VARIATION OF THE CARBON ISOTOPE COMPOSITION IN SOME NATURAL PROCESSES

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A Thesis
Submitted to the Faculty of Arts and Science
in partial fulfilment of the requirements
for the degree
Master of Science

McMaster University
October, 1955

MASTER OF SCIENCE (1955)
(Chemistry)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Variation of the Carbon Isotope Composition in Some Natural Processes.

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SUPERVISION: Professor H. G. Thode

NUMBER OF PAGES: vi, 51

SCOPE AND CONTENTS:

The variation in the carbon isotope composition of the cap rock of Texas and Louisiana sulphur wells was investigated by means of a simultaneous collection mass spectrometer. These rocks showed anomalously large depletions in C^{13}. The isotope depletion in the decarboxylation of pyruvic acid, both by chemical means and by bacteria, was measured and the isotope composition of the carbon dioxide released was found to be similar to that of the cap rock. The hypothesis is advanced that the carbonate of the cap rock may have originated by the precipitation of carbon dioxide released in the bacterial decarboxylation of an organic substrate.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Professor H. G. Thode for his advice and direction in this investigation. The author would also like to thank Drs. W. Fleming, R. Farquhar, and C. McMullen for their cooperation and advice.

The financial assistance of the Research Council of Ontario is gratefully acknowledged.

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INTRODUCTION

Previous investigations by many workers have shown wide variations in the abundances of the isotopes in naturally occurring samples. These variations, referred to as isotope effects, can be brought about by equilibrium or unidirectional processes. Thus, a study of natural samples can yield information a bout the origin and subsequent physical and chemical changes of the material being investigated. A study of reactions in the laboratory shows that isotope effects can be obtained in chemical reactions and these effects can be used to elucidate the mechanisms of such reactions.

The results of isotope analyses on naturally occurring carbon samples show certain definite regularities. Organic sources have a C^{12}/C^{13} ratio about 3 per cent higher than limestones. However, organic sources overlap in their ratios with other sources such as igneous rock, so that a clear-cut decision on the origin of a particular carbon sample cannot always be made on the basis of its isotope constitution alone.

Sulphur isotope studies on the sulphur wells of Louisiana and Texas point to a bacterial reduction of sulphate to sulphur as the mechanisms by which the sulphur is formed. Preliminary isotope studies on the calcitic limestone cap rock of these wells indicate that this limestone is also of organic origin. These carbon isotope analyses have been repeated and extended.

Historical

The discovery of isotopes was intimately connected with the work begun on radioactivity at the turn of the century. As new members of the radioactive sequences were discovered, it was found in some cases that two or more of the atoms in the series had exactly the same chemical properties (1). The possibility of such a situation had been suggested as far back as 1886 by Sir William Crookes (2): "I conceive therefore that when we say the atomic weight of, for instance, calcium is 40, we really express the fact that, while the majority of calcium atoms have atomic weight 40, there are not a few which are represented by 39 or 41."

Several examples of this phenomenon were known among the radioactive atoms. Attention was drawn to this matter in 1909 by Strömholm and The Svedberg (3). In 1910 Soddy (4) coined the name isotopes.

J.J. Thompson (5) in 1913 verified Soddy's conclusions by separating the mass components of neon. Thompson's apparatus utilized parallel electric and magnetic fields. A fine stream of ions was directed into the fields. Ions of the same mass to charge ratio and of the same velocity would be focussed as a spot on a photographic plate. However, Thompson's source produced ions with a spread of 1000 e.v. so that the images were drawn out into a series of parabolas. In this way Thompson detected an ion of mass 22 for which no element existed, and he concluded it was a form of neon. This result, which was confirmed by Aston (6) in the same year, showed that isotopes were present in the lighter elements.

In 1919 Aston produced the first mass spectrograph. He arranged electric and magnetic fields in succession so that the dispersion produced by the electric field was just compensated by the dispersion produced by the magnetic field. All particles with the same mass to charge ratio, but with different velocities within a broad velocity spectrum, were focussed to the same line on a photographic plate. This type of apparatus was improved by Bainbridge, Dempster, Jordan and others. It is best suited for accurate measurements of atomic masses.

An apparatus suitable for making abundance measurements was developed by Dempster. In this machine, the ions are accelerated by an electric field. They pass normally into a magnetic field, are bent through 180° and brought to a focus. Dempster (1918) was the first to use the focussing properties of a magnetic field. The focussing of sector magnetic fields was discussed by Stevens in 1934 (7). This type of instrument was developed by Bleakney (8), Tate and Smith (9) and Nier (10,11) particularly, who from 1936 onwards introduced many refinements and simplifications.

The variation in the abundances of the isotopes of an element is usually quite small with the exception of the lighter elements. In 1925 Briscoe (14) was able to report a slight variation in the atomic weight of boron taken from different geological sources.

The rare isotopes of the light elements had escaped detection in the mass spectrograph. Their presence was established by studies of molecular spectra. In 1929, O^{17} and O^{18} were discovered by Giaque and Johnston (15), C^{13} by King and Birge (16) and N^{15} by Naude (17). Birge and Menzel (18) were the first to predict that hydrogen should have a rare isotope of mass two. Urey (19) in 1931 discovered deuterium and separated it from hydrogen by distillation. It was found that due to the large mass difference between deuterium and hydrogen, their chemical properties were quite different. By the methods of statistical mechanics, Urey and Rittenberg (20) calculated the equilibrium constants for the exchange of hydrogen and deuterium in molecular hydrogen and the hydrogen halides. The equilibrium constants were not equal to one, indicating that isotope fractionation could be expected. This prediction was verified experimentally by these authors (21). Urey and his co-workers extended the calculations of equilibrium constants to reactions involving other light elements, namely lithium, boron, carbon, nitrogen and oxygen (22). However, relative abundance studies of hydrogen, nitrogen and oxygen by Urey and Bradley (23) and Urey and Murphy (24) and on oxygen by Urey et al (25) in 1934 did not show any variation within the experimental error. Their experimental accuracy was ± 2 per cent. On the other hand, accurate density studies by Dole (26) on samples of water prepared with oxygen from various sources did show variations in the oxygen isotope ratio up to about 4 per cent.

To measure these small differences it was necessary to develop mass spectrometers of higher precision. In 1932 Bleakney had made the first major changes in Dempster's design by using a solenoidal magnetic field -- an innovation which is now of historical interest only. Bleakney was also the first to use a source system in which an electron beam was lined up by the main magnetic field, enabling the use of longer ion source slits with a corresponding increase in intensity. This principle was developed by Nier into the present source system.

All spectrometers previous to 1940 were 180° deflection instruments. Nier (13) developed a sector field instrument in which the ions were deflected through 60° (and later through 90° in Hipple's (27) instrument of 1942).

The resolution of 180° instruments is the same as that of the 60° and 90° instruments, since it depends only on the slit dimensions and the radius of curvature. The spherical aberration is also the same. However, the sector field instruments have the advantage that a much smaller magnet can be used. This gain is partly offset by certain minor problems. The sector field instrument requires a much more critical alignment of source and collector slits and of the magnet iself with respect to the tube. The source requires an auxiliary magnetic field to define the electron beam. There are also the effects of a fringe field. These are minor points and most instruments of recent design are of the sector field type. This system enables the measurement of isotope variations with an accuracy of ± 0.1 per cent.

Further refinements were made in the method of measuring and handling the samples. Simultaneous collection was introduced by a student of Dempster in 1938 for the measurement of nickel isotopes. In this method the two isotopes whose ratio is to be measured are collected simultaneously and the signal from the less abundant isotope is compared with a fraction of the signal from the more abundant one. This method was improved upon by Nier (13). McKinney et al (28) developed a new technique for the rapid comparison of two samples using electromagnetic valves. These two improvements eliminate many of the errors associated with variations of the source conditions and of the sample gas. A machine used for determining sulphur isotopes by this technique is described by Wanless and Thode (29). An accuracy of ± 0.02 per cent is obtainable in routine work.

The high precision now obtainable in the measurement of isotope abundances is also due to a large extent to the development of stable electronic circuits for detecting and amplifying the ion beam, regulated power supplies, and the replacement of galvanometers by recording potentiometers.

Since 1939 a large number of papers have appeared on the variation of isotope abundances in nature. Urey and his co-workers have studied the exchange equilibria of O^{18} between carbonate ion and water, and carbon dioxide and water (30). For example, in the equilibrium at 0° for the reaction,

$$\tfrac{1}{2}\, CO_2^{16} \text{ gas} + H_2O^{18} \text{ liq.} \longrightarrow \tfrac{1}{2}\, CO_2^{18} \text{ gas} + H_2O^{16} \text{ liq.}$$

the O^{18} concentrates in the CO_2 by an excess of 4.6 per cent. Urey

(31) has utilized the exchange equilibrium of O^{18} in the precipitation of carbonate ion to determine the temperature of sea water in remote geological times. Dole (32) has continued his extensive studies on O^{18} in the atmosphere and in the ocean (33).

