THE FRACTIONATION OF SULFUR ISOTOPES IN THE PLANT METABOLISM OF SULFATES

By

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A Thesis

Submitted to the Faculty of Arts and Sciences

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

October, 1952

The author of this thesis holds the following degree:

Bachelor of Science, Honour Chemistry and Physics, 1949
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Scope and contents of this thesis:

The isotopic fractionation of sulfur in the plant metabolism of sulfate by mustard plants was investigated. A method of analysis of plant material suitable for this investigation was developed. An apparatus was devised to grow mustard plants by aqueous culture under sterile conditions.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. H.G. Thode and Dr. H. Kleerekoper for their help and direction in this work, and Dr. R.P. Graham and Dr. S. Kirkwood for their helpful discussions on the thesis. Also, we thank Mr. W.H. Fleming for assistance in the mass spectrometric analyses.

We wish to acknowledge financial assistance from the National Research Council of Canada.

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INTRODUCTION

Recent studies of the isotope abundances in nature suggest that sulfur isotope fractionation occurs in biological processes, and an attempt to elucidate the part played by plant metabolism of sulfur compounds is necessary.

The discovery of isotopes in 1910 has been attributed to Soddy [1] and independently to Fajans [2]. In the study of radioactive elements, it was found that elements could exist in several forms, identical chemically but differing in mass and radioactive properties. Soddy concluded that this occurrence of two or more species of the same element was common to the periodic classification, and he designated these species "isotopes" from the Greek word meaning "same place" with reference to the periodic table.

In 1914 Richards and Lembert [3] reported variations in the atomic weights of lead associated with ores of radioactive elements. Thus it was early known that the isotopes of radioactive origin varied in their abundance. Briscoe [4] in 1925 reported variations in the atomic weight of boron. This evidence was somewhat doubtful however, and considerable evidence was accumulated to show that isotopes had identical chemical properties and that their concentrations remained constant in nature.

The heavy isotopes of the lighter elements had escaped detection in early mass spectrographic work. However, in 1929 by means of molecular spectra, the heavy isotopes of oxygen were discovered [5] immediately followed by the discovery of the nitrogen and carbon isotopes [6,7]. Finally

in 1931, deuterium, the heavy hydrogen of mass 2, was discovered by Urey, Brickwedde, and Murphy [8]. They also discovered that the vapour pressures of deuterium and hydrogen differed when they separated deuterium from liquid hydrogen by fractional distillation. Later Washburn and Smith [9] separated the hydrogen isotopes by the distillation and the electrolysis of water.

In 1933 Urey and Rittenberg [10], using the methods of statistical mechanics calculated the equilibrium constants for deuterium - hydrogen exchange reactions:

$$H_2 + D_2 \rightleftharpoons 2HD \qquad (i)$$

$$HBr + D_2 \rightleftharpoons DBr + HD \qquad (ii)$$

$$HI + D_2 \rightleftharpoons DI + HD \qquad (iii)$$

which were experimentally confirmed [11 - 13]. Thus it was shown that the chemical and physical properties of the isotopes did differ.

The fact that the hydrogen isotopes differed markedly in their chemical properties suggested to Urey that the isotopes of other light elements might also differ in their chemical properties. Urey and Grief [14] studied isotope exchange reactions of the type

$$\tfrac{1}{2}CO_2^{16} + H_2O^{18} \rightleftharpoons \tfrac{1}{2}CO_2^{18} + H_2O^{16} \qquad K_{25^\circ C} = 1.046 \qquad (iv)$$

Equilibrium constants for exchanges involving lithium, boron, carbon, nitrogen and oxygen were calculated. The calculated equilibrium constant for reaction (iv) was found to be 1.046 at 25°C, indicating a four per cent enrichment of O^{18} in carbon dioxide over that in water. This result was experimentally confirmed by Weber, Wahl and Urey [15]. Other equilibrium constants for carbon, nitrogen and sulfur have also been experimentally confirmed [16 - 20].

