.028	1.003
$\frac{1}{2}\text{CO}_2^{16} + \text{H}_2\text{O}^{18} \rightleftharpoons \frac{1}{2}\text{CO}_2^{18} + \text{H}_2\text{O}$	1.064	1.054	1.014
$\frac{1}{2}\text{O}_2^{16} + \text{H}_2\text{O}^{18} \rightleftharpoons \frac{1}{2}\text{O}_2^{18} + \text{H}_2\text{O}^{16}$	1.024	1.020	1.003
$\text{Li}^6\text{H} + \text{Li}^7 \rightleftharpoons \text{Li}^7\text{H} + \text{Li}^6$	1.028	1.025	1.008

For a recent investigation of some reactions important in the concentration of the heavy isotopes of nitrogen and sulphur, reference is made to the work of Thode, Graham, and Ziegler (1945).

The magnitude of the change in activation energy brought about by the isotopic zero-point energy differences is well illustrated for the process of differential absorption of the two hydrogen isotopes in palladium. From the temperature coefficient for absorption of normal isotopic  $H_2$ , Farkas and Farkas

hydrogen isotopes. Studies on the isotopes of lithium (Taylor and Urey, 1938) and potassium (Brewer, 1939) have also been reported in which appreciable isotopic separation has been found. The phenomenon is identical with the process of base exchange used for softening water on a commercial scale. Certain minerals such as zeolites or clays are especially effective as agents for accomplishing base exchange. Thus, when a solution of lithium chloride is percolated through a column of sodium zeolite, the sodium ion is

TABLE 2  
(After Thode *et al.*)

Reaction	Equilibrium Constant 298.1° K
$N^{14}H_4^+ + N^{15}H_2 \rightleftharpoons N^{15}H_4^+ + N^{14}H_2$ (sol) (gas) (sol) (gas)	$1.031 \pm .022$
$N^{14}H_3 + N^{15}H_3 \rightleftharpoons N^{15}H_3 + N^{14}H_3$ (aq) (gas) (aq) (gas)	$1.007 \pm .002$
$NaHS^{32}O_3 + S^{34}O_2 \rightleftharpoons NaHS^{34}O_3 + S^{32}O_2$ (sol) (gas) (sol) (gas)	$1.019 \pm .002$
$NaHS^{32}O_3 + S^{36}O_2 \rightleftharpoons NaHS^{36}O_3 + S^{32}O_2$ (sol) (gas) (sol) (gas)	$1.043 \pm .004$

(1935, p. 122) report an over-all activation energy for atomic absorption of some 16,500 calories. The zero-point energy of  $\frac{1}{2}H_2$  is given as 405 calories higher than  $\frac{1}{2}HD$  which in turn is 485 calories higher than  $\frac{1}{2}D_2$ . Thus, the light hydrogen requires 890 calories less energy for activation than the heavy.

If exchange is carried out in a two-phase system in such a manner that equilibrium fractionation is multiplied many times over, bulk separation of the isotopic constituents can be effected. By use of two-phase counter-current methods similar to the process of fractional distillation of chemical mixtures great success has been attained in making available commercial amounts of isotopic material (Urey, 1939, pp. 59-60).

A special case of importance to geochemists arises when the equilibrium involves exchange of isotopic components between liquid and solid. Preferential absorption phenomena have been mentioned above for the case of the

displaced by the lithium ion, the light isotope of the lithium ( $Li^6$ ) being more effective than the heavier one ( $Li^7$ ). In this manner, Taylor and Urey find that samples of lithium can be secured in which the concentration of heavier isotope is changed as much as 25 per cent. Similar results have been obtained with potassium and nitrogen isotopes, the solute in the latter case being ammonium chloride. However, in potassium and nitrogen, it is the heavier isotope that is concentrated on the zeolite. Hence, chemical equilibrium is involved rather than merely a kinetic phenomenon.

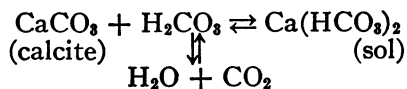
Of the many other methods proposed for separation of isotopes, a few may be mentioned briefly here. Utilization of the difference in photochemical activation of isotopic constituents has been suggested (Hartley, Ponder, Bowen, and Merton, 1922). As an example, consider the molecule phosgene which contains the isotopic combinations

$\text{COCl}^{35}\text{Cl}^{35}$ ,  $\text{COCl}^{35}\text{Cl}^{37}$  and  $\text{COCl}^{37}\text{Cl}^{37}$ . For each of these molecules, a different hyperfine structure will obtain in the absorption spectrum. By picking out absorption lines belonging to one or another of these molecules and irradiating with monochromatic light corresponding to these lines, it should be possible to excite one isotope of chlorine preferentially. This should result in preferential photochemical action on this isotope. It has been reported that phosgene decomposed photochemically in this manner yields chlorine with a combining weight of 35.428 as compared with the normal 35.455 (Kuhn and Martin, 1932). As will be noted, the rigorous requirement of monochromatic light corresponding to sharp absorption lines due to isotopic components renders the importance of this phenomenon in geochemical isotope fractionation very dubious.

Another possibility from a geochemical standpoint is the process of fractional crystallization since it is related to the slow deposition of ocean salt and the general process of sedimentation and production of salt deposits by evaporation. However, no laboratory separation of isotopes has been achieved in this manner. Robinson and Briscoe (1925) have recrystallized ammonium bromide some

2700 times without obtaining any appreciable separation of bromine isotopes in the "head" or "tail" fractions.

Dempster (*in* Cook, 1943) has made the interesting suggestion that in the formation of long, narrow stalactites a cyclic deposition and redissolving of calcium carbonate takes place in which the carbonate enters into the following equilibrium:



The possibility exists that the heavier calcium isotopes tend to concentrate at the top of the stalactite, the lighter ones migrating preferentially to the bottom. An investigation of such stalactites would seem to be indicated.

The rhythmic supersaturation and deposition of ions in a colloidal system which is the basis for Liesegang ring formation have also been suggested as a process for isotope separation (Lindemann, 1921). "Liesegang" banding in rocks is the geochemical analogue. Here again no encouragement is derived from laboratory experience, which shows no isotope separation for migration of isotopic ions through gelatin (Aston, 1933, p. 232; Murrmann, 1923).

## GEOCHEMICAL CONSIDERATIONS

In a survey of the material available on isotope separations carried out in the laboratory, the conclusion is reached that only under highly specialized conditions can one expect to detect significant changes in isotopic distribution through the agency of purely physicochemical processes. A number of possibilities have already been indicated in the foregoing sections. Cook (1943) has discussed the present status of geological factors in isotope separation emphasizing the importance of cyclic phenomena and the necessity for absence of mixing which obviates the separations that might be obtained. The possibility that isotopic separation might occur during formation of extrusive rocks appears remote on these grounds. Other phenomena involved in vulcanism, such as fractional crystallization in volcanic magma under the influence of gravity, appear to have

more likelihood of exhibiting isotope effects (Bowen, 1928, p. 32). An investigation of those igneous rocks presumably formed to some extent by gravitative settling is suggested by Cook.<sup>1</sup>

However, the exchange equilibria discussed above represent the most favorable mechanism for isotope separation by geochemical systems. The efficacy of base exchange has been demonstrated in the laboratory. The widespread occurrence of zeolites and clays, therefore, takes on significance from this standpoint. Brewer (1939) has found that when sodium chloride solution is passed through a column of potassium-containing zeolitic greensand, a preferential desorption

<sup>1</sup> Formations of this nature are found in Canada and South Africa; cf. Coleman (1924), Daly and Molen-graaff (1924).



of the potassium occurs with the greensand becoming impoverished in the light isotope. It can be imagined that a similar process could be operative on a geologic scale in a stratum of porous material (i.e., volcanic in origin) through which calcium-bearing ground water is conducted for considerable distances. The calcium isotopes might exhibit preferential absorption along such a stratum. In the case of clay deposits, which are more common than zeolites, similar phenomena might be encountered. It is known that cations are absorbed preferentially in certain clays, an analogous process to chromatographic separation of inorganic ions on alumina (Schwab and Jockers, 1937). Thus, hydrogen replaces calcium which in turn replaces magnesium, and so on down to sodium which shows the least absorptive

power (Kelley, 1939, p. 455). In bentonite, which is a clay possessing very high exchange capacity, the aluminum ion present in the clay lattice can be replaced by ions of magnesium or iron. By analogy with the behavior of potassium in greensands, one can expect isotope effects for many metal ions in bentonite and clays in general. The common occurrence of beds of bentonite throughout the United States affords a material for investigation of isotopic separation. Presumably, the mechanism here would also involve the action of ground water percolating through the bentonite bed; sampling at different localities on the same bed should be sufficient to reveal the effect if it exists.

Other examples of the action of chemical exchange reactions are best discussed under the specific cases treated later in this report.

### BIOGEOCHEMICAL EFFECTS

The isotopic separations effected by biological organisms represent a special case of the physicochemical systems which have been discussed. The difference in degree that has been ascribed to biological systems by Vernadsky can only result from the setting up of conditions peculiarly favorable for the operation of processes such as diffusion, evaporation, and differentiation in reaction rates inside the organisms. Thus, for example, organisms present fat or protein surfaces to an essentially aqueous environment. Such two-phase systems can achieve isotopic separation over and above that in the surrounding medium by mechanisms based on differential solubility or simple exchange. It must be admitted, therefore, that isotope differentiation may be more characteristic of living systems than inert systems. Nevertheless, there exists a wealth of evidence to indicate that it can occur in both cases. To establish a criterion for distinguishing bio-active and bio-inert categories on the basis of isotope separation there will be required a systematic investigation of all factors operative both in the organism examined and its environment. In many cases, where an effect is found, it may be difficult to exclude purely

physical fractionation as operative.