Thode et al (34) found a 3.5 per cent variation in the isotopes of boron. This result is significant for nuclear physics since boron standards are used for comparing neutron capture cross sections.

Carbon Isotope Variations

Variations in carbon isotopes were first measured mass spectrometrically by Nier and Gulbranson (35) in 1939, and by Nier and Murphy (36) in 1941. Their results are given in terms of the ratio of C^{12} to C^{13}. The relative abundances can be measured more accurately than the absolute ratio of C^{12} to C^{13}. It is perhaps more convenient to refer the values to some arbitrary standard. Nier has determined the absolute C^{12}/C^{13} ratio very accurately (37), using a sample of Solenhofen limestone. His result agrees quite well with the value he originally gave for this sample in his 1941 paper. For our purposes, Nier and Murphy's results are listed as

$$\text{per cent } C^{13} \text{ depletion} = \frac{C^{12}/C^{13}\text{ sample} - C^{12}/C^{13}\text{ standard } 100}{C^{12}/C^{13}\text{ standard}}$$

where C^{12}/C^{13} standard = 88.89 as obtained by Craig. Nier's results can be converted to this scale from Craig's determination of Nier's Solenhofen limestone. The results of Wickman, Von Ubish and

Baertsche are also referred to in this manner, based on Craig's determination of the standards used by these workers.

Certain general trends are evident from their work. Limestones have a C^{13} depletion in the range -0.1 to +0.6. Organic sources, such as plants, petroleum and coal, range from 2.7 to 4.4. Igneous rocks, meteorites and diamonds form a loose group from 0.1 to 3.5. Some of Nier's results are listed in Table I.

Although there is a fairly wide range in each group, especially the third one, it would appear that organic carbon has a 3 per cent higher C^{13} depletion than limestone. However, it overlaps with the values from igneous sources. The general pattern has been confirmed by later investigators.

Wickman et al (38) have analyzed carbonate minerals. Their results fall in the range of from -0.9 to -0.1. Further work by Wickman and Von Ubish (39) shows that the carbonate minerals such as dolomite and magnesite have the same isotope range as the limestones from which they are derived.

Wickman and Blix (40) have studied limestones and graphites from the same locality. They find that for five pairs of samples the graphite is depleted in C^{13} by up to 1.5 per cent.

Wickman (41) has also analyzed over 100 plants of various species. They range from 1.1 to 2.8. Craig (42), in a broad study of carbon variations, has verified and extended the numbers obtained by Nier and Wickman. Craig's results were obtained on a simultaneous collection type machine (28). Twenty-one marine limestones fell in the range -0.2 to +0.4 (Table II). However, limestones in general show a range from -1.0 to +0.4. This range coincides fairly well with that found by Nier. Also Baertsche (43) has analyzed carbonate rocks. His results ranged from about -0.3 to +0.8.

According to Craig (42), land plants, wood and coal fall in the range 2.1 to 2.4, whereas marine plants and marine invertebrates are much lower, from 0.8 to 1.8.

Although the results of Wickman (41) and Craig agree as to the range of the depletion for plants, the authors place different interpretations on the data. Wickman postulates a local carbon cycle which adds an extra fractionation to the carbon ratio in regions where conditions are suitable. Desert plants and plants grown in very windy areas show a smaller depletion in C^{12}. Wickman supports this theory by data on four desert plants which are low in C^{13} and by rain forest trees which are high in this isotope. Craig (44) maintains there is no such correlation and that Wickman's mechanism will not work.

Igneous rocks appear to be about 2.5 per cent depleted in C^{13} with respect to average limestone. It is difficult to decide what the primordial ratio should be. The igneous rocks show roughly the same ratios as do organic carbon. The possibility that the low C^{13} value of igneous rocks is due to organic impurities must be considered, since the carbon content of the rocks is low (i.e. less than 0.1 per cent).

TABLE I*

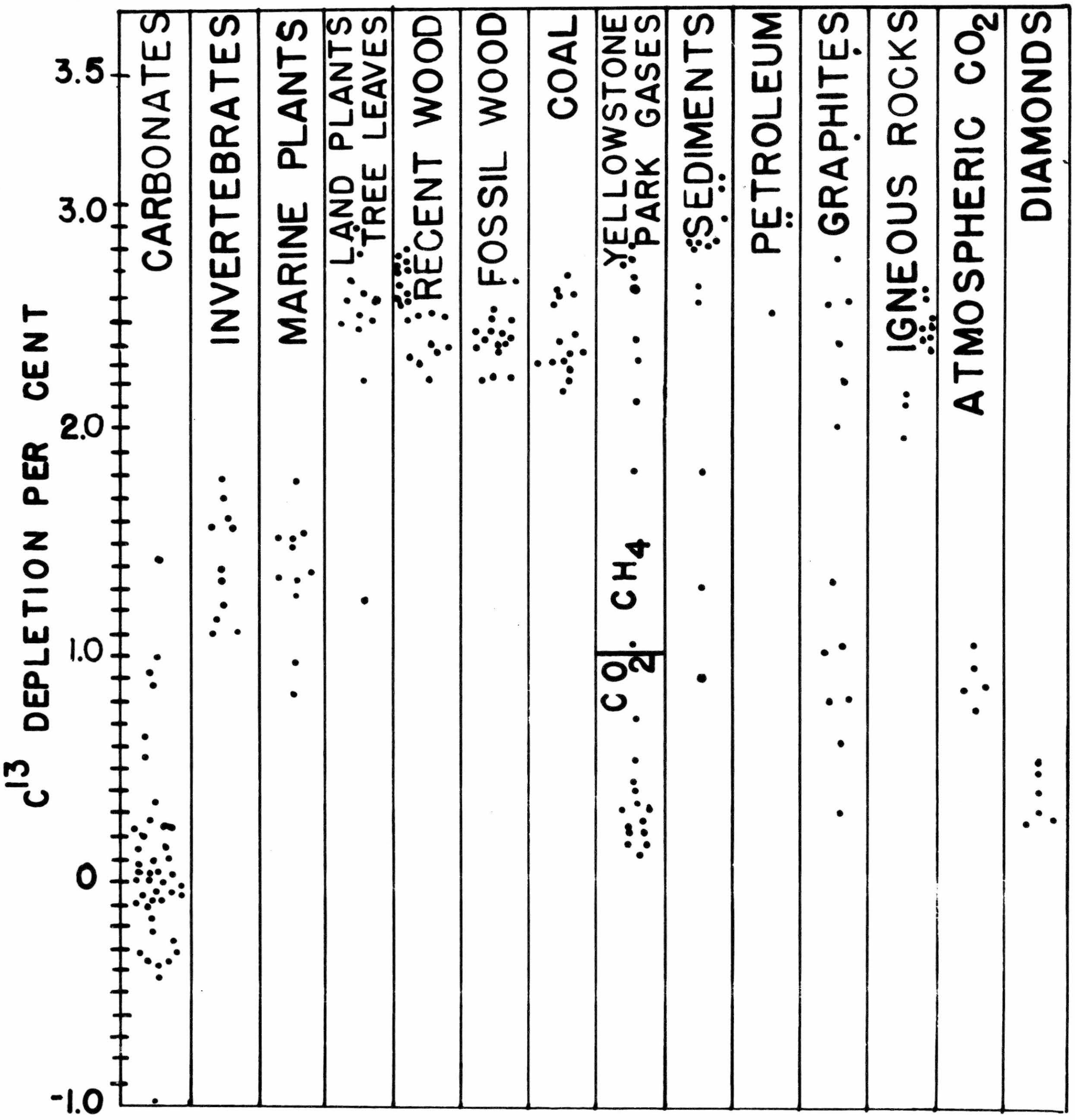
Carbon Isotope Distribution

ORIGIN	NO. OF SAMPLES	SOURCE	C^{13} DEPLETION %**	AVERAGE
Limestone	10	Limestones	-0.1 - +0.6	0.3
	10	Humic coal	2.7 - 3.5	3.3
Organic	7	Wood	3.0 - 3.7	3.3
	6	Petroleum	3.5 - 4.4	4.1
	2	Graphite	1.1 - 1.5	1.4
Igneous	7	Meteorites	1.0 - 3.5	2.7
	1	Diamonds	0.1	0.1

* From Nier, A. O. et al, J. A. C. S. 61, 697 (1939), and Phys. Rev. 59, 771 (1941).

** Values recorded are % differences from standard carbonate $\frac{C^{12}}{C^{13}} = 88.89$

TABLE II



(FROM CRAIG GEO. ET COSMO. ACTA
VOL. 3 60 1953)

Geologists at present do not agree on what represents true primordial terrestrial carbon or truly igneous carbon. In this connection graphites show a range of composition of 3.5 per cent which is considerably greater than any other group. This may be due to the derivation of some graphites from organic carbon while others come from carbonates. Such a hypothesis is supported by the results of Craig's analyses, which showed that graphites from schists are much lighter than graphites from gneisses and pegmatites. Organic carbon will react with both water and carbon dioxide at temperatures at which metamorphosis takes place, and the resulting isotopic composition of the graphite will depend on the temperature and the degree to which equilibria obtain among the intermediates.