From these studies, it was clear that the isotopes of the lighter elements do differ in their chemical properties and can be fractionated by chemical means. Material, highly enriched in C^{13}, N^{15}, or S^{34}, has been produced (16, 18, 21, 22) using isotopic exchange in equilibrium systems.

It has been well established in theory and by experiment that there is isotope fractionation in equilibrium processes, but this fractionation can occur in unidirectional processes as well. In 1934 Geroe and Schmidt (23) showed deuterides to be more stable than hydrides. Differences in the rates of reaction of deuterium-containing and hydrogen-containing molecules were reported (24 - 26). In 1948 Stevenson et al (27), studying the dissociation of propane-1-C^{13} by electron impact, reported a preferential rupture of the $C^{12} - C^{12}$ bond over the $C^{12} - C^{13}$ bond. The pyrolysis of the same material showed a similar result (28). Similar evidence was presented in the decomposition of oxalic acid (29), and in the decarboxylation of malonic acid reported by Bigeleison and Friedman (30), and Lindsay, Bourns and Thode (31). This confirmed earlier work by Yankwich and Calvin (32) who used malonic acid labelled with C^{14} in one carboxyl group to show that $C^{12} - C^{12}$ bonds were more easily ruptured than $C^{12} - C^{14}$ bonds. There is some theoretical basis for these results since theory predicts that the heavier isotope will form the stronger bond.

Stacey and Bourns (33), studying the deamination of phthalimide reported a nitrogen isotope effect. The results suggested that both the formation and rupture of the C - N bonds must be taken into account to explain the effect.

Since isotopes differ somewhat in their chemical properties we can

expect isotope fractionation in chemical processes both in the laboratory and in nature. However, early work showed little or no variation in isotopic abundances. Thus Urey and co-workers (34, 35), investigating the isotopic constitution of hydrogen, nitrogen and oxygen, reported no variation beyond the limits of experimental error, 10 per cent. However, Briscoe et al (36), with accurate density measurements, made a comprehensive survey on water from various sources. The variations in density they found were assumed to be due to variations in the isotopic constitution of hydrogen. This was the first report of any variations in the relative abundances of the isotopes.

But recent work has shown that the variations in the isotope abundances of the elements are more widespread. Thode, Macnamara and Collins (35) in a mass spectrometric survey of boron samples have found variations up to 3.5 per cent in the B^{10}/B^{11} ratio.

The reports of isotope fractionation in oxygen-exchange reactions suggested that slight differences in water densities, reported by Briscoe (36) might be attributed to variations in the abundances of oxygen isotopes as well. An extensive investigation of the isotopic constitution of oxygen in samples of air, water and minerals by Dole and co-workers (20, 38, 42), using a very sensitive density method, showed variations up to three per cent. Smith and Thode (43), by mass spectrometer study, have confirmed these results and found further variations up to four per cent. The results of these investigations are outlined below.

Table I

Distribution of O^{18} in Nature

	Source	Relative Abundance of O^{18}
Water:	Fresh water, Lake Ontario	1.00
	Ocean water	1.009
	Atmospheric water vapour over Lake Ontario	0.991
	Glacier water, Lake Louise	0.977
	Dead Sea water	1.020
Air:	Atmospheric O_2	1.033
		1.03
		1.03
	Atmospheric CO_2	1.040
Rocks:	Limestone	1.039
	Cuprite O_2	1.00
	Swedish Magnetite, Fe_2O_4	1.00
	Iron Ores	1.00
	Photosynthetic Oxygen	1.006

Although the oxygen of the atmosphere was found by Dole [38] and Vrooman [44] to be enriched about 3.1 per cent in O^{18} the cause is as yet uncertain. It is generally assumed that atmospheric oxygen is produced in photosynthesis according to the general reaction.