To test the validity of Vernadsky's criterion, it may prove advisable to confine future investigations to the case of hydrogen, since for this element the differences in chemical properties of the two isotopes are sufficiently great to promise unambiguous correlations of isotope distribution with biochemical activity. This is not to say that investigation of all elements other than hydrogen should be excluded. Regardless of whether correlations between biochemical activity and isotopic concentration are found or not, the occurrence of such phenomena may give valuable information on the nature of purely geochemical phenomena. A general investigation of isotope effects using the refined assay methods now available is most certainly indicated. Such a research should be undertaken without the preconceived notion that all effects found must of necessity be uniquely biological in origin. The interplay between the bio-inert and bio-active components in the production of isotopic phenomena can be best appreciated by reference to specific examples which are treated in the next section.

# ISOTOPIC DIFFERENTIATION IN THE BIOSPHERE

## HYDROGEN AND OXYGEN

STUDIES ON THE DENSITY FLUCTUATIONS of water from various natural sources supply the bulk of data available. The numerous review articles may be consulted<sup>1</sup> for complete coverage of the literature. In this section, results from a few representative researches will be presented.

It is necessary to treat hydrogen and oxygen together since in most of the researches conducted on isotopic content of natural waters, no isolation of hydrogen and oxygen effects is reported.

Hydrogen consists of two stable isotopes with masses 1 and 2, called "protium" and "deuterium," respectively.<sup>2</sup> The protium-deuterium ratio in "normal" water, that is, distilled fresh water, is difficult to define since all samples prepared undergo some fractionation during distillation. The original value, 5000, adopted by the International Committee on Atomic Weights (Baxter, Hönigschmid, and LeBeau, 1938) appears to be in error, since samples prepared by procedures free of isotopic effects give values close to 6900 (Hall and Jones, 1936; Gabbard and Dole, 1937; Swartout and Dole, 1939). As pointed out below, relative densities are not affected by this uncertainty, since the various standards employed do not appear to differ markedly in absolute isotope content.

Commercial tank oxygen is a ternary mixture of isotopes with masses 16, 17, and 18, for which percentage abundances are given as 99.76, 0.041, and 0.20, respectively (Smythe, 1934; Murphey, 1941). The mass ratios found by Murphey (1941) are, for  $O^{16}/O^{18}$ ,  $500 \pm 15$ , and, for  $O^{18}/O^{17}$ ,  $49 \pm 0.2$ . Enrichment of heavy isotope in oxygen is credited to the  $O^{18}$  isotope entirely because of the very low abundance of  $O^{17}$ .

It is usually assumed that the major contribution to the change in density noted for any given water sample arises from con-

centration of the hydrogen isotopes. That this is erroneous has been pointed out by several writers (Gilfillan, 1934; Dole, 1936). Much of the work discussed in this section should be repeated using a precision spectrometer to determine quantitatively the contribution of each element to the total isotopic effect.

Another complication which is less serious arises from the use of different standards in the various laboratories devoted to this work. Tap water from the locality in which the research is conducted is the usual standard. Such water is derived in the main from fresh surface water which is a condensate of atmospheric water and so should show little variation in isotopic content. This has been confirmed by a comparison of tap waters from a representative sampling of laboratories throughout the world (Greene and Voskuyl, 1939). The fluctuations found hardly exceed 0.1 $\gamma$  unit (cf. table 3). Comparisons of results gotten in different localities can be made with an uncertainty of this order of

TABLE 3  
COMPARISON OF TAP WATERS  
(After Greene and Voskuyl)

Source	$\Delta d$ (in $\gamma$ units) Standard: Cambridge, Mass., Water
Cambridge, Mass., U. S. A.	0
Osaka, Japan	+0.09
London, England	+0.13
Columbus, Ohio, U. S. A.	+0.02
Washington, D. C., U. S. A.	+0.15
Average	+0.02

magnitude. However, if more precise experiments involving distinctions in hydrogen and oxygen effects are contemplated, a reproducible precision standard is required. Precautions to exclude isotopic effects arising from chemical purifications employed are described by Dole (1936).

<sup>1</sup> Compare Reitz (1938) for a general review and bibliography.

<sup>2</sup> The discovery that "tritium" (hydrogen of mass 3) is radioactive (cf. Alvarez and Cornog, 1939) appears to dispose of the possibility that a stable hydrogen of mass 3 exists.

With the reservations implied above, the results obtained on geochemical isotope effects in natural waters show, in general, the following:

1. Sea water is denser than fresh water; the density increment is in the neighborhood of  $2\gamma$  units, varying with locality.

2. No general increase in density is found in deep-sea water compared to surface water, indicating hydrostatic equilibrium has not been reached. As discussed previously, a sedimentation of isotopes should occur under the influence of gravity.<sup>1</sup>

3. Samples of water from hydrated minerals are denser than fresh water.

4. Samples obtained from bio-active or bio-inert specimens show invariably an increase in heavy isotope content.

Illustrative of the first point are the data exhibited in table 4 (Emel  us *et al.*, 1934). Spring waters derived from sources as widely

TABLE 4  
(After Emel  us *et al.*)

Source	$\Delta d$ ( $\gamma$ units)
London water (stand.), Eng.	0
S. Wales spring, Eng.	0
Dew pond, Dorset, Eng.	-0.63
Dew pond, Salisbury, Eng.	+0.42
Sumatra spring	0
Pangong Lake, Tibet, alt. 13,500 ft.	+1.47
Dead Sea, Palestine	+2.52

separated as Sumatra and England have the same density. Dew ponds, presumably formed by condensation of atmospheric water, should give a negative  $\Delta d$  value. While this is true for the Dorset pool, it appears that  $\Delta d$  for the pool in Salisbury is positive. However, the effects, while greater than the assigned experimental error ( $0.1\gamma$ ), are small. The authors consider the Dorset value significant and are inclined to ascribe the Salisbury result to "accident." The Tibetan lake water has a density value consistent with concentration of heavy isotope by evaporation,

<sup>1</sup> Gilfillan (1934) states that a depth of 4500 meters sea water should be heavier than at the surface by some  $7\gamma$  units owing to separation of various isotopic species in the gravitational field.

as has water from the Dead Sea. The evaporation in the former case is much less than for the salt lake, but it is suggested that the increased efficiency in isotope separation due to the low pressure at the high altitude counteracts this partially. However, Hutchinson (1937) points out that Pangong Lake has undoubtedly overflowed at some stage in its history. Of the whole group of lakes in this region, Tso Kar has never overflowed, according to the same author. This lake contains some 7.9 per cent total solids as compared with Lake Pangong which has a solid content of 1.3 per cent. Hence, an investigation of  $\Delta d$  values for Tso Kar as well as the other lakes would be of considerable interest.

Similar results have been obtained by other workers. Thus, Gilfillan (1934) reports that Atlantic sea water (latitude  $36^\circ 27'$  N., longitude  $68^\circ 38'$  W.) shows a density excess over distilled laboratory water of  $2.3\gamma$ . He finds in this case that virtually all the effect noted can be ascribed to concentration of  $O^{18}$ . Wirth, Thompson, and Utterback (1935), using Washington State river water, report a somewhat lower value for water taken from the Pacific and Indian oceans ( $1.4\gamma$ ). Water from the land-locked Baltic Sea is less dense than Pacific water owing to extensive dilution by fresh water from the rivers. Riesenfeld and Chang (1936) also find ocean water to be  $1.5\gamma$  units denser than land water which in turn is 1 to  $2\gamma$  units heavier than snow or rain water.

Data on the variation in isotope content with depth of sample are not so uniform in agreement. Gilfillan (1934) fails to find a variation in the Atlantic Ocean at latitude  $36^\circ 27'$  N., longitude  $68^\circ 38'$  W. Wirth, Thompson, and Utterback (1935) find the same to hold true for the Pacific and Indian oceans with the exception of the North Pacific in the Bering Strait region. They ascribe the effect noted (some  $0.5\gamma$  for a depth of 200 meters) to the unique lack of vertical circulation in these waters. A large effect is reported for Lake Baikal water by Vereř  agin, Gorbov, and Mendelevjev (1934) who find that water taken from a depth of 1200 meters shows an increase in density over surface water of  $24\gamma$ . This very large effect is quite astonishing inasmuch as one might expect mixing to occur at such depths.

Hutchinson (private communication) suggests that a study of deep water from tropical stagnant lakes such as Lake Tanganyika and Lake Nyassa in Africa would be interesting in connection with these results, since Beauchamp (1940) has shown that in these lakes the deep water is static, oxygen free, and apparently cut off from surface water.