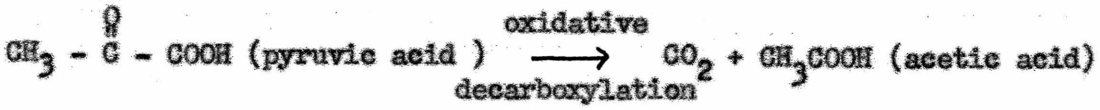
The possibility of obtaining very light samples by starting with organic carbon will be referred to later in this thesis.

Sulphur Isotopes and the Sulphur Wells

Thode and co-workers have published extensive investigations of the variation of sulphur isotopes. The range of values is about 9.0 per cent in the ratio of S^{34} to S^{32}. In general, sulphates are enriched in the heavy isotope, while sulphides and free sulphur are depleted in S^{34}. The ratio of S^{32}/S^{34} in meteoritic sulphur falls very close to the average value for terrestrial sulphur and is very nearly constant in all the samples studied to date. The results of 18 analyses of meteorites by Warren and Thode (45) were identical within the experimental error of the instrument, i.e. to 0.02 per cent. Thode, Kleerkoper and McElcheran (46) found a 1.0 per cent depletion of S^{34} in the hydrogen sulphide produced by the bacterial reduction of sulphate. Also, MacNamara and Thode (47) have studied the sulphur and sulphate associated in a lake in Africa in which bacterial action is known to take place. Their results show the sulphur to be depleted by 3.2 per cent in S^{34} as compared to the sulphate.

Thode, Wanless and Wallouch (48) have studied the abundance of S^{34} in native sulphur deposits in Texas and Louisiana salt domes. In these wells native sulphur is found in a matrix of calcitic limestone which usually lies between layers of gypsum and anhydrite, all of which form the cap rock of a salt dome. These salt domes are considered to be offshoots of bedded salt deposits formed and forced upwards by pressure in Pleistocene times. The anhydrite cap is of normal marine crystallization and is thought by some to have been forced up ahead of the salt. Small amounts of calcite and sulphur occur in the anhydrite but these decrease rapidly with depth. The free sulphur in the calcitic limestone was found to be depleted by 3.9 per cent on the average with respect to the sulphate. The sulphide showed an additional depletion of 1.0 per cent. These results, taken with those for the bacterial reduction of sulphate, support the hypothesis that bacteria have been responsible for the production of free sulphur in these deposits. The S^{32}/S^{34} ratio for the associated gypsum and anhydrite shows little evidence of sulphur isotope fractionation. Their ratios are higher and in the range of sea water sulphate. Large variations in the S^{32}/S^{34} ratio were found within a single drill core. This again points to local low temperature action, namely by bacteria. Further evidence for the bacterial hypothesis can be obtained from a study of the carbon isotopes of the calcite in which the free sulphur is embedded. The sulphate reducing bacteria (Vibrio desulphuricans) require a substrate containing some reduced carbon compound, which is oxidized to obtain free energy for the sulphate reduction.

In laboratory experiments, Vibrio desulphuricans can be grown on lactic or pyruvic acid. The substrate is oxidized to carbon dioxide and acetic acid. The carbon dioxide which is produced could conceivably form the secondary limestone which is found associated with the sulphur. The process could be summarized as follows:



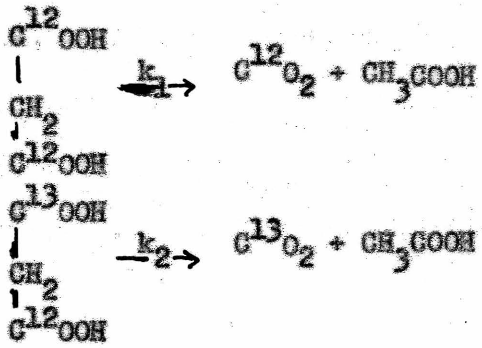
$$CH_3 - \overset{\overset{O}{\parallel}}{C} - COOH \text{ (pyruvic acid) } \xrightarrow[\text{decarboxylation}]{\text{oxidative}} CO_2 + CH_3COOH \text{ (acetic acid)}$$

$$CO_2 + H_2O + CaSO_4 \longrightarrow CaCO_3 + H_2SO_4$$

If so, this limestone will have a C^{12} content characteristic of organic carbon rather than that of ordinary limestone, i.e. it will be about 3.0 per cent depleted in C^{13}. Preliminary results obtained by Thode, Wanless and Wallouch (48) for the analysis of seven samples of carbonate from sulphur wells do indeed show a C^{13} depletion of 3.3 to 5.5 per cent over the value for the limestone standard used by Craig. The largest depletion for limestones reported by Craig is 1.4 per cent. The sulphur well limestones would appear to have had a different mode of formation or a different history from the limestones examined by all other investigators. A further study of these secondary limestones is reported in this thesis. The only result quoted by Craig in the range of these limestones is for a graphite schist of Laramie Range, Wyoming.

This investigation was undertaken to provide further information on the isotopic constitution of these wells. The C^{13} depletions of 3.3 to 5.0 per cent obtained by Thode et al (48) range up to a whole per cent above any natural carbon previously reported, so that some mechanism is necessary to account for this extra enrichment.

Isotope effects in unidirectional chemical reactions have been extensively investigated in the past few years. In the decarboxylation of acids a depletion of C^{13} in the initial fraction of the carbon dioxide has been found by several investigators. For example, the intermolecular isotope effect for malonic acid at 131° was found to be 1.034 by Lindsay, Bourns and Thode (49). The number quoted is the ratio of the rate constants k_1/k_2 of the reactions.



$$\begin{array}{l} C^{12}OOH \\ | \\ CH_2 \\ | \\ C^{12}OOH \end{array} \xrightarrow{k_1} C^{12}O_2 + CH_3COOH$$

$$\begin{array}{l} C^{13}OOH \\ | \\ CH_2 \\ | \\ C^{12}OOH \end{array} \xrightarrow{k_2} C^{13}O_2 + CH_3COOH$$

This deviation of the ratio of the rate constants from unity is referred to as an isotope effect and its physical significance is that the C^{12}/C^{13} ratio of the initial fraction of carbon dioxide liberated is 1.034 times that of the C^{12}/C^{13} ratio of the carbon in the carboxyl group of the acid. This mechanism might then account for the particularly light value of our carbon. In this connection, the isotope effect in the oxidative decarboxylation of pyruvate was measured chemically and also enzymatically using a culture of *Vibrio desulphuricans*.

Theoretical

Isotope fractionation can be brought about either by chemical equilibria which favour an isotope in a particular compound or by unidirectional processes.

(a) Isotope Exchange Reactions

Urey and Grieff (50) in 1934 showed that the equilibrium constants for isotope exchange reactions involving the light elements differ from unity and that therefore fractionation of the isotopes could be expected in equilibrium processes. Urey (51) in a general paper has presented a great many of these calculations.

Consider the isotope exchange reaction $aA_1 + bB_2 = aA_2 + bB_1$ (1) where the subscripts 1 and 2 refer to light and heavy isotopes present in the molecule. Then K, the equilibrium constant, is given by,

$$K = \left(\frac{A_2}{A_1}\right)^a \Big/ \left(\frac{B_2}{B_1}\right)^b \qquad (2)$$

From classical thermodynamics we get

$$RT \ln K = -\Delta F^o \qquad (3)$$

where F^o is the free energy of a constituent at unit pressure in its standard state.

The free energies can be evaluated from statistical mechanics:

$$F = E_o + RT \ln N - RT \ln Q \qquad (4)$$

where N = Avogadro's number

Q, the partition function, is given by

$$Q = \sum_i^{\infty} g_i \, e^{-E_i/kT}$$

g_i = degeneracy of the state with energy e_i

T = absolute temperature

k ~~Q~~ = Boltzmann's constant

ϵ_i = an energy state of the molecule

The summation is to be taken over all the energy states (translational, rotational, vibrational, electronic and spin).

E_o = zero point energy of the molecule

Substituting (4) in (3) and simplifying, we get

$$K = \left(\frac{Q_{A_2}}{Q_{A_1}}\right)^a \Big/ \left(\frac{Q_{B_2}}{Q_{B_1}}\right)^b \quad e^{\frac{-aE_{oA_2} - bE_{oB_1} + aE_{oA_1} + bE_{oB_2}}{RT}} \qquad (5)$$

In this case it is convenient to take the reference point for the energy as the minimum of the potential energy curve. We assume this curve is the same for isotopic species, so the exponential factor becomes unity and

$$K = \left(\frac{Q_{A_2}}{Q_{A_1}}\right)^a \Bigg/ \left(\frac{Q_{B_2}}{Q_{B_1}}\right)^b \tag{6}$$

In the case of an ideal diatomic molecule the Q's can be evaluated. We assume that there is no interaction between the various kinds of energy, so that the Q for the molecule is the product of the Q's for translational, rotational, vibrational, electronic and nuclear spin levels. Since nuclear spins are unchanged in a chemical reaction they can be neglected in calculating the partition function ratios.