$$nCO_2 + nH_2O \longrightarrow (CH_2O)_n + O_2$$

Hence Dole [38] suggested that the atmospheric oxygen originated from the CO_2 oxygen during photosynthesis as the latter is normally enriched in O^{18} by four per cent (see equation (iv) above). But further work by Dole [42] showed that the evolved oxygen had an O^{18} content nearly the same as that of the water in the plant or nutrient solution. This was confirmed by Ruben et al [45] who showed, in tracer work with O^{18}, that the evolved oxygen did not come from CO_2. Some explanations [46, 47] have been advanced on the basis that atmospheric oxygen for the most part does not originate in photosynthesis. However, Kamen and Barker [48] have shown that oxygen is produced from plants at a rate sufficient to supply all the atmospheric oxygen every 2,000 years. They point out that the results are not necessarily contradictory but suggest as an explanation a molecular oxygen-water exchange:

$$\tfrac{1}{2}O_2{}^{16}\ (gas) + H_2O^{18}(gas) \rightleftharpoons \tfrac{1}{2}O_2{}^{18}(gas) + H_2O^{16}(gas) \qquad (v)$$

Although the exchange constants are 1.017 at 0°C and 1.014 at 25°C Urey [49] postulated the exchange occurring between molecular oxygen as ozone - formed by ultraviolet radiation - and water vapour at the low temperatures of the Upper Stratosphere. At these low temperatures the calculated equilibrium constant for this exchange is in accord with the observed enrichment factor.

The equilibrium constant for the isotopic exchange of oxygen when calcium carbonate crystallizes slowly from aqueous solution, i.e.,

$$1/3\ (CO_3{}^{16})^{=} + H_2O^{18} \rightleftharpoons 1/3\ (CO_3{}^{18})^{=} + H_2O^{16}$$

has been shown in theory and experiment to vary with temperature, with values of 1.036 at 0°C and 1.022 at 25°C. Hence the O^{18} content of calcium carbonate deposited by marine animals should vary with temperature. Urey et al [50]

predicted that a determination of the O^{18} content of the carbonate in fossils would give information on geological temperatures. Their results show that some measurements of past temperatures is possible with the " O^{18} thermometer ".

The determination of the O^{18} content of rocks has enabled Baertschi (51) to distinguish igneous rocks from meta-sedimentary rocks since sedimentary rock has a greater O^{18} content.

A systematic survey of variations in the isotopic constitution of carbon was completed by Nier and co-workers (18, 52). The results indicated a preferential abundance of C^{13} in limestone and other inorganic sources of carbon and the opposite for carbon of organic material. Rankama (50), using mass spectrometric analyses of Neir suggested that the original C^{12}/C^{13} ratio before or during early geological history was within the range of values for meteoritic carbon. The abundances of carbon isotopes in petroleum has been investigated by West (53). The results of these investigations are presented in Table II.

Table II

Relative Abundances of Carbon Isotopes in Nature

Source	Ratio C^{12}/C^{13}
Limestone and $CaCO_3$	87.9 - 89.5
Igneous Carbon (diamond, graphite calcite)	89.0 - 90.2
Carbon Dioxide (air and soil)	89.9 - 91.5
Meteoric Carbon	89.4 - 92.0
Animal Carbon	90.1 - 92.5
Bituminous Sediments	90.3 - 92.7
Vegetable Carbon	90.6 - 93.1
Petroleum	91.2 - 92.4

The high value of C^{12}/C^{13} ratios for organic material has been used by Rankama[55] to determine the organic origin of pre-Cambrian sediments and thus has suggested the earliest record of life to be 600 - 700 million years ago.

Urey[56] has found that some fractionation of the carbon isotopes occurs in plant photosynthesis. For example, algae, simple plants, contain 2.97 per cent less C^{13} than the carbonate of the nutrient in which they grew. He suggested that in photosynthesis reactions occur which favour the lighter isotope, and thus C^{12} was concentrated in the plant. This is in accord with the general distribution of C^{13} in nature.

There has been little investigation of the natural abundances of nitrogen isotopes. As mentioned above, Murphy and Urey[35] in 1932 could report only that the isotopic abundances of nitrogen were constant within ten per cent. But recently, Schoenheimer and Rittenberg[57] reported as high as 0.008 per cent excess N^{15} in amino acids as compared to atmospheric nitrogen.