It appears that for the oceans hydrostatic equilibrium has not been reached even at depths at which convection is not operative. Water taken from sea organisms should parallel in density that of the depth in which they live, since it has been shown that algae (Reitz and Bonhoeffer, 1934), bacteria (Cavanagh, Horiuti, and Polanyi, 1934), fish (Hevesy and Hofer, 1934), and mammals (Smith, Trace, and Barbour, 1936) rapidly equilibrate deuterium, either gaseous or aqueous.<sup>1</sup>

Mineral water of hydration shows an increased density in some cases. It is found (Emeléus *et al.*, 1934; Riesenfeld and Chang, 1936) that for the minerals *rasorite* ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 4\text{H}_2\text{O}$ ) and *tincal* ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) the bound water is 6.30 and 2.94 $\gamma$  units denser, respectively, than distilled laboratory water. These minerals are formed presumably by deposition during evaporation in salt lakes, and hence it is to be expected the bound water will be heavy. The difference found between the decahydrate and tetrahydrate is due possibly to slow dehydration of the former which is assumed to be the parent substance. In contrast to these results, sulfate water of crystallization shows no increase in density over that of the mother liquor (Erlenmeyer and Gartner, 1934; Friend, 1934) when the salt is crystallized from a mixture of light and heavy water.

Water from bio-active sources (vegetables and animals) is reputed to show marked density excess over natural surface water in many cases. Emeléus *et al.* (1934) give values of  $\Delta d$  (table 5) for a long list of vegetables. Density excesses from 1 to 4 $\gamma$  units are found. The authors point particularly to the case of the tomato which is known to show high evaporation and for which an appreciable positive  $\Delta d$  would be anticipated. Such an

increase in density of tomato water is found. Furthermore, the English variety grown under forced conditions in enclosed spaces where the evaporation rate is lessened shows a smaller  $\Delta d$  than the variety grown in the Canary Islands which, being raised in the open, can evaporate more of its water. Riesenfeld and Chang (1936) find similar

TABLE 5  
(After Emeléus *et al.*)

Source	$\Delta d$ ( $\gamma$ units)
Orange, S. Africa	0.84
Coconut milk	0, -0.2
Grapefruit, Cuba	-0.6, -0.8
Lemon, Italy	+1.47
Melon, Rumania	+0.2
Marrow, England	+1.05
Pineapple, Hawaii	+2.94
Pineapple, S. Africa	+2.73
Pomegranate, Palestine	+1.26, +0.84
Tomato, England (forced)	+2.52, +2.94
Tomato, Canary Islands	+3.78
Turnip, England	-0.2
Cabbage, England	+1.47, +2.10
Rhubarb, England	0
Onion, Holland	+0.42

results for plants in general. Washburn and Smith find that the sap of the willow tree has a positive  $\Delta d$  of 2.8 $\gamma$ , and the dry wood a positive  $\Delta d$  of 5.4 $\gamma$ .<sup>2</sup> Sucrose gives a water on combustion with a high density of 8.6 $\gamma$  units. Emeléus *et al.* (1934), Morita and Titani (1936a), and Morita, Goto, and Titani (1938) show that sucrose decomposed in deuterium-free hydrogen gives water with a density excess of 4.6 $\gamma$ , presumably owing to an increased  $\text{O}^{18}$  content. Apparently, much of the effect noted in cellulose and cellular sap by the other workers may be due in large part to concentration of the heavy oxygen isotopes rather than heavy hydrogen.

On waters of animal origin, no general agreement is noted. In table 6 some preliminary results from the work of Emeléus *et al.*<sup>3</sup> are tabulated. Other workers find no

<sup>2</sup> Confirmed by Emeléus *et al.* (1934) for English specimens.

<sup>3</sup> Emeléus *et al.* (1934). A value for "animal water" is also given by Riesenfeld and Chang (1936) ( $\Delta d = \pm 1.5\gamma$  referred to "land water") which is in accord with these results.

<sup>1</sup> A study of the mechanism whereby *Proteus vulgaris* catalyzes the isotopic equilibrium between hydrogen and water has been published by Hoberman (1942).

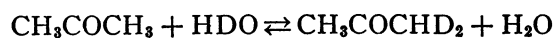


significant differences between distilled tap water and water from various biological fluids including those shown in table 6 (Stewart and Holcomb, 1934; Breusch and Hofer, 1934). While investigations on this point have not been sufficiently extensive to warrant general conclusions, it appears that significant isotopic fractionation in bio-active sources has not been established.

TABLE 6  
(After Emeléus *et al.*)

Source	$\Delta d$ ( $\gamma$ units)
Human urine	0
Human milk	+2.31
Human blood	+1.05
Cow's milk	-0.84
Ox blood	+2.52
Standard London tap water	(0)

In interpreting the data obtained on waters from bio-active sources, most of the investigators are inclined to ascribe the increase in density to evaporation. Negative values for  $\Delta d$  would not be accounted for on this basis. Furthermore, the lack of precise data on the contribution of each of the isotopes involved renders uncertain the mechanism operative. It is to be expected that chemical exchange reactions play an important role in establishing differentiation in the different tissues and fluids of biological systems. From the purely physicochemical viewpoint, the major difference between bio-active and inert bodies is that the former supply particularly advantageous conditions for exchange equilibria and finely balanced discrimination in activation potentials. The predominance of exchange equilibria in bio-active systems in itself may be the most important single factor in establishing isotope effects. It will be recalled in this connection that many model reactions for this type of isotopic differentiation are at hand. Thus, the reaction



is found to have an equilibrium constant of 2 at room temperature (Halford, Anderson, and Bates, 1934). The difference in energy

required to activate deuterium as compared to protium would lead to the expectation that protium would be used in preference to deuterium in hydrogen transfer equilibria. In biological oxidation, a similar case can be set forth for the slower utilization of the heavier oxygen isotopes. In examining results such as those on the wood of the willow tree, it is important, therefore, to know to what extent the different isotopes of hydrogen or oxygen are contributing to the increase in density of the water obtained.

The possibility that an excess of deuterium in water may be biologically significant is to be expected in principle, since the ability of dehydrogenases to transport protium in biological oxidation and fermentation is known to be much greater than for deuterium. This follows from the lower activation energy of the protium isotope. Thus, Pacsu (1934) finds that fermentation by yeast cells in nearly pure  $\text{D}_2\text{O}$  is nine times slower than in ordinary water. Deuterium in high concentrations has been shown by numerous authors to be a respiratory poison (Lewis, 1933; Taylor, Swingle, Eyring, and Frost, 1933), the effect presumably arising from the inactivation of the dehydrogenases. The influence of water containing a slight excess (up to 1 per cent) of deuterium would not be expected to be appreciable extrapolating, from data furnished by these investigators working at high deuterium levels. However, Barnes<sup>1</sup> and coworkers present a strong case for significant biological effects in the presence of low concentrations such as 0.6 per cent deuterium. The main effects noted are an increase in dry weight of yeast grown in 0.06 per cent isotope water, retardation of enzymatic hydrolysis by systems such as amylase and zymase, and stimulation of cellular growth. Other authors (Macht and Davis, 1934) fail to note the expected fermentation increase in yeast maintained in a glucose medium containing 0.06 per cent deuterium water. Barnes suggests that the discrepancy arises from the fact that in these experiments the cells are not incubated in the heavy water prior to the test. It is claimed that zymase shows no retardation in hydrolysis

<sup>1</sup> Consult Barnes and Jahn (1934) for a complete account and bibliography.

of glucose in the presence of 0.06 per cent deuterium water if the enzyme is not incubated first in the isotope water (Barnes and Larson, 1933). The possibility that organisms grow better in the presence of a slight excess of deuterium as compared to

deuterium-free water leads to the intriguing speculation that small variations in deuterium content brought about through astrophysical means<sup>1</sup> may play a role in the evolution and development of terrestrial organisms.

## THE ISOTOPIC COMPOSITION OF ATMOSPHERIC OXYGEN

The oxygen of the atmosphere is derived mainly from the photosynthetic process in green plants.<sup>2</sup> It is to be expected that such oxygen should have the same  $O^{18}$  content as that of sea water for the following reasons:

1. In experiments with the green alga, *Chlorella pyrenoidosa* (Ruben, Randall, Kamen and Hyde, 1941), and two land plants, sunflower and *Coleus* (Dole and Jenks, 1944), it is found that the  $O^{18}$  content of the evolved oxygen is determined by the isotope content of the water in which the organisms are bathed. There is no dependence on the  $O^{18}$  content of the carbon dioxide available.

2. The major fraction (over 80 per cent) of photosynthesis in the biosphere is carried on by marine organisms.

The information available in the distribution of  $O^{18}$  in the biosphere may be summarized as follows (Birge, 1941):

1. Fresh-water oxygen has the same  $O^{18}$  content as that in rocks and ores devoid of carbonates.

2. Sea water has an excess of  $O^{18}$  compared to fresh water. As discussed in the previous section, various investigators find density excesses ranging from 1.3 to 2.3 $\gamma$  units. As Gilfillan points out on the basis of data given by Washburn, Smith, and Frandsen (1933), nearly all of this effect can be accounted for

by fractionation of the oxygen isotopes during evaporation.

3. Water prepared from oxygen derived from carbonate rocks is denser than fresh water by some 8 $\gamma$  units.

4. Water prepared from atmospheric oxygen is denser than fresh water by 6.6 $\gamma$  units.

A full discussion of difficulties that arise when these data are interpreted on the basis of the photosynthetic origin of atmospheric oxygen is available (Kamen and Barker, 1945). It will suffice to present a summary of these difficulties in this section.

Attention is called first to the exchange reactions that are most probably involved. In table 7 are tabulated four reactions with the corresponding equilibrium constants for temperatures of  $0^\circ$  and  $25^\circ$  C., as calculated by Urey and Greiff (1935). It will be noted that when gaseous carbon dioxide or oxygen is equilibrated with water, the latter is impoverished in  $O^{18}$ . Thus, in reaction 2, water prepared from the carbon dioxide should be denser by some 10 $\gamma$  units than the water in equilibrium with it. This has been confirmed experimentally by Webster, Wahl, and Urey (1935). The other reactions have not been studied experimentally.