The values for the translational energy levels (E_i) can be obtained by solving the Schrödinger equation for a particle in a box. Similarly, the rotational and vibrational levels are obtained from the solutions for the rigid rotator and the harmonic oscillator.

This gives, for the diatomic case,

$$\frac{Q_2}{Q_1} = \left(\frac{M_2}{M_1}\right)^{3/2} \left(\frac{I_2}{I_1}\right) \left[\frac{e^{-u_2/2}\,(1 - e^{-u_1})}{e^{-u_2/2}\,(1 - e^{-u_2})}\right] \frac{\sigma_1}{\sigma_2} \tag{7}$$

where u = fundamental vibration frequency

$$u_i = \frac{hcw_i}{kt}$$

1_1 and 1_2 are the moments of inertia

M_1 and M_2 are the molecular weights

The terms in the brackets arise from the translational, rotational and vibrational parts respectively. σ refers to the symmetry numbers. In diatomic and polyatomic molecules certain atoms may be identical. We define the symmetry number σ as the number of ways in which a molecule may be rotated into positions which would be different if the identical atoms were distinguishable. If there is a comparatively large number of rotational levels, then there will be only $\frac{1}{\sigma}$ as many states in the symmetric as in the non-symmetric molecule. Only $\frac{1}{\sigma}$ of the terms in the sum for the rotational partition function will appear, thus the rotational partition function must be divided by the symmetry number.

For a polyatomic molecule we have

$$\frac{Q_2}{Q_1} = \frac{\sigma_1}{\sigma_2} \left(\frac{A_2\ B_2\ C_2}{A_1\ B_1\ C_1}\right)^{1/2} \left(\frac{M_2}{M_1}\right)^{3/2} \prod_{i}^{3n-6} \left\{ \frac{e^{-u_{2_i}/2}\ (1-e^{-u_{1_i}})}{(1-e^{-u_{2_i}})\ e^{-u_{1_i}/2}} \right\} \quad (8)$$

where A, B and C are the principal moments of inertia. The product is taken over the degrees of vibrational freedom. The moments of inertia can be eliminated by a theorem due to Teller and Redlich which states, for the diatomic case,

$$\frac{I_2}{I_1}\left(\frac{M_2}{M_1}\right)^{3/2}\left(\frac{m_1}{m_2}\right)^{3/2n}\frac{u_1}{u_2}=1 \qquad (9)$$

We multiply equation (7) by $\left(\frac{m_1}{m_2}\right)^{3/2n}$, where m_1 and m_2 are the atomic weights of the isotopes being exchanged. The right side of equation (7) is multiplied and divided by u_1/u_2. We define new partition functions Q_2^* and Q_1^* so that

$$\frac{Q_2^*}{Q_1^*}=\left(\frac{m_1}{m_2}\right)^{3/2}\frac{Q_2}{Q_1} \qquad \therefore \frac{Q_2^*}{Q_1^*}=\frac{o_1}{o_2}\,\frac{u_2}{u_1}\,\frac{e^{-u_2/2}}{1-e^{-u_2}}\,\frac{1-e^{-u_1}}{e^{-u_1/2}} \qquad (10)$$

The equilibrium constant can now be calculated from a knowledge of the fundamental vibration frequency only. Urey has carried out such calculations (51) and finds fairly good agreement with experiment.

(b) Unidirectional Processes

The theory has been put into its most satisfactory state by Bigeleisen and Mayer (52) and Bigeleisen (53). Consider a chemical reaction in which A_1,B,C.... are converted to A_2,B,C...., where A_1 and A_2 are isotopic molecules. The rate constants are given by the absolute rate theory,

$$k_1=\frac{K_1C_1^*}{C_{A_1}C_B\cdots\cdot}\left(\frac{kT}{2\pi m_1^*}\right)^{1/2}\frac{1}{\delta_1}$$

$$(11)$$

$$k_2=\frac{K_2C_2^*}{C_{A_2}C_B\cdots\cdot}\left(\frac{kT}{2\pi m_2^*}\right)^{1/2}\frac{1}{\delta_2}$$

as given by Gladstone, Laidler and Eyring (54), where

K = transmission co-efficient

C = concentration

$*$ = refers to activated complex

m^*= effective mass of the complex along the co-ordinate of decomposition. In this work, it is usually taken as the reduced mass of the atoms at the ends of the bond being broken.

δ = length of the top of the potential energy barrier.

For isotopic molecules $\delta_1/\delta_2 = 1$

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \; \frac{C_1^*}{C_2^*} \; \frac{C_{A_2}}{C_{A_1}} \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \qquad (12)$$

This can be rewritten:

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \; \frac{f}{f^*} \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \qquad (13)$$

$$\text{where } f = \frac{\sigma_1}{\sigma_2} \prod_{i}^{3n-6} \frac{u_i}{u_i + \Delta u_i} \; e^{\Delta u_i/2} \; \frac{\left[1-e^{-(u_i + \Delta u_i)}\right]}{(1-e^{-u_i})} \qquad (14)$$

which is just the function used in the previous section

$$u_i = h\nu_i/kT$$

$$u_i = \frac{h}{kT}\left[\nu_{i_1} - \nu_{i_2}\right]$$

A similar expression obtains for f^*.

If Δu_i is small, equation (13) can be written as:

$$\frac{k_1}{k_2} = \frac{\sigma_1 \sigma_2^*}{\sigma_2 \sigma_1^*} \quad \frac{K_1}{K_2} \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \left[1 + \sum_i^{3n-6} G(u_i) \Delta u_i - \sum_i^{3n^1-6} G(u_i^*) \Delta u_i^* \right] \quad (15)$$

where $G(u_i) = \frac{1}{2} - \frac{1}{u_i} + \frac{1}{e^{u_i}-1}$ and is referred to by Bigeleisen as the free energy function.

In this form the ratio of the rate constants can be determined if the vibrational frequencies of the molecule and complex are known. Since these are not usually known, some estimation must be made by theory. A guess must also be made as to the structure of the complex.

In a review paper (55), Bigeleisen discusses the agreement between theory and experiments up to that date (1952). In view of the approximations necessary to obtains a result, the agreement is quite good for C^{13} effects at least.

EXPERIMENTAL

Preparation of Carbon Dioxide Samples From Sulphur Well Carbonates

The rocks studied, calcitic limestones containing crystals of sulphur, occur as the cap rock of the salt domes of Louisiana and Texas. The samples were obtained from sulphur well drill cores. With the exception of wells 3 and 7, the samples used were small pieces of rock about 25 gm. in weight. The samples from wells 3 and 7 were already finely powdered. In general, two analyses were made on each well, using two separate pieces of rock from which to prepare the samples.

The material was first ground to a powder with a mortar and pestle and then extracted with carbon disulphide to remove the sulphur. The limestone was filtered off and washed with carbon disulphide, leaving a powder ranging in colour from grey to white. About 250 mg. of the powder samples was placed in a nichrome boat and roasted at 450° for one hour in a stream of nitrogen to remove organic material.

About 50 to 75 mg. of the sample was placed in a cup in flask A (see Fig. I), which contained 30 ml. of concentrated sulphuric acid. After the flask was pumped out for one hour to remove any gases present, the cup was rotated to place the sample in the sulphuric acid. Two samples which were prepared using decomposition times of 2½ and 24 hours gave identical isotope analyses. In view of this, 3 hours were allowed for the decomposition, and at