Since differences in chemical properties of isotopes will depend on the percentage mass difference, one would expect little fractionation of the isotopes of the heavier elements in chemical processes. However, where there are a large number of isotopes with a widespread in mass, isotope fractionation is possible for many of the heavier elements. Recently, Graham, Macnamara, MacFarlane and Crocker[58] reported a mass spectrometric investigation of the isotopic constitution of germanium. The Ge^{70}/Ge^{76} ratios for four different samples showed variations up to 0.7 $\pm$ 0.01 per cent. Less accurate are the results of Duckworth and Hogg[59] who reported no

variation in Cu^{63}/Cu^{65} ratios within one-half per cent, the limit of precision on their double-focussing mass spectrograph. Similarly the results of Valley and Anderson[50] on the isotopic abundances of iron are not accurate as they could report only that there was no variation within a few per cent.

Variations in the Isotopic Constitution of Sulfur

The first isotopic abundance determination on sulfur was reported by Aston[61] in 1927 when he showed the presence of three isotopes in the proportions of 96 parts S^{32}, 1part S^{33} and 3 parts S^{34}. In 1938 Nier[62] reported the presence S^{36} in natural sulfur and gave the isotope concentrations, good to two per cent:

Mass	32	33	34	36
Concentration	95.1%	0.74%	4.2%	0.016%

In 1945, Thode, Graham and Ziegler[63] determined the equilibrium constants for the isotope exchange between SO_2 gas and bisulfite solution:

$$S^{34}O_2 + HS^{32}O_3^- \rightleftharpoons S^{32}O_2 + HS^{34}O_3^- \qquad K_{25^\circ C} = 1.019$$

$$S^{36}O_2 + HS^{32}O_3^- \rightleftharpoons S^{32}O_2 + HS^{36}O_3^- \qquad K_{25^\circ C} = 1.039$$

Previously, Thode, Gorham and Urey[22] had concentrated the heavy sulfur isotopes by chemical exchange methods. Hence variations in the isotopic constitution of sulfur seemed likely.

An extensive investigation of the isotopic constitution of sulfur has been reported by Thode and co-workers[64 - 72]. The results, reported as S^{32}/S^{34} ratios are summarized in a graph in figure 1[70]. Although

DISTRIBUTION OF S^{34} IN TERRESTRIAL AND METEORITIC SULFUR

S^{34}/S

21.60 21.80 22.00 22.20 22.40 22.60 22.80 23.00

FRESH WATER SULFATE

SEA WATER SULFATE

GYPSUM & ANHYDRITE

PYRITE MINERALS

19 SULFIDE ORES OF IGNEOUS ORIGIN

IGNEOUS ROCKS

12 SAMPLES OF METEORITIC SULFUR

VOLCANIC

ORGANIC

NATIVE SULFUR

ORGANIC SULFUR

SULFURETTED WATER (H_2S)

SEDIMENTARY SULFIDE

variations in the sulfur isotopes were reported as S^{32}/S^{34} ratios, Macnamara[70] has shown there are corresponding variations, according to the mass difference, in the isotopes of masses 33 and 36.

Table III

Comparison of S^{32}/S^{33} and S^{32}/S^{34} Ratios

Sample	S^{32}/S^{33}	S^{32}/S^{34}
1	127.3±0.1	22.66±0.02
2	125.2±0.1	21.93±0.02
Percentage Difference	1.68	3.33

A doubling of the mass difference results in a double change in the isotopic ratio. Although the S^{32}/S^{36} ratios were not measured because of the very low concentration of S^{36} (0.017 per cent) the variations were expected to double approximately those of the S^{32}/S^{34} ratios. This assumption is supported by the previously mentioned work of Thode, Graham and Ziegler [63].

From the graph in figure 1, it is seen that from the lowest value for sulfates to the highest for sedimentary sulfides represents an overall variation of about 8.6 per cent in S^{34}. Sulfates, whether as gypsum deposits or in solution were found[65] enriched in S^{34}. On the other hand sulfuretted water (water containing H_2S) was depleted in S^{34}. Later work[72] showed that sedimentary sulfides, with the widest range of values for any one source of sulfur, were also depleted in S^{34}. The sulfur of pyrite minerals was reported[65] to have widespread variations, embracing the mean isotopic abundance.