From reaction 2, it can be predicted that the oxygen of carbonate rocks will yield water with a density excess over fresh water of some 8–10 $\gamma$  units. Experimentally, such water is actually found to be 8 $\gamma$  units denser than fresh water (Dole and Slobod, 1940). The prediction based on the assumption that calcareous rocks of marine origin are in isotopic equilibrium with carbonate from which they are deposited is thus borne out. While no direct experimental evidence is at hand on the relative  $O^{18}$  content of dissolved and gaseous carbon dioxide in equilibrium, the results on the  $O^{18}$  content of the carbonate

<sup>1</sup> Goldsmith (1934) finds that, owing to the nuclear instability of deuterium at elevated temperatures, the D/H ratio in stars can fall as low as 1/600,000. Thus, the presence of deuterium in higher concentrations as on the earth could effect the development of terrestrial organisms by making it necessary for them to adapt to such concentrations. Conversely, the present D/H ratio for earth waters may be a result of organic action to some extent.

<sup>2</sup> Gaffron (1944). A summary of available evidence is given by Kamen and Barker (1945) and in the recent monograph by E. I. Rabinowitch (1945, p. 10).

rocks would seem to indicate that the  $O^{18}$  content of gaseous carbon dioxide is not changed appreciably on solution in water. An experimental study of carbonate precipitated from equilibrated carbon dioxide water systems should shed light on this point.

When the  $O^{18}$  content of atmospheric oxygen is considered on the basis of reaction 4, which dictates the final  $O^{18}$  content of the atmosphere, a discrepancy is noted. The enrichment in  $O^{18}$  content of gaseous oxygen predicted from reaction 4 amounts to 1 $\gamma$  unit only. Nevertheless, atmospheric oxygen yields water some 6.6 $\gamma$  units denser than fresh water (Dole, 1935; Swartout and Dole, 1939; Morita and Titani, 1936). This effect,

units difference in density between water prepared from atmospheric oxygen and fresh water. The remaining 3.6 $\gamma$  units remain to be explained.

A number of possibilities present themselves:

1. Some of the photosynthetic oxygen arises from the heavy oxygen of the atmospheric carbon dioxide. This is invalidated by the experimental observation that the oxygen evolved by photosynthetic plants shows the same  $O^{18}$  content as the water in the medium bathing the plants.

2. The calculated equilibrium constant for reaction 4 is in error. This is doubtful, since the physical constants required for calcula-

TABLE 7  
EQUILIBRIUM CONSTANTS OF SOME OXYGEN EXCHANGE REACTIONS  
(After Urey and Greiff)

Reaction	Equilibrium Constant	
	0° C.	25° C.
(1) $^{16}_{(g)}CO_2 + ^{18}_{(g)}H_2O \rightleftharpoons ^{18}_{(g)}CO_2 + ^{16}_{(g)}H_2O$	1.128	1.110
(2) $^{16}_{(g)}CO_2 + ^{18}_{(l)}H_2O \rightleftharpoons ^{18}_{(g)}CO_2 + ^{16}_{(l)}H_2O$	1.097	1.080
(3) $^{16}_{(g)}O_2 + ^{18}_{(g)}H_2O \rightleftharpoons ^{18}_{(g)}O_2 + ^{16}_{(g)}H_2O$	1.048	1.041
(4) $^{16}_{(g)}O_2 + ^{18}_{(l)}H_2O \rightleftharpoons ^{18}_{(g)}O_2 + ^{16}_{(l)}H_2O$	1.020	1.012

first noted by Dole and independently by Morita and Titani, has been amply confirmed by a number of other investigators (Greene and Voskuyl, 1936; Hall and Johnston, 1936; Jones and Hall, 1937; Smith and Matheson, 1936). Gilfillan (1934) finds that sea water is denser than fresh water by 2.3 $\gamma$  units. From his data and the work of Washburn, Smith, and Frandsen, it is reasonable to assume 2 $\gamma$  units are due to the concentration of  $O^{18}$  in sea water arising from fractionation during evaporation. Thus, we can account for 3 $\gamma$

tion of this equilibrium are known quite accurately. Furthermore, a similar calculation for reaction 2 has been verified experimentally. An experimental study of reaction 4 is rendered difficult by the slow rate at which equilibrium is attained. However, it is possible that organisms such as *Chlorella* may catalyze the reaction.<sup>1</sup>

3. One might suppose an enrichment

<sup>1</sup> Preliminary evidence that this may be true has been presented by Dole and Jenks (1944).

taking place in the upper stratosphere involving the vapor phase reaction 3 which has a more favorable equilibrium constant than reaction 4. It is difficult to see how any such effect would help in this situation, since such vapor diffusing downward into the region directly over the ocean where samples are collected would equilibrate according to reaction 4. Thus, any gain in  $O^{18}$  content attained by means of reaction 3 would be obviated.

4. The experiments on the isotopic content of photosynthetic oxygen have not been carried out using marine algae. These may not handle oxygen the same way as do *Chlorella* and the two land plants, sunflower and *Coleus*. While this possibility seems remote, it should be investigated.

5. A gravitational settling of the heavy oxygen may be contributing to the increased density of  $O^{18}$  at the surface of the sea. The maximum increase due to such an effect might be as high as  $2\gamma$  units (Dole, 1936). However, convection should abolish most of this effect. A study of the  $O^{18}$  content of the atmosphere at different altitudes should be undertaken to see to what extent gravitational settling is occurring.

6. The preferential utilization of  $O^{16}$  by respiratory organisms may result in a gradual

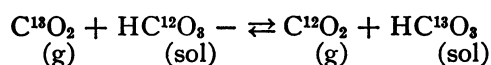
increase in the  $O^{18}$  content of the atmosphere.<sup>1</sup> At present no evidence exists as a basis for supposing a difference in oxidation rates of organic substrates by the different oxygen isotopes. Careful experiments to determine whether such a difference exists should be carried out. In this connection, it would be of interest to examine iron ores that have been subjected to extensive weathering. An increase in  $O^{16}$  content should be found, if a difference in ease of oxidation by the oxygen isotopes exists. Such studies are available (Dole and Slobod, 1940) for certain iron rocks from the Great Lakes region. In these rocks no isotope effect relative to water is found. However, there exists the possibility that such ores have been formed by precipitation and so show the same isotope content as in Great Lakes water.

7. There is the possibility that when the atmosphere was first formed, a large fractionation of the oxygen isotopes occurred. It seems most unlikely that such an initial concentration can have survived the enormous dilution with photosynthetic oxygen over a period of some  $10^9$  years, especially when it is remembered that a complete turnover of all atmospheric oxygen is estimated to occur through photosynthesis every few thousand years.

## CARBON

Carbon consists of a binary mixture of isotopes with masses 12 and 13. The ratio  $C^{12}/C^{13}$  is given by Nier and Gulbransen (1939) as  $90 \pm 2$ . These authors have investigated four kinds of carbon samples classified according to order of formation: (1) igneous carbons, (2) limestones, (3) plant forms, and (4) miscellaneous. Their results are reproduced in table 8. The accuracy given for these ratios is  $\pm 0.5$  per cent. All samples are measured as carbon dioxide.

The most notable result is the apparent enrichment of heavier isotope occurring in the limestones. This can be explained by noting that the equilibrium



lies somewhat to the right (Urey and Greiff,

1935). If the limestones are formed by deposition from carbonate in equilibrium with atmospheric carbon dioxide, then one can expect an increase in  $C^{13}$  content of limestone carbon as compared to that of igneous carbon or carbon from carbon dioxide. The miscellaneous data fit well with this conclusion. Thus, we would expect the sodium carbonate sample with the low ratio (88.3) to have been prepared from a limestone. This was found to be the case.

These observations have been extended to include effects of age in a single series of

<sup>1</sup> Rabinowitch (1945) suggests a similar hypothesis, picturing a situation in which oxygen is produced in photosynthesis indiscriminately from  $H_2O^{18}$  and  $H_2O^{16}$ , whereas in a thermal back reaction, the heavy isotope reacts more slowly, thus leading to a gradual accumulation of  $O^{18}$  in the atmosphere.

observations in limestone (Murphey and Nier, 1941). Limestones ranging in age from pre-Cambrian to modern show no trend in  $C^{13}$  content with age. The same conclusion is reached for various coals and woods. Just as in the earlier work, there is a concentration of  $C^{13}$  in limestones, whereas plant forms show a preference for  $C^{12}$ .

From table 8 one notes that the mean value of the ratio  $C^{12}/C^{13}$  for carbon from natural

1800 and 2200 grams per  $cm^2$  (Goldschmidt, 1937; Noddack, 1937). Clearly the geochemical estimates are not in conflict with the divergence in isotopic ratio. Further refinements in values for the isotopic ratio may prove of considerable value in controlling more exactly geochemical estimates.