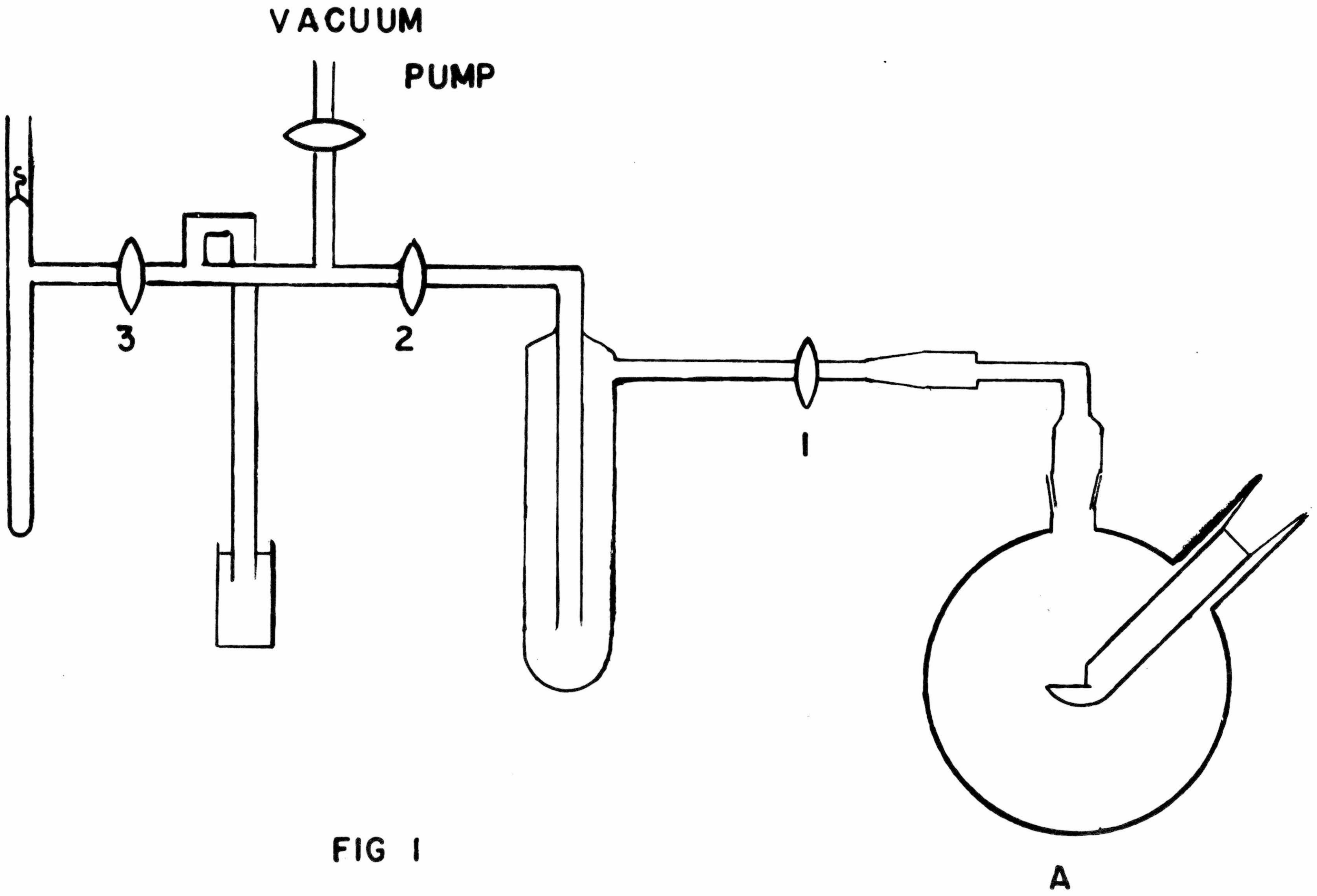


FIG I

the end of this period stop cock #2 was closed, liquid air was placed around the trap and the reaction flask was flamed gently to remove gases from the solution. Stop cock #1 was closed and the liquid air Dewar flask was replaced with a dry ice-acetone mixture and the carbon dioxide was allowed to distill into a sample tube immersed in liquid air so as to remove any water from the carbon dioxide samples.

One carbon dioxide sample was prepared using 100 per cent phosphoric acid and compared with a sample from the same source of rock made up using sulphuric acid. The isotope analyses were the same within the limits of the experimental error.

Samples which were inspected in the spectrometer did not show any impurities in the region adjacent to masses 44 and 45. This method of preparation was applied to all the rock samples with the exception of sample #3 from the Garden Island dome. It will be discussed in the next section.

Oxidative Decarboxylation of Pyruvic Acid

Attempts were made to purify the free acid as obtained from BDH by vacuum distillation, but it was found that the free acid would not keep pure, so it was converted to the sodium salt. The acid was dissolved in 10 volumes of 95 per cent ethanol and the solution with mechanical stirring was neutralized by the slow addition of a solution containing 1 volume of saturated sodium hydroxide in 10 volumes of 95 per cent ethanol. The white precipitate which formed was filtered and immediately redissolved in a minimum of hot water. The solution was cooled and one volume of cold 95 per cent ethanol was added to reprecipitate the pyruvate which was filtered off, dried, and stored in the refrigerator.

The decarboxylation was carried out in the apparatus shown in Figure II. The pyruvate was dissolved in a few millilitres of boiling water and placed in the reaction flask which was then immersed in a bath of boiling water. The system was flushed with carbon dioxide free nitrogen obtained by passing tank nitrogen through solutions of pyrogallol, concentrated sulphuric acid and potassium hydroxide for five minutes. A tenfold excess of sodium periodate in boiled water was added from the funnel, causing the evolution of carbon dioxide which was swept through a scrubbing column containing carbonate free sodium hydroxide. An additional trap containing potassium hydroxide was placed after the scrubbing column to be sure that no carbon dioxide could diffuse into the system.

Calvin's procedure (56) was used in the precipitation of the samples. The sodium hydroxide solution was prepared by adding sodium hydroxide pellets to water in a ratio of 1 to 1. Considerable heat was evolved which allowed the sodium hydroxide to dissolve. On cooling, some of the sodium hydroxide and most of the carbonate precipitated, and the solution was filtered on a large sintered crucible directly into the suction flask in which it was stored.

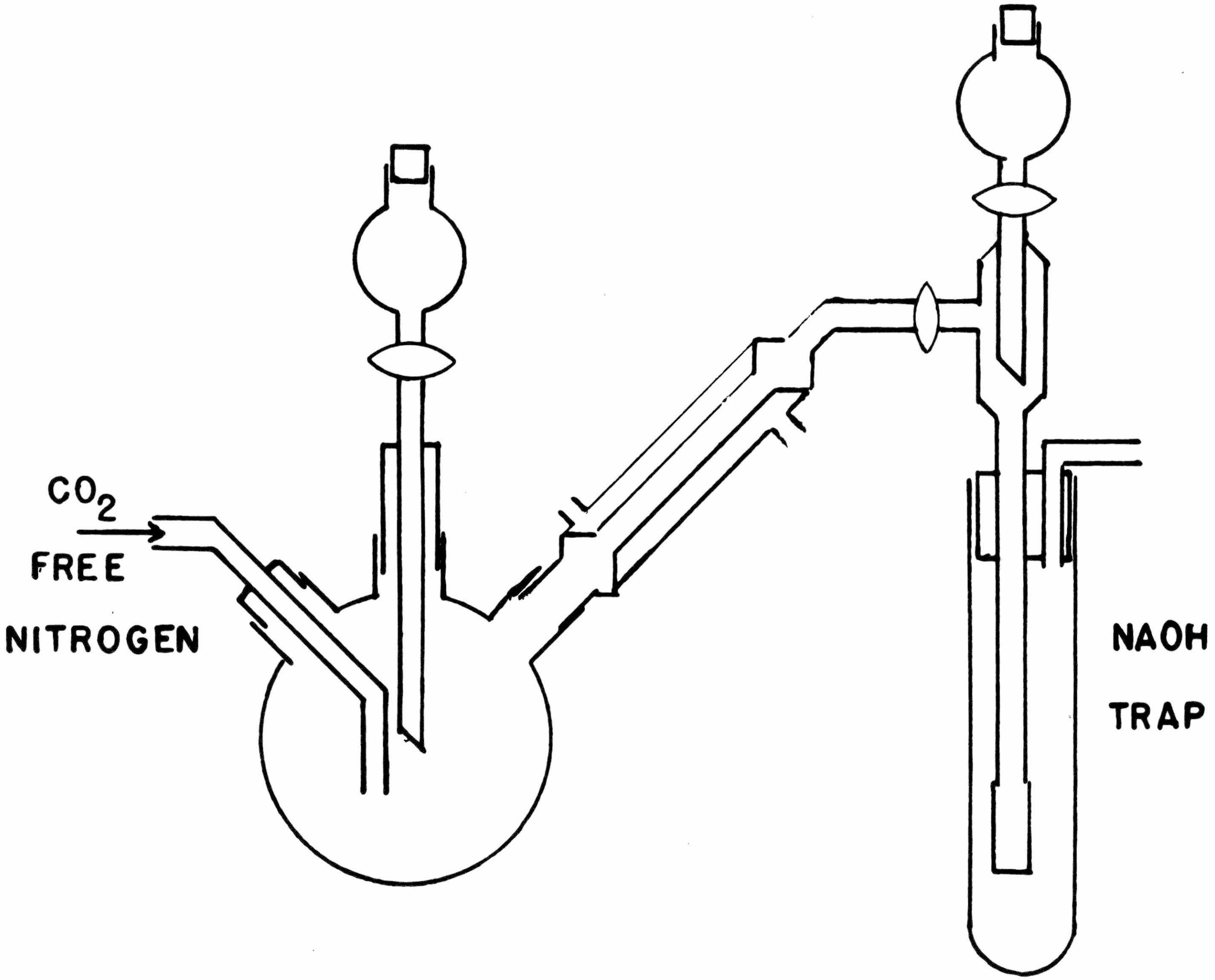


FIG II

When sodium hydroxide was removed for use, the air necessary to replace the solution was allowed to enter through the side arm via a drying tube. With these precautions, the carbonate blank was found to be about 1 mg./millimole of sodium hydroxide.