The  $C^{12}/C^{13}$  ratio in animal or bacterial tissues does not appear to fluctuate markedly. At present, there is some evidence that the

TABLE 8  
 $C^{12}/C^{13}$  RATIO FOR VARIOUS SOURCES OF CARBON  
(After Nier and Gulbransen)

Sample	Source	Age ( $\times 10^6$ yrs.)	Average $C^{12}/C^{13}$
1. Igneous C			
Graphite	Ceylon	500 +	89.8
Meteorite	Tennessee	500	89.3
Diamond	South Africa	60?	89.0
2. Limestones, $CaCO_3$			
Grenville	New York	1200	87.9
Ordovician	Vermont	380	88.5
Clam shell	Boston	Present	88.6
3. Plant Sources			
Anthracite coal	Tennessee	250	91.8
Wood	Mass. pine	Present	91.5
4. Miscellaneous			
Dry ice	Virginia coal	230 +	91.2
Oil	West Texas	200 +	91.2
Air	Massachusetts	Present	89.8
$Na_2CO_3$	Michigan limestone	300 +	88.3
Clam flesh	Boston	Present	90.1

carbonates, namely, 88.4, differs negatively from the ratio for primary carbon sources, namely, 89.4, by approximately one percentage unit. The value of the mean ratio for plant carbon, namely, 91.4, differs positively by two percentage units. These results, if substantiated, can be used to check geochemical estimates of reduced carbon in sediments as compared with the carbon in carbonate sediments. Thus, one could predict a ratio of about 2:1 for the amount of  $CO_2$  fossilized as  $CaCO_3$  to that fossilized as carbonaceous matter. The best figure, derived from Clarke (1904) for the reduced carbon in sediments would appear to be 1100 grams per  $cm^2$  of earth's surface. The carbon of carbonate sediments is estimated as between

$C^{13}$  content in animal tissue averages somewhat lower than that in normal sodium bicarbonate (Swendseid, Barnes, Hemingway, and Nier, 1942). However, no detectable difference is found between  $C^{13}$  abundance in enzyme preparations of *Micrococcus lysodeikticus* and normal bicarbonate (Krampitz, Wood, and Werkman, 1943). Systematic discrimination such as appears to occur in the spectrometric assay of the potassium isotopes (see below) may account for the observations in animal tissue. At any rate, a careful study of the  $C^{13}$  content in the various organs of animal systems is indicated.

A few words concerning the case of nitrogen should be included. This element, which consists of two isotopes,  $N^{14}$  and  $N^{15}$ , ex-

hibits a normal  $N^{15}$  abundance of 0.368 atom per cent (Schoenheimer and Rittenberg, 1939). The sole reference to isotopic fluctuations in bio-active sources is that of Schoenheimer and Rittenberg (1939) who find several amino acids which have  $N^{15}$  contents in

excess of atmospheric nitrogen ranging up to 0.008 atom per cent. The authors are not prepared to state whether this small difference is a reflection of metabolic activity or simple physical exchange.

### POTASSIUM

Potassium is a ternary mixture with isotopic masses 39, 40, and 41. The isotope of mass 40 is radioactive, and its abundance in normal potassium is about 0.012 per cent. The ratio  $K^{39}/K^{41}$  for the two stable isotopes is given as  $14.12 \pm 1.28$  (Cook, 1943).

Data on the variation in isotopic ratio of potassium from various natural sources have been presented by Brewer (1936, 1937, 1938) and Brewer and Baudisch (1937). Cook has failed to verify a number of these observations. Table 9 is reproduced from an article

by Brewer. From this table it is noted that while little deviation occurs in minerals, plant materials show marked fluctuations. Kelp, grown in water in which the ratio is 14.20, shows a ratio of 13.75. Kelp thus assimilates some 6 per cent more  $K^{41}$  than  $K^{39}$  on the basis of these data. Cook, on the other hand, finds no marked decrease in the  $K^{39}/K^{41}$  ratio for a variety of kelps as well as a large number of minerals and fossils. The deviations reported by Cook are less than 1 per cent in the isotope ratio. Furthermore, an examina-

TABLE 9  
ISOTOPE RATIOS IN POTASSIUM FROM VARIOUS NATURAL SOURCES  
(After Brewer)

Sample	Description	$K^{39}/K^{41}$
Minerals:		
Pacific Ocean water	Surface	14.20
Pacific Ocean water	2500 meters depth	14.20
Wyoming shale	—	14.23
Wyomingite	Lava	14.25
Hawaiian basalt	Lava	14.11
Puerto Rico clay	Colloidal	14.20
N. Carolina clay	Red	14.20
Plants:		
Tobacco	Leaf and stock	14.20
Algae	Fresh water	14.20
Maple	—	14.20
Rose leaves	Grown in clay	14.23
Rose leaves	Grown in fertilizer soil	13.70
Potato vines	Small	14.20
Potato vines	Mature	13.77
Kelp	—	13.75
Animals:		
Muscle tissue	Beef	14.21
Bone marrow	Human	13.90
Bone marrow	Horse	13.92
Bone marrow	Bull	13.80
Bone marrow	Veal	13.70
Brain	Rat	14.22
Heart	Auricle	14.28
Pituitary gland	Pork	14.17
Liver	Beef	14.20

tion of rocks from different geological eras (early pre-Cambrian to Tertiary) shows no measurable change in the potassium isotope ratio within the obtainable accuracy of 1 per cent. Cook ascribes observed fluctuations to isotope effects of the ion source in the spectrometer and exhibits considerable experimental evidence as a basis for this contention.

To these results may be added the observation of Jacques<sup>1</sup> who finds a lowered  $K^{39}$  content in the sap of the algae *Valonia* and *Nitella* as compared with their environment (Bermuda sea water). He proposes a mechanism based on differential solubility in the system cellular sap-water analogous to the system guaiacol-water. Data obtained with this model system are not conclusive.

Lasnitzki (1939) and Lasnitzki and Brewer (1941, 1942) present data on the isotope content of various animal tissues with emphasis on a lowered  $K^{41}$  content in tumor tissue or other rapidly growing tissue. From the results of Cook, fossil bone shows no excess  $K^{41}$ , whereas new bone is found by Brewer and Lasnitzki to have a considerable deficiency in  $K^{41}$ . The fluctuations are considered significant by the writers, although an examination of their results indicates that many of the effects found lie well within the limit of error assigned by Cook in his work on the isotope ratio in kelp and fossils.

In view of such discrepancies, no conclusions on the significance of the potassium work can be drawn. More experiments designed to eliminate isotopic fractionation in the ion source are urgently required.

Some controversy on the abundance of  $K^{40}$  in living cells as compared to mineral potassium has existed since the claim by Ernst that the  $K^{40}/K^{39}$  ratio is higher in living tissue (Ernst, 1934). The measurement of this isotope is accomplished by radioactive assay and so is not subject to the criticism levelled at the researches with the stable isotopes. Fenn, Bale, and Mullins (1941) present strong evidence that the  $K^{40}$  content in human ash is some 1–2 per cent less abundant than in potassium from commercial sources.

It would be premature to attempt an

<sup>1</sup> Jacques (1940); the determinations of  $K^{39}/K^{41}$  ratio are due to Brewer.

analysis of these data in their present state. However, it is not out of place to recall to the reader the striking analogy between this question of biological separation of potassium isotopes and accumulation of potassium in the protoplasm of living cells. This accumulation occurs in the presence of sodium; in fact sodium is usually present in excess of potassium in the external medium. Spiegelman and Reiner conclude that no satisfactory mechanism based on strictly physical potentials involving preferential permeability can be formulated (Spiegelman and Reiner, 1942) (Steinbach, 1940). They reason that a chemical mechanism must be operative since the energy necessary to concentrate potassium in the presence of sodium (10 k. cal. per mole) while much greater than the energy available from electrical membrane potentials can be supplied by numerous chemical reactions. Many model systems similar to that tried by Jacques (see above) are known in which potassium and sodium show differential partition (Shedlovsky and Uhlig, 1934; Osterhout, Kamerling, and Stanley, 1934). In addition there are the observations in which preferential absorption of inorganic ions is observed in simple chemical systems such as clay-water. Selective accumulation of potassium is also reported in myosin (Mullins, 1942).

Drawing on the analysis of Spiegelman and Reiner, it seems certain that a separation of potassium isotopes cannot be expected on the basis of mobility differences, electrostatic barriers, or simple forces set up by two-phase systems. Brewer and Lasnitzki point out that if a chemical equilibrium exists in which potassium is combined with a large molecule inside the cell, and is present as the ion outside, a difference in mobility is set up for the two isotopes sufficient to explain the small accumulation of  $K^{39}$  inside the cell. However, the mechanism of simple base exchange as in the preferential absorption of  $K^{41}$  by greensand can also be invoked. Thus, the cell membrane could be considered to possess a surface on which  $K^{41}$  or  $K^{40}$  is more strongly bound than  $K^{39}$ . This might be tested by investigating the specific activity of the cell wall as compared to protoplasm after exposure to  $K^{40}$ .



## SUMMARY

IN THIS REVIEW, material on the separation and assay of isotopes is presented. All of the available evidence on isotope effects in the biosphere is collected and discussed. The reader will find that while on the one hand a large fund of data is available on the laboratory separation and assay of isotopes, there is a paucity of reliable data on geochemical isotope effects. This situation arises from the fact that only recently have assay methods reached the precision required for application

to geochemistry. The special role of biological systems in affecting isotope separations still remains to be established. Preliminary data already reveal such effects for hydrogen, oxygen, and carbon, and perhaps potassium. It can hardly be doubted that careful systematic studies with the newer techniques available will contribute much more valuable data, as well as resolve anomalies and uncertainties now existing in present data.

## BIBLIOGRAPHY

- ALVAREZ, L. W., AND R. CORNOG  
1939. Helium and hydrogen of mass 3. *Phys. Rev.*, vol. 56, p. 613.
- ASTON, F. W.  
1933. *Mass spectra and isotopes*. London, Ed. Arnold and Co.
- BABCOCK, H. D.  
1929. The constitution of oxygen. *Nature*, vol. 123, p. 761.
- BAINBRIDGE, K. T., AND E. B. JORDON  
1936. Mass spectrum analysis. 1. The mass spectrograph. 2. The existence of isobars of adjacent elements. *Phys. Rev.*, vol. 50, pp. 282-296.
- BARNES, T. C., AND T. L. JAHN  
1934. Properties of water of biological interest. *Quart. Rev. Biol.*, vol. 9, pp. 292-341.
- BARNES, T. C., AND E. J. LARSON  
1933. Further experiments on the physiological effect of heavy water and of ice water. *Jour. Amer. Chem. Soc.*, vol. 55, pp. 5059-5060.
- BAXTER, G. P., O. HÖNIGSCHMID, AND P. LEBEAU  
1938. Eighth report of the Committee on Atomic Weights of the International Union of Chemistry. *Jour. Amer. Chem. Soc.*, vol. 60, pp. 737-745.
- BEAMS, J. W.  
1938. High speed centrifuging. *Rev. Mod. Phys.*, vol. 10, pp. 245-263.
- BEAUCHAMP, R. S. A.  
1940. Chemistry and hydrography of Lakes Tanganyika and Nyasa. *Nature*, vol. 146, p. 253.
- BIRGE, R. T.  
1941. The general physical constants as of August, 1941 with details on the velocity of light only. *Repts. on Progress in Physics*, vol. 8, pp. 90-134.
- BLEAKNEY, W.  
1932. The ionization potential of molecular hydrogen. *Phys. Rev.*, vol. 40, pp. 496-501.  
1936. The mass-spectrograph and its uses. *Amer. Phys. Teacher*, vol. 4, pp. 12-23.
- BOWEN, N. L.  
1928. *Evolution of igneous rocks*. Princeton, New Jersey, Princeton University Press.
- BREUSCH, F., AND E. HOFER  
1934. Über das verhältnis des schweren wassers zum leichten in organismus. *Klin. Work.*, vol. 13, pt. 2, pp. 1815-1816.
- BREWER, A. K.  
1936. The abundance ratio of the isotopes of potassium in mineral and plant sources. *Jour. Amer. Chem. Soc.*, vol. 58, pp. 365-370.  
1937. Abundance ratio of the isotopes of potassium in animal tissues. *Ibid.*, vol. 59, pp. 869-872.  
1938. The isotopes of potassium. *Jour. Indus. and Eng. Chem.*, vol. 30, pp. 893-896.  
1939. Isotopes of potassium in phosphate rocks and soils. *Jour. Amer. Chem. Soc.*, vol. 61, pp. 1597-1599.
- BREWER, A. K., AND O. BAUDISCH  
1937. Isotopes of potassium and lithium in Saratoga mineral water and crysozoön. *Jour. Amer. Chem. Soc.*, vol. 59, pp. 1578-1579.
- BRÖNSTED, J. N., AND G. HEVESY  
1920. The separation of the isotopes of mercury. *Nature*, vol. 106, p. 144.  
1921. The separation of the isotopes of chlorine. *Ibid.*, vol. 107, p. 619.
- CAVANAGH, B., J. HORIUTI, AND M. POLANYI  
1934. Enzyme catalysis of the ionization of hydrogen. *Nature*, vol. 133, p. 797.
- CHAPMAN, S.  
1916. On the kinetic theory of a gas. Part II. A composite monatomic gas: diffusion, viscosity, and thermal conduction. *Phil. Trans.*, vol. 217A, pp. 155-197.
- CLARKE, F. W.  
1904. *Analyses of rocks from the laboratory of the United States Geological Survey, 1880 to 1903*. U. S. Geol. Surv., Bull. 228.
- CLUSIUS, K., AND G. DICKEL  
1938. Neues verfahren zur gasentmischung und isotopentrennung. *Naturwissenschaften*, vol. 26, p. 546.
- COLEMAN, A. P.  
1924. Geology of the Sudbury nickel deposits. *Econ. Geol.*, vol. 19, pp. 565-576.
- COOK, K. L.  
1943. The relative abundance of the isotopes of potassium in Pacific kelps and in rocks of different geologic ages. *Phys. Rev.*, vol. 64, pp. 278-293.
- CRIST, R. H., G. M. MURPHY, AND H. C. UREY  
1933. The isotopic analysis of water. *Jour. Amer. Chem. Soc.*, vol. 55, pp. 5060-5061.
- DALY, R. A., AND G. A. F. MOLENGRAAFF  
1924. Structural relations of the Bushveld igneous complex. *Transvaal Jour. Geol.*, vol. 32, pp. 1-35.

- DEMPSTER, A. J.  
 1918. A new method of positive ray analysis. *Phys. Rev.*, vol. 11, pp. 316-325.  
 1938. The atomic masses of the heavy elements. *Ibid.*, vol. 53, pp. 64-75.
- DOLE, M.  
 1935. The relative atomic weight of oxygen in water and air. *Jour. Amer. Chem. Soc.*, vol. 57, p. 2731.  
 1936. The relative atomic weights of oxygen in water and in air. A discussion of the atmospheric distribution of the oxygen isotopes and of the chemical standard of atomic weights. *Jour. Chem. Phys.*, vol. 4, pp. 268-275.
- DOLE, M., AND G. JENKS  
 1944. Isotopic composition of photosynthetic oxygen. *Science*, vol. 100, p. 409.
- DOLE, M., AND R. J. SLOBOD  
 1940. Isotopic composition of oxygen in carbonate rocks and iron oxide ores. *Jour. Amer. Chem. Soc.*, vol. 62, pp. 471-479.
- EMELÉUS, H. J., F. W. JAMES, A. KING, T. G. PEARSON, R. H. PURCELL, AND H. V. A. BRISCOE  
 1934. The isotopic ratio in hydrogen: A general survey by precise density comparisons upon water from various sources. *Jour. Chem. Soc., London*, vol. 136B, pp. 1207-1219.
- ENSKOG, D.  
 1911. Bemerkungen zu einer fundamentalgleichung in der kinetischen gastheorie. *Phys. Zeitschr.*, vol. 12, pt. 2, pp. 533-539.
- ERLENMEYER, H., AND H. GARTNER  
 1934. Experiments on heavy water. *Nature*, vol. 134, p. 327.
- ERNST, E.  
 1934. Die speicherung des radioaktiven kalium-isotops durch die organe. *Naturwissenschaften*, vol. 22, p. 479.
- FARKAS, A., AND L. FARKAS  
 1933. Some experiments on heavy hydrogen. *Nature*, vol. 132, p. 894.  
 1935. Orthohydrogen, parahydrogen and heavy hydrogen. Cambridge, England, Cambridge University Press.
- FENN, W. O., W. F. BALE, AND L. J. MULLINS  
 1941. The radioactivity of potassium from human sources. *Jour. Gen. Physiol.*, vol. 25, pp. 345-353.
- FRIEND, J. N.  
 1934. Heavy water and water of crystallization. *Nature*, vol. 134, p. 463.
- FRIVOLD, O. E.  
 1920. Dichtebestimmungen an lösungen, nebst bestimmung der dichteunterscheide zwischen wasser und wasser mit luft gesättigt. *Phys. Zeitschr.*, vol. 21, pp. 529-534.
- GABBARD, J. L., AND M. DOLE  
 1937. A redetermination of the deuterium-protium ratio in normal water. *Jour. Amer. Chem. Soc.*, vol. 59, pp. 181-185.
- GAFFRON, H.  
 1944. Photosynthesis. In *Encyclopedia Britannica*.
- GIAUQUE, W. F., AND H. L. JOHNSTON  
 1929. An isotope of oxygen. *Nature*, vol. 123, p. 318.
- GILFILLAN, E. S., JR.  
 1934. The isotopic composition of sea water. *Jour. Amer. Chem. Soc.*, vol. 56, pp. 406-408.
- GILFILLAN, E. S., JR., AND M. POLANYI  
 1933. Mikropyknometer zur bestimmung von verschiebungen im isotopenverhältnis des wassers. *Zeitschr. f. Phys. Chem.*, vol. 166A, pp. 254-256.
- GOLDSCHMIDT, V. M.  
 1934. Drei vorträge über geochemie. *Geol. Fören. Förhandl.*, vol. 56, pp. 385-427.
- GOLDSMITH, H. H.  
 1934. Disintegration of  $H^2$  and the stellar abundance of  $H^2$  and  $H^3$ . *Phys. Rev.*, vol. 46, pp. 78-79.
- GREENE, C. H., AND R. J. VOSKUYL  
 1936. Explanation of the relatively large concentration of  $O^{18}$  in the atmosphere. *Jour. Amer. Chem. Soc.*, vol. 58, pp. 693-694.  
 1939. The deuterium-protium ratio. 1. The densities of natural waters from various source. *Ibid.*, vol. 61, pp. 1342-1349.
- GRÓH, J., AND G. HEVESY  
 1920. Die selbstdiffusionsgeschwindigkeit des geschmolzenen bleis. *Ann. der Phys.*, ser. 4, vol. 63, pp. 85-92.
- HALFORD, J. O., L. C. ANDERSON, AND J. R. BATES  
 1934. Introduction of deuterium atoms into acetone. *Jour. Amer. Chem. Soc.*, vol. 56, pp. 491-492.
- HALL, N. F., AND T. O. JONES  
 1936. A redetermination of the protium-deuterium ratio in water. *Jour. Amer. Chem. Soc.*, vol. 58, pp. 1915-1919.
- HALL, W. H., AND H. L. JOHNSTONE  
 1936. The influence of combustion conditions on the density of water formed from commercial hydrogen and oxygen. *Jour. Amer. Chem. Soc.*, vol. 58, pp. 1920-1922.
- HARKINS, W. D., AND B. MORTIMER  
 1928. The separation of isotopes and a further