The carbon dioxide collected in the sodium hydroxide trap as sodium carbonate was transferred to a 250 ml. erlenmeyer flask. Any sodium hydroxide solution inside the scrubber was removed by forcing water though it. The sodium carbonate was precipitated by the addition of an equimolal amount of ammonium chloride and a twofold excess of barium chloride. The flask was then stoppered and the air above the solution removed by suction. After being allowed to stand for fifteen minutes, the precipitate was filtered on a large medium cintered glass funnel and washed with boiling water. The filtration and washing time was less than two minutes. The barium carbonate obtained in this manner was dried at 120° and the yield determined. It was converted to carbon dioxide in the same manner as the rock samples.

A blank run on this apparatus gave about 4 mg., which represented less than 3 per cent of the yield. For the complete decarboxylation, yields of 101 per cent to 105 per cent were obtained.

The sample of rock from Garden Island dome #3 was an anhydrite (calcium sulphate), containing less than 2 per cent calcium carbonate. It was prepared twice by the method described for the rock samples, but was found to be contaminated each time, yielding a very low C^{12}/C^{13} ratio. About 5 gm. of this rock was placed in the system used for the decarboxylations, 50 ml. of sulphuric acid was added from the dropping funnel, and the reaction was allowed to proceed for three hours. Only about 100 mg. of precipitate was obtained, so that the blank for this run was about 10 per cent.

Bacterial Carbon Dioxide

The *Vibrio desulphuricans* strain used in the other sulphur isotope experiments of Thode and co-workers was grown on a medium containing a known amount of pyruvate, plus inorganic salts. The bacteria were grown in a litre flask held in a constant temperature bath at 25°. The system was swept with carbon dioxide free nitrogen and the carbon dioxide which evolved was collected in sodium hydroxide and precipitated as before. The apparatus was identical with that used for the decarboxylation, except that the three-necked flask and condenser were replaced with a litre erlenmeyer flask containing the bacteria culture.

Mass Spectrometry

The mass spectrometer was a modification of the one described by Wanless and Thode (29). It was a conventional 90° instrument modified to permit simultaneous collection and measurement of two isotopes by a null method. An adjustable slit system enabled the collection of the common pairs of isotopes, 28/29, 44/45 and 64/66, corresponding to nitrogen carbon and sulphur analyses. The collector assembly was constructed so that only the ion currents of the masses in question struck the collector electrodes. The tube was stainless steel with end flanges cut to accommodate "O" ring gaskets of natural rubber. These flanges were provided with a water-cooling system to permit baking of the tube.

The ion current from the more abundant peak, denoted by i_1, is amplified by a DC amplifier and the output of this amplifier applied across a 10,000 ohm put and take potentiometer. The current i_2 from the other peak is amplified by a vibrating reed electrometer and this current is balanced by a fraction X of the voltage developed across the put and take potentiometer. Thus for a balance there is zero input to the vibrating reed. The output of the reed is fed into a Leeds and Northrup recorder. For zero signal input to the recorder the pen is set at the centre of the chart by a test voltage. With the system at balance the pen remains in the centre of the chart. Distance across the chart is expressed as an isotope ratio difference by moving the potentiometer off the balance value. A standard sample of carbon dioxide is used to provide the initial balance. The displacement obtained by changing X to some new value X^1 is measured. This distance is converted to an isotope ratio difference using the equation

$\frac{i_1}{i_2} = \frac{R_2}{R_1} \frac{1}{X}$ as given by Wanless and Thode.

$\frac{i_1}{i_2}$ = ratio of the standard gas

R_2 = cross-over resistor

R_1 = grid leak resistor of the DC amplifier

The new setting of the potentiometer X^1 would correspond with the hypothetical ratio

$${\frac{i_1}{i_2}}^1 = \frac{R_2}{R_1}\,\frac{1}{X^1}$$

$$\frac{\frac{i_1}{i_2} - {\frac{i_1}{i_2}}^1}{\frac{i_1}{i_2}} \text{ X } 100 = \frac{\frac{R_2}{R_1}\,\frac{1}{X} - \frac{R_2}{R_1}\,\frac{1}{X^1}}{\frac{R_2}{R_1}\,\frac{1}{X}} \text{ X } 100 = \frac{\frac{1}{X} - \frac{1}{X^1}}{\frac{1}{X}} \text{ X } 100$$

The left hand side is the per cent difference in isotope ratio with respect to the standard gas. This corresponds to a distance d on the recorder chart. The unknown sample is switched into the spectrometer using an electromagnetic valve, causing a new displacement of the chart d^1.

The isotope ratio difference of this sample from the standard is then $\frac{d^1}{d}\,\frac{\frac{1}{X} - \frac{1}{X^1}}{1/X} \text{ X } 100$ expressed as a per cent.

This method is not accurate if the sample differs by more than about one per cent from the standard. In this case the system must be rebalanced when the sample is added.

The time necessary for equilibrium to obtain when a new sample is switched in depends on the speed of the main pump and on the waste sample line pump, as well as on the size of the gas inlet lead. Using a large Martin mercury pump with a capacity of 67 litres per second, the time was 10 seconds, while with a smaller pump it was greatly increased.

It was not possible to remove completely the 44 and 45 residual peaks. This may have been due in part to the choice of gaskets. A small correction was made for the residual peaks. There was also a leakage through the valves of the order of one per cent; however, since all samples measured differed by 1.5 per cent or less in their isotope ratio, the correction for leakage was 0.03 per cent or less.

The reproducibility claimed for this instrument is ± 0.02 per cent. It depends on the actual peak heights used since this controls the sensitivity, i.e. the per cent isotope difference per centimetre displacement on the chart. In most of the work a reproducibility of this order was obtained. The decarboxylation samples were run using the slower pump. The sample pressures employed had to be reduced and the reproducibility was about ± 0.05 per cent.

In this work the abundances were determined from the 44/45 ratio, $\frac{44}{45} = \frac{C^{12}\,O^{16}\,O^{16}}{C^{13}\,O^{16}\,O^{16} + C^{12}\,O^{16}\,O^{17}}$

The O^{17} abundance determined by Nier (37) is 0.0374 per cent. A correction factor based on this value was applied to all values, namely that the measured values were multiplied by 1.067. (A similar correction was applied by Craig (42)).

RESULTS AND DISCUSSION

Results

Rock Analyses

The C^{12}/C^{13} ratios for six samples taken from five sulphur wells were reported by Thode, Wanless and Wallouch (48). These analyses were repeated and extended. Samples from twelve sulphur wells were analyzed and the results are shown in Table III. The results are tabulated as per cent C^{13} depletion over the limestone standard adopted by Craig (42). Craig gives this standard a value of 88.89 based on Nier's determination of Solenhofen limestone. None of Craig's standard was available, but barium carbonate provided by Wickman and marble provided by Baertsche were used as a reference. Both these standards have been analyzed by Craig so that our numbers were converted to Craig's values. A value of +1.11 per cent depletion was adopted for Wickman's carbon with respect to Craig's standard. Craig gives the value +1.07 per cent for this sample (57).

To determine the reproducibility of the instrument, a sample was kept and re-run several times. The results are shown in Table IV. The reproducibility of a sample preparation was determined by making up two carbon dioxide samples from the same rock samples. The results are shown in Table V. It is seen that the values check about as closely as the reproducibility of the instrument will permit.

A series of samples was prepared from the #10 core to determine the variations through the limestone layer (Table VI). Considering also the data in Table III, only the #10 and #6 wells show an appreciable variation in isotope ratios.

In the preliminary work of Thode, Wanless and Wallouch, samples were prepared by a different technique involving conversion to $BaCO_3$ and reprecipitation. Samples of carbon dioxide were made from their $BaCO_3$ preparations and compared with samples from the respective wells prepared by the present technique. It is seen from the results given in Table VII that the differences between the two sets of values are no greater than the variations found between different samples from a particular sulphur well core.

The sulphur bearing limestone appears as part of a cap rock over the salt layers. Part of the limestone cap may be free from sulphur. Gypsum and anhydrite layers are usually found adjacent to the limestone layer. Limestone in these adjacent layers might be expected to have a high C^{13} depletion.

To determine whether this is the case, three core samples were obtained from two wells consisting of cores from barren limestone, sulphur bearing limestone and anhydrite. However, both the "barren" limestone and anhydrite showed traces of sulphur.

From the Garden Island dome three samples were prepared, one from each layer. From the Chacahoula dome two samples were prepared from each layer. Samples were taken from opposite ends of the cylindrical core samples, a distance of about 15 cm., to see if there was a variation across the core. In addition, a sample was made up from gypsum from #5 well. These results are shown in Table VIII. The results from Garden Island anhydrite appear anomalous. This sample was purified by precipitation as $BaCO_3$ and, due to the very low yield of carbon dioxide from this sample, it could be contaminated with up to 10 per cent of carbon dioxide from the atmosphere.