- separation of mercury by evaporative diffusion. *Phil. Mag.*, ser. 7, vol. 6, pp. 601-631.
- HARMSSEN, H., G. HERTZ, AND W. SCHÜTZE  
1934. Weitere versuche zur isotopentrennung. Reindarstellung des schweren wasserstoffs durch diffusion. *Zeitschr. f. Physik*, vol. 90, pp. 703-711.
- HARTLEY, H., A. O. PONDER, E. J. BOWEN, AND T. R. MERTON  
1922. An attempt to separate the isotopes of chlorine. *Phil. Mag.*, ser. 6, vol. 43, pp. 430-435.
- HERTZ, G.  
1932. Ein verfahren zur trennung von gasförmigen isotopengemischen und seine anwendung auf die isotopen des neons. *Zeitschr. f. Physik*, vol. 79, pp. 108-121.  
1934. Ein verfahren zur trennung von isotopengemischen durch diffusion in strömendem quecksilberdampf. *Ibid.*, vol. 91, pp. 810-815.
- HEVESY, G.  
1927. The radioactivity of potassium. *Nature*, vol. 120, pp. 838-839.
- HEVESY, G., AND E. HOFER  
1934. Diplogen and fish. *Nature*, vol. 133, pp. 495-496.
- HEVESY, G., AND F. A. PANETH  
1938. Radioactivity. London, Oxford University Press.
- HOBERMAN, H. D.  
1942. Biological catalysis of the exchange reaction between water and hydrogen. [Ph.D. dissertation.] New York, Columbia University.
- HUFFMAN, J. R., AND H. C. UREY  
1937. Separation of oxygen isotopes by a fractionating column. *Jour. Indus. and Eng. Chem.*, vol. 29, pp. 531-535.
- HUGHES, A. L., AND U. ROJANSKY  
1929. On the analysis of electronic velocities by electrostatic means. *Phys. Rev.*, vol. 34, pp. 284-290.
- HUTCHINSON, G. E.  
1937. Limnological studies in Indian Tibet. *Internatl. Rev. Hydrobiol. Hydrog.*, vol. 35, pp. 133-176.
- JACQUES, A. G.  
1940. Separation of potassium isotopes in Valonia and Nitella. *Jour. Gen. Physiol.*, vol. 23, pp. 741-742.
- JEVONS, W.  
1932. Report on band-spectra of diatomic molecules. London, The Physical Society.
- JONES, T. O., AND N. F. HALL  
1937. The relative atomic weights of oxygen from air and water determined by an interchange reaction. *Jour. Amer. Chem. Soc.*, vol. 59, pp. 259-261.
- KAMEN, M. D., AND H. A. BARKER  
1945. Inadequacies in present knowledge of the relation between photosynthesis and the  $O^{18}$  content of atmospheric oxygen. *Proc. Natl. Acad. Sci.*, vol. 31, pp. 8-15.
- KEESOM, W. H., H. VAN DIJK, AND J. HAANTJES  
1934. On the separation of neon into its isotopic components by means of rectification. *Proc. K. Akad. Wetensch., Amsterdam*, vol. 37, p. 615.
- KELLEY, W. P.  
1939. Base exchange in relation to sediments. In Trask, Parker D. (editor), Recent marine sediments. Tulsa, Oklahoma, American Association of Petroleum Geologists.
- KING, A. S., AND R. T. BIRGE  
1929. An isotope of carbon, mass 13. *Nature*, vol. 124, p. 127.
- KRAMPITZ, L. O., H. G. WOOD, AND C. H. WERKMAN  
1943. Enzymic fixation of carbon dioxide in oxalacetate. *Jour. Biol. Chem.*, vol. 147, pp. 243-253.
- KUHN, W., AND H. MARTIN  
1932. Photochemische trennung von isotopen. *Naturwissenschaften*, vol. 20, p. 772.
- LASNITZKI, A.  
1939. On the radioactivity of potassium in tumor tissue. *Amer. Jour. Cancer*, vol. 35, pp. 225-229.
- LASNITZKI, A., AND A. K. BREWER  
1941. Isotopic constitution of potassium in animal tumors and muscle from tumor-bearing animals. *Cancer Res.*, vol. 1, pp. 776-778.  
1942. The isotopic constitution of potassium in normal tissues and cancer from human subjects. *Ibid.*, vol. 2, pp. 494-496.
- LEWIS, G. N.  
1933. The biochemistry of water containing hydrogen isotope. *Jour. Amer. Chem. Soc.*, vol. 55, pp. 3503-3504.
- LEWIS, G. N., AND D. B. LUTEN  
1933. The refractive index of  $H_2O^{18}$ , and the complete isotope analysis of water. *Jour. Amer. Chem. Soc.*, vol. 55, pp. 5061-5062.
- LEWIS, G. N., AND R. T. MACDONALD  
1933. Some properties of  $H^2H^2O$ . *Jour. Amer. Chem. Soc.*, vol. 55, pp. 3057-3059.
- LINDEMANN, F. A.  
1921. Discussion on isotopes. *Proc. Roy. Soc., London*, vol. 99A, pp. 102-104.

- LINDEMANN, F. A., AND F. W. ASTON  
1919. The possibility of separating isotopes. *Phil. Mag.*, ser. 6, vol. 37, pp. 523-535.
- MACHT, D. I., AND M. E. DAVIS  
1934. Some pharmacological experiments with deuterium. *Jour. Amer. Chem. Soc.*, vol. 56, p. 246.
- MATTAUCH, J.  
1936. A double-focusing mass spectrograph and the masses of  $N^{15}$  and  $O^{18}$ . *Phys. Rev.*, vol. 50, pp. 617-623.
- MATTAUCH, J., AND H. EWALD  
1944. Über eine neue methode zur messung relativer häufigkeiten von isotopen. Die isotopenverteilung und das atomgewicht von hafnium. *Zeitschr. f. Physik*, vol. 122, pp. 314-356.
- MORITA, N., K. GOTO, AND T. TITANI  
1938. Über den gehalt der kohlenhydrate an schwerem sauerstoff. *Bull. Chem. Soc. Japan*, vol. 13, pp. 99-110.
- MORITA, N., AND T. TITANI  
1936. Erzeugung des leichten wassers und bestimmung der deuterium-konzentration im normalen wasser. *Bull. Chem. Soc. Japan*, vol. 11, pp. 403-413.  
1936a. Über den gehalt der kohlenhydrate an schwerem sauerstoff. *Ibid.*, vol. 11, pp. 695-699.
- MULLIKEN, R. S.  
1922. The separation of isotopes by thermal and pressure diffusion. *Jour. Amer. Chem. Soc.*, vol. 44, pp. 1033-1051.  
1922a. The separation of isotopes by distillation and analogous processes. *Ibid.*, vol. 44, pp. 2387-2390.  
1923. The separation of isotopes. Application of systematic fractionation to mercury in a high-speed evaporation-diffusion apparatus. *Ibid.*, vol. 45, pp. 1592-1604.
- MULLINS, L. J.  
1942. Selective accumulation of potassium by myosin. *Fed. Proc.*, pt. 2 (Fed. Amer. Soc. Exper. Biol.), vol. 1, p. 61.
- MURMANN, ERNST  
1923. Versuche zur Trennung von Elementen und Isotopen durch Diffusion. *Oesterreichische Chem.-Zeitg.*, new ser., vol. 26, pp. 14-15.
- MURPHEY, B. F.  
1941. Relative abundance of the oxygen isotopes. *Phys. Rev.*, vol. 59, p. 320.
- MURPHEY, B. F., AND A. O. NIER  
1941. Variations in relative abundance of the carbon isotopes. *Phys. Rev.*, vol. 59, pp. 771-772.
- NAUDE, S. M.  
1930. The isotopes of nitrogen, mass 15, and oxygen, mass 18 and 17, and their abundances. *Phys. Rev.*, vol. 36, pp. 333-346.
- NIER, A. O.  
1937. A mass-spectrographic study of the isotopes of Hg, Xe, Kr, Be, I, As, and Cs. *Phys. Rev.*, vol. 52, pp. 933-937.
- NIER, A. O., AND E. A. GULBRANSEN  
1939. Variations in the relative abundance of the carbon isotopes. *Jour. Amer. Chem. Soc.*, vol. 61, pp. 697-698.
- NODDACK, W.  
1937. Der kohlenstoff im haushalt der natur. *Angew. Chem.*, vol. 50, pp. 505-510.
- OSTERHOUT, W. J. V., S. E. KAMERLING, AND W. M. STANLEY  
1934. The kinetics of penetration. VI. Some factors affecting penetration. VII. Molecular versus ionic transport. *Jour. Gen. Physiol.*, vol. 17, pp. 445-480.
- PACSU, E.  
1934. Mutarotation of  $\alpha$ -d-glucose in deuterium water. *Jour. Amer. Chem. Soc.*, vol. 56, pp. 745-746.
- RABINOWITCH, E. L.  
1945. Photosynthesis and related processes. New York, Inter-science Publishers, Inc.
- RAYLEIGH, LORD  
1896. Theoretical considerations respecting the separation of gases by diffusion and similar processes. *Phil. Mag.*, ser. 5, vol. 42, pp. 493-498.
- REITZ, O.  
1938. Reaktionsgeschwindigkeiten in schwerem wasser. *Zeitschr. Elektrochem.*, vol. 44, pp. 72-81.
- REITZ, O., AND K. F. BONHOEFFER  
1934. Über den einbau von schwerem wasserstoff in wachsende organismen. *Naturwissenschaften*, vol. 22, p. 744.
- RICHARDS, T. W., AND J. W. SHIPLEY  
1912. A new method for the quantitative analysis of solutions by precise thermometry. *Jour. Amer. Chem. Soc.*, vol. 34, pp. 599-603.
- RIESENFELD, E. H., AND T. L. CHANG  
1936. Über den gehalt an HDO und  $H_2O^{18}$  in regen und schnee. *Ber. Deutsche Chem. Gesellsch.*, vol. 69, pt. 2, pp. 1305-1307.
- ROBINSON, P. L., AND H. V. A. BRISCOE  
1925. A redetermination of the atomic weight of bromine. The inseparability of the isotopes by fractional crystallisation.