Decarboxylation of Pyruvic Acid

Three runs were made of the total decomposition of sodium pyruvate, allowing three-quarters of an hour for the reaction to go to completion. The yields and ratios are as shown in Table IX. For partial decomposition the reaction was allowed to go for three to five minutes. The yields and the ratios are as shown in Table IX.

Bacterial Carbon Dioxide

A sample of carbon dioxide from the bacteria culture, taken fifty-seven hours after the inoculation of the medium, was analyzed. The result appears in Table IX. The bacteria died and attempts to regrow them on pyruvic acid were unsuccessful.

TABLE III

Carbon Isotope Abundance Data For Texas and Louisiana Sulphur Wells

CORE NO.	LOCATION	SAMPLE NO.	C^{13} DEPLETION%*
1	Hoskins Mount, Brazoria Co., Texas	1	4.58[2]
		2	4.77
2	Clemens Dome, Brazoria Co., Texas	1	4.32
		2	4.29[2]
3	Starks Dome, Calcasieu Parish, Louisiana	1	4.83
		2	4.80
4	Piedras Pintas Dome, Duval Co., Texas	1	2.48[1]
		2	2.43
5	Long Point Dome, Ft. Bend Co., Texas	1	3.47
		2	3.47
6	Moss Bluff Dome, Liberty Co., Texas	1	3.35
		2	3.02
		3	3.03[2]
7	Boling Dome, Wharton Co., Texas	1	4.12[2]
		2	4.19
8	Lake Washington, Plaquenimes Parish, Louisiana	1	4.18
		2	4.20
9	Orchard Dome, Ft. Bend Co., Texas	1	4.18
		2	4.94
10	Spindletop Dome	1	4.80[3]
11	Garden Island Bay, Plaquenimes Parish, Louisiana	1	4.14
12	Chacahoula Dome, Lafourche Parish Louisiana	1	4.56
		2	4.56

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* Values recorded are % difference from standard carbonate $C^{12}/C^{13} = 88.89$

[1] Average of two runs

[2] Samples prepared by initial precipitation as barium carbonate, followed by decomposition in sulphuric acid

[3] Average of six samples

TABLE IV

Reproducibility of Spectrometer*

DATE OF DETERMINATION	C^{13} DEPLETION %**
May 23	4.99
" 26	4.98
" 29	4.97
June 3	5.00
" 11	4.99
" 24	4.98
" 25	4.94
	4.977 ± 0.013 (a.d.)

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* Source of sample - #10 well

** Values recorded are % differences from standard carbonate C^{12}/C^{13} = 88.89

TABLE V

Duplicate Preparations From Same Source

SOURCE	SAMPLE PREPARATION NO.	C^{13} DEPLETION %*
Well #2	1	4.30
	2	4.28
Well #4	1	2.49
	2	2.41
Well #9	1	3.93
	2	3.95
Well #10	1	4.99
	2	4.99
	3	4.98
Well #11 (barren limestone)	1	2.91
	2	2.97

---

* Values recorded are % differences from standard carbonate C^{12}/C^{13} = 88.89

TABLE VI

Variation in C^{13} Isotope Depletion in #10 Well Core*

TOTAL VARIATION	C^{13} DEPLETION %**
0.29%	4.99
	4.77
	4.95
	4.73
	4.75
	4.80
	4.68
	4.803

* Samples taken at random from rock about 10 cm. X 15 cm.

** Values recorded are % differences from standard carbonate C^{12}/C^{13} = 88.89

TABLE VII

Comparison of Results Obtained by
$BaCO_3$ Technique[1] and Roasting Technique[2]

SOURCE	C^{13} DEPLETION % PREVIOUS METHOD	C^{13} DEPLETION %* PRESENT METHOD
Well #1	4.58	4.77
Well #6	3.03	3.02 3.34
Well #7	4.12	4.19
Well #10	4.82	4.99 to 4.68

[1] Purification by initial precipitation as $BaCO_3$ followed by decomposition to CO_2 with sulphuric acid

[2] Purification by extracting with carbon disulphide, roasting and decomposing with sulphuric acid

* Values recorded are % differences from standard carbonate C^{12}/C^{13} = 88.89

TABLE VIII

Variation in C^{13} Concentration Between Sulphur Bearing Limestone and Carbonate in Adjacent Formation

SOURCE		SAMPLE NO.	C^{13} DEPLETION %
Well #11	Barren limestone 1867' to 1870'	1	2.89
	Sulphur bearing 1885' to 1888'	1	4.14
	Anhydrite 1912' to 1922'	1	4.45
Well #12	Barren limestone 2327' to 2337'	1 2	3.85 3.14
	Sulphur bearing 2367' to 2377'	1 2	4.58 4.58
	Anhydrite 2387' to 2397'	1 2	4.35 4.40
Well #5	Sulphur bearing 718'	1	3.77
	Gypsum 833'	1	3.28

TABLE IX

C^{12}/C^{13} Isotope Effect In the Decarboxylation of Pyruvic Acid

SAMPLE	YIELD	C^{12}/C^{13} RATIO*	R	RATIO OF RATE CONSTANTS k_1/k_2
Total decarboxylation	105.0%	91.83		
	101.0%	91.79		
	105.0%	91.89		
		91.837 ±0.04 (a.d.)		
Partial decarboxylation	12.6%	93.19	1.0147	1.0157
	19.4%	93.30	1.0159	1.0178
	12.8%	93.21	1.0150	1.0163
				1.0166 ±0.0008 (a.d.)
Bacterial decarboxylation	54.0%	92.74	1.00983	1.0150

* Referred to Craig's carbonated standard $C^{12}/C^{13} = 88.89$

$$R = \frac{C^{12}/C^{13} \text{ for partial decarboxylation}}{C^{12}/C^{13} \text{ for complete decarboxylation}}$$

Discussion

Rock Analyses

The sulphur bearing limestones vary considerably in their C^{13} depletion, showing a total spread of 2.8 per cent. The average depletion was 4.11 per cent. All the samples analyzed show a greater C^{13} depletion by 0.8 per cent or more than any limestones analyzed to date.

This fact points to a unique origin for this limestone. Indeed, carbonates which have been formed from the precipitation of carbonate ion from solution could not have such a large C^{13} depletion. Also within the #10 well for which a number of different samples were analyzed (see Table VI), there is a variation of about 0.3 per cent in the samples. Craig's (42) earlier studies on limestones from related sources do not show a comparable variation and none would be expected in the precipitation of carbonates from solution.

The work by Thode et al (48) on the sulphur compounds found in these wells indicates that the sulphur has been produced by the reduction of sulphate by bacterial action. Since this process is known to involve the production of carbon dioxide, the anomalous isotope depletion values for these limestones can be readily explained by supposing that they have been formed from carbon dioxide liberated in the decomposition of an organic substrate by these same bacteria. The organic origin of the carbon dioxide would lead us to expect the C^{13} depletion of the limestones to be of the order of 3 per cent in keeping with the values found by Craig for organic sources.

The C^{13} depletions for samples taken at different depths (see Table VIII) show a definite decrease in the barren limestone above the sulphur bearing layer. However, the depletions are still in the range of those found for organic carbon. A dilution of the carbonate by marine precipitation might be expected in the barren limestone region, and this would serve to lower the C^{13} depletion.

There appears to be no correlation between the carbon fractionation shown for these wells and the sulphur fractionation reported by Thode et al. This is not surprising since the controlling factor for the carbon isotope case is probably the presence of a large reservoir of organic material as a substrate, whereas for the sulphur case the ratio will depend on the amount of sulphate present, and these factors are unrelated -- the bacteria will not grow without both being present, of course -- as far as occurrence in nature.

Hanna (58) in discussing the origin of these wells believes that the salt originally came from a sedimentary salt series. It was later thrust up with respect to the surrounding sediments.

The leading theories for the origin of the cap rock are as follows:

1) It was deposited as part of the original salt series and was

pushed ahead of the salt to its present position.

2) Only the anhydrite was primary; the gypsum, limestone, et cetera are alteration products of the anhydrite.
3) The cap was precipitated on the salt core from intermingling of circulatory waters.
4) Anhydrite in the salt core has been concentrated at the periphery of the salt. This was brought about by the solution of large quantities of salt. The anhydrite was later altered to gypsum, limestone, et cetera.

Theories #1 and #2 are held to be unlikely since no mechanism is known by which a 900 feet thick slab of anhydrite could be lifted 20,000 to 30,000 feet through sediments by the salt. Theory #1 is discounted by our results since carbonate of this isotopic composition could not be obtained from sedimentation. Theory #3 is also discounted on the same basis. With regard to theory #4, the objections are raised that there is not enough anhydrite present in the salt core to account for the thickness of anhydrite and that a sufficient amount of circulating water is not available to remove the great amount of salt. However, water saturated with salt is found in connection with salt domes (59) and salt marshes are also found associated with many salt domes, indicating that a great deal of salt has been removed in solution.