- Jour. Chem. Soc., London, vol. 127, pp. 138-150.
- RUBEN, S., M. RANDALL, M. D. KAMEN, AND J. L. HYDE  
1941. Heavy oxygen ( $O^{18}$ ) as a tracer in the study of photosynthesis. Jour. Amer. Chem. Soc., vol. 63, pp. 877-879.
- SCHOENHEIMER, R., AND D. RITTENBERG  
1935. Deuterium as an indicator in the study of intermediary metabolism. Jour. Biol. Chem., vol. 111, pp. 163-181.  
1939. Protein metabolism. General considerations in the application of isotopes to the study of protein metabolism. The normal abundance of nitrogen isotopes in amino acids. *Ibid.*, vol. 127, pp. 285-290.
- SCHWAB, G., AND K. JOCKERS  
1937. Anorganische chromatographie. Naturwissenschaften, vol. 25, p. 44.
- SEABORG, G. T.  
1940. Artificial radioactivity. Chem. Rev., vol. 27, pp. 199-285.
- SHEDLOVSKY, T., AND H. H. UHLIG  
1934. On quaiacol solutions. II. The distribution of sodium and potassium quaiacolate between quaiacol and water. Jour. Gen. Physiol., vol. 17, pp. 563-576.
- SHERWIN, C. W.  
1940. The cross section for change of charge and ionization by high-velocity metallic ions in hydrogen and helium. Phys. Rev., vol. 57, pp. 814-821.
- SHERWIN, C. W., AND A. J. DEMPSTER  
1941. The relative abundance of the calcium isotopes. Phys. Rev., vol. 59, p. 114.
- SMITH, E. R., AND H. MATHESON  
1936. Difference in atomic weight of oxygen from air and from water. Jour. Res. Natl. Bur. Stand., vol. 17, pp. 625-628.
- SMITH, P. K., J. TRACE, AND H. G. BARBOUR  
1936. The fate of deuterium in the mammalian body. Jour. Biol. Chem., vol. 116, pp. 371-380.
- SMYTHE, W. R.  
1926. A velocity filter for electrons and ions. Phys. Rev., vol. 28, pp. 1275-1286.  
1934. The isotopic ratio in oxygen. *Ibid.*, vol. 45, pp. 299-303.
- SMYTHE, W. R., AND J. MATTAUCH  
1932. A new mass spectrometer. Phys. Rev., vol. 40, pp. 429-433.
- SODDY, F.  
1911. The chemistry of mesothorium. Jour. Chem. Soc., London, Phil. Trans., vol. 99, pp. 72-83.
- SPIEGELMAN, S., AND J. M. REINER  
1942. A kinetic analysis of potassium accumulation and sodium exclusion. Growth, vol. 6, pp. 367-389.
- STEINBACH, H. B.  
1940. Electrolyte balance of animal cells. Cold Spring Harbor Symposium, vol. 8, pp. 242-254.
- STEWART, W. W., AND H. HOLCOMB  
1934. Biological separation of heavy water. Jour. Amer. Chem. Soc., vol. 56, pp. 1422-1423.
- STRAUS, H. A.  
1941. A new mass spectrograph and the isotopic constitution of nickel. Phys. Rev., vol. 59, pp. 430-433.
- SWARTOUT, J. A., AND M. DOLE  
1939. The protium-deuterium ratio and the atomic weight of hydrogen. Jour. Amer. Chem. Soc., vol. 61, pp. 2025-2029.
- SWENDSEID, M. E., R. H. BARNES, A. HEMINGWAY, AND A. O. NIER  
1942. Formation of acetone bodies from acetic acid. Jour. Biol. Chem., vol. 142, pp. 47-52.
- TAYLOR, H. S., W. W. SWINGLE, H. EYRING, AND A. H. FROST  
1933. The effect of water containing the isotope of hydrogen upon fresh water organisms. Jour. Chem. Phys., vol. 1, p. 751.
- TAYLOR, T. I., AND H. C. UREY  
1938. Fractionation of the lithium and potassium isotopes by chemical exchange with zeolites. Jour. Chem. Phys., vol. 6, pp. 429-438.
- THODE, H. G., R. L. GRAHAM, AND J. A. ZIEGLER  
1945. A mass spectrometer and the measurement of isotope exchange factors. Canadian Jour. Res., vol. 23B, pp. 40-47.
- TUCHOLSKI, F.  
1934. Increase of the percentage of diplogen in water during very slow evaporation. Nature, vol. 134, p. 29.
- UREY, H. C.  
1939. Separation of isotopes. Repts. on Progress in Physics, vol. 6, pp. 48-77.
- UREY, H. C., F. G. BRICKWEDDE, AND G. M. MURPHY  
1932. A hydrogen isotope of mass 2. Phys. Rev., vol. 39, pp. 164-165.  
1932a. A hydrogen isotope of mass 2 and its concentration. *Ibid.*, vol. 40, pp. 1-15.
- UREY, H. C., AND L. J. GREIFF  
1935. Isotopic exchange equilibria. Jour. Amer. Chem. Soc., vol. 57, pp. 321-327.
- VEREŠČAGIN, G., A. GORBOV, AND I. MENDELEJEV  
1934. Sur l'existence de l'eau de densité anormale dans des conditions naturelles.

- Compt. Rendus Acad. Sci. URSS, new ser., 1934, vol. 2, pp. 134-137.
- VERNADSKY, W. I.
1938. On some principal problems of biogeochemistry. Bull. Acad. Sci. URSS, Cl. Sci. Math. Nat., Sér. Geol., 1938, no. 1, Russian text: pp. 19-33, English text: pp. 33-34.
1944. Problems of biogeochemistry. II. The fundamental matter-energy difference between the living and the inert natural bodies of the biosphere. Trans. Connecticut Acad. Arts and Sci., vol. 35, pp. 483-517.
- WASHBURN, E. W., E. R. SMITH, AND M. FRANDSEN
1933. The isotopic fractionation of water. Bur. Stand. Jour. Res., vol. 11, pp. 453-462.
- WEBSTER, L. A., M. H. WAHL, AND H. C. UREY
1935. Fractionation of the oxygen isotopes in an exchange reaction. Jour. Chem. Phys., vol. 3, p. 129.
- WIRTH, H. E., T. G. THOMPSON, AND C. L. UTTERBACK
1935. Distribution of isotopic water in the sea. Jour. Amer. Chem. Soc., vol. 57, pp. 400-404.

### ADDENDUM

After the present work was read in page proof, a paper by A. P. Vinogradov and R. V. Teis ("Isotopic composition of oxygen of different origin," 1941, Comptes Rend. (Doklady) Acad. Sci. URSS, vol. 33, pp. 490-493) was received, which had not been previously available in America. These authors found  $d$  of 4.5 units, referred to a standard prepared from Moscow river water, for water derived from the photosynthetic oxygen of *Helodea canadensis*. Atmospheric oxygen was found to give water of excess density 7.3 units referred to the same standard, in good agreement with previous investigations. The density excess of water prepared from the oxygen of the sodium bicarbonate used as a carbon source for the plant was found to be 11.6 units. The photosynthetic oxygen evidently had an isotopic composition which was not identical with that of the oxygen of fresh water, though nearer to it than to that of

the sodium bicarbonate employed. The difference between photosynthetic and freshwater oxygen implied in this work is of the correct order of magnitude to explain the anomaly in the isotopic composition of atmospheric oxygen, but in view of the work of Ruben, Randall, Kamen, and Hyde on *Chlorella* and of Dole and Jenks on land plants, an explanation of the animals based on the findings of Vinogradov and Teis cannot be accepted unreservedly until more work has been done on a variety of plants.

A note has also appeared by A. S. Knox ("Pollen radioactivity," 1945 [*sic*, issued in 1946], Pollen and Spore Circular no. 12) which states that Tripp estimates the potassium content of fresh ragweed pollen, on the basis of  $K^{40}$  radioactivity, to be  $160 \times 10^{-3}$  gr per gr. This value seems very high and may imply a concentration of  $K^{40}$  in such material.