Our results support the theory that the limestone was not obtained by precipitation from solution. The only origin seems to be via a transformation of the anhydrite. Most students of salt dome petrology have accepted the theory of alteration for the origin of the gypsum and limestone cap rock, and our isotope studies support this theory.

Decarboxylation of Pyruvic Acid

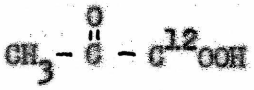
From the data in Table VII the isotope effect can be readily calculated:

$$\frac{k_1}{k_2} = \frac{\ln (1 - rf)}{\ln (1 - f)}$$

where r = ratio of C^{12}/C^{13} values for partial and total decomposition

f = yield for partial decomposition

k_1 = specific rate constant for the decarboxylation of



$$CH_3 - \overset{O}{\overset{\|}{C}} - C^{12}OOH$$

For the chemical reaction we obtain:

$$\frac{k_1}{k_2} = 1.0166$$

For the bacterial reaction we obtain:

$$\frac{k_1}{k_2} = 1.0150$$

Since the bacterial reaction is considerably more complex and the determination subject to larger errors, the check between the two results is fairly good.

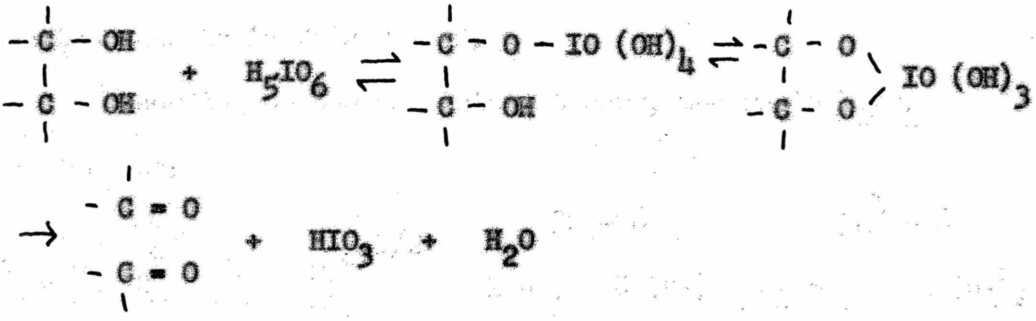
According to the Bigeleisen theory, the ratio k_1/k_2 is given by:

$$\frac{k_1}{k_2} = \frac{\sigma_1\ \sigma_2^*}{\sigma_2\ \sigma_1^*}\ \frac{K_1}{K_2}\ \left(\frac{m_2^*}{m_1^*}\right)^{1/2} \left[1 = \sum_i^{3n-6} G\,(u_i)\,\Delta\, u_i - \sum_i^{3n^1-6} G\,(u_i^*)\,\Delta\, u_i^*\right]$$

K_1/K_2, which is the ratio of the transmission coefficients, can be taken as unity. The term $\frac{\sigma_1\ \sigma_2^*}{\sigma_2\ \sigma_1^*}$ is also unity since there is no symmetry present. The term involving $G(u_i^*)$ is often set equal to zero when using this theory, since it depends on the force constant of the bond and as the activated complex is supposed to decompose as a result of the first vibration along the reaction co-ordinate the force constant must be very small. The minimum value of k_1/k_2 would be obtained if the term in $G(u_i)$ were also zero. In this case,

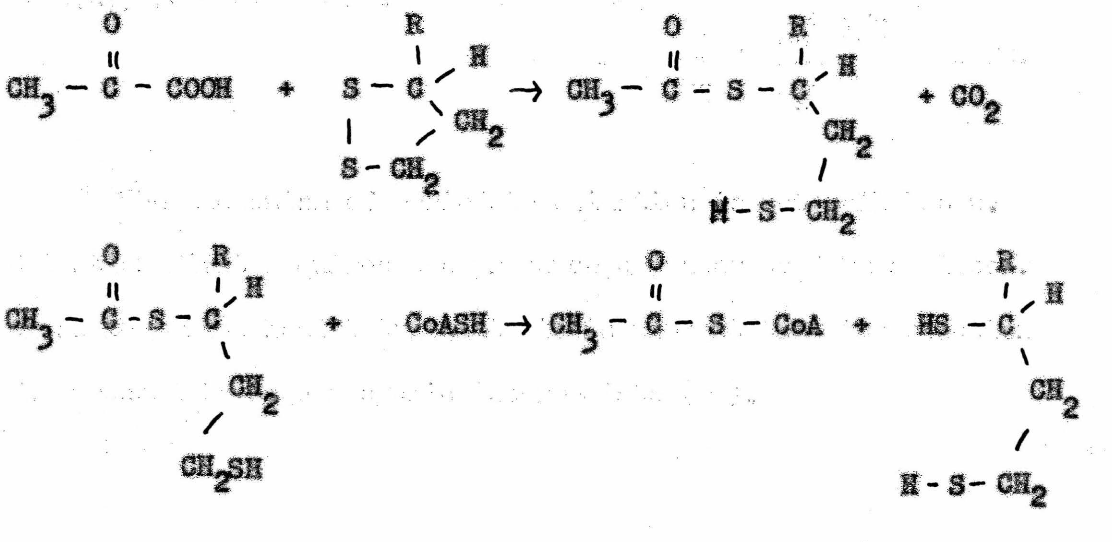
$$\frac{k_1}{k_2} = \frac{m_2^*}{m_1^*}^{1/2} = 1.020.$$

The mechanism of periodate oxidation is not well known. Oxidation first requires the presence of water to form a glycol. By analogy with lead tetracetate it is thought that the reaction may proceed through a cyclic intermediate (60).



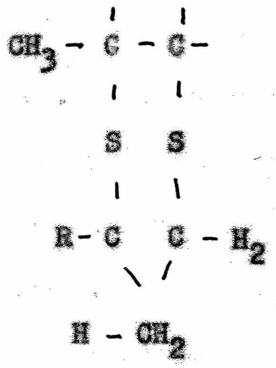
The first and second steps are equilibrium reactions so that no isotope effect is expected. The rate determining step involves a cyclic intermediate which would strain the carbon carbon bond and thus decrease the G(u) term so that a value close to 1.020 might be expected. However, it does not seem likely that the formation of such a cyclic intermediate would decrease the isotope effect to a value below 1.020.

In the biochemical case, the reaction of pyruvic acid in the Krebs cycle proceeds somewhat as follows (61):



CoASH stands for co-enzyme A. R stands for $(CH_2)_4$—COOH, the active agent being lipoic acid.

We cannot be sure that such a reaction would occur in anaerobic bacteria. The mechanism of the reaction is not known, but the products indicate it cannot be a simple bond-breaking reaction. Since the isotope effects are nearly the same, one might be tempted to postulate a cyclic intermediate.



The two sulphur atoms would act as the oxidizing agent in place of the two oxygen atoms in the previous reaction.

We notice that the value of the C^{12}/C^{13} ratio for the carboxyl group of pyruvic acid corresponds to a depletion of 3.00 per cent with respect to the standard limestone. If the hypothesis of bacterial action is correct, we would expect that due to a dilution with ordinary carbonate the depletion would range from a value below 3.00 per cent, but above ordinary carbonates to a value approaching 4.7 ± 0.1 per cent. That this is the case can readily be seen from the data. However, four analyses from #9 and #10 wells are just above this range.

The actual substrate for the bacteria need not be and most probably was not sodium pyruvate, so that we cannot expect complete agreement. However, our results indicate that it is possible to obtain carbon dioxide from the decomposition of organic matter which will have a C^{13} depletion as high as 5.0 per cent. This carbon dioxide could have formed the secondary limestone present in these wells by alteration of the $CaSO_4$, a proposal which is supported by geological evidence. It is difficult to imagine any process, other than the bacterial decomposition of organic matter, which could produce such a large C^{13} depletion.

SUMMARY

Carbon isotope ratios for twelve Louisiana and Texas sulphur wells were obtained. These ratios were all from 0.8 to 3.3 per cent higher than those of any other limestones reported to date.

It is believed that these carbonates were deposited from carbon dioxide evolved by sulphate reducing bacteria. The organic origin of the carbon dioxide would partly account for the high C^{12}/C^{13} ratios. However, an additional one or two per cent is necessary. It was thought possible that this could arise from a kinetic isotope effect in the production of the carbon dioxide in the decarboxylation of the substrate by the bacteria. The isotope effect for such a reaction was measured and found to be of the proper order or magnitude.

Further evidence for the hypothesis was obtained by analyses of the neighbouring rock layers. The rocks above the sulphur bearing layer were found to be 0.5 to 1.0 per cent lower in C^{12}/C^{13} but still in the range of organic carbon, as would be expected on this hypothesis.

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