Macnamara and Thode[66] have found the isotopic constitution of meteoritic sulfur to be remarkably constant. Twelve samples were reported to have S^{32}/S^{34} ratios agreeing to 0.1 per cent. The samples were taken from siderite and stony meteorites, which are considered to represent the core and crust of a plant once existing in our solar system. It is also interesting to note that the S^{32}/S^{34} values for meteoritic sulfur lie midway in the range of values for terrestrial sulfur. These facts have lead Macnamara and Thode to suggest that the isotopic ratio obtained for meteoritic sulfur is the primordial value for the sulfur of our solar system, and that the values for terrestrial sulfurs above and below this base value have occurred through isotopic fractionation. They also suggested that the isotopic content of meteoritic sulfur might be taken as the basis for the absolute abundance of sulfur isotopes given in Table IV[66].

Table IV

Absolute Isotopic Abundance of Sulfur

Mass	Concentration (%)
32	95.018
33	0.750
34	4.215
36	0.017

Recent work in this laboratory[69] indicates that the S^{32}/S^{34} ratios obtained for sulfur of igneous origin are somewhat lower than those for meteoritic sulfur, although the values are fairly constant. However, with about 0.6 per cent variation, the spread of values for igneous rock sulfur does overlap the meteoritic value. The igneous sulfides show only a 0.3

per cent variation with two exceptions. Investigations of the isotopic abundance of native sulfur deposits show variations up to 4.5 per cent with free sulfur of organic origin depleted in S^{34} in contrast to that of volcanic origin.

In general the sulfates were enriched in S^{34} whereas sulfur of organic compounds was depleted in S^{34}. This enrichment in sulfates and depletion in organic sulfur parallels the results for the isotopic variation of carbon, wherein the organic carbon was depleted in the heavier isotope as compared to inorganic sources of carbon. Sulfides were also found to be depleted in S^{34}. Sea water sulfates were reported[67] to have a comparatively constant isotopic content, although definite differences were noted between samples from the Atlantic and Pacific Oceans. On the other hand gypsum deposits showed[67] variations as high as 2.5 per cent.

An interesting relation occurs between the fresh water sulfates and the hydrogen sulfide of sulfuretted water samples. Although these were present together in sulfur wells, the sulfate was enriched over the hydrogen sulfide in S^{34} from 2 - 4 per cent.[65] The results are listed below in Table V[65]. Also to be noted is that the free sulfur

Table V

A Comparison of Isotope Abundance Data for $SO_4^=$, S^o, and H_2S Present Together in Well Water

Sample	Dorchester, Ont.	Tillsonburg, Ont.	Port Ryersee Ont.	Port Stanley Ont.
	S^{32}/S^{34} Ratios			
H_2S	22.705±0.01	22.180±0.01	22.400±0.01	22.15±0.01

Table V (Cont'd.)

A Comparison of Isotope Abundance Data for $SO_4^=$, S^o, and H_2S Present Together in Well Water

Sample	Dorchester, Ont.	Tillsonburg, Ont.	Port Ryersee Ont.	Port Stanley Ont.
	S^{32}/S^{34} Ratios			
$SO_4^=$	21.735±0.01	21.715±0.01	21.650±0.01	21.585±0.01
S^o	22.705±0.005	22.285±0.01	-	-

present had the same isotopic content as the hydrogen sulfide.

Widespread variations in the isotopic abundance of pyrite sulfur was reported by Thode, Macnamara and Collins[65]. No correlation between the deposition age and isotopic content of samples was found. Although no general correlation was found between isotopic content and the crystallizing temperature of the pyrite, as measured by a pyrite geothermometer, definite correlation of these two factors was noted within single samples.

Natural Processes and the Sulfur Cycle

Undoubtedly the variations in the isotopic abundances of terrestrial sulfur has come about through isotope fractionation in geological or biological processes, since the calculations of Tudge and Thode[71] indicate that the isotopic compounds of sulfur do differ in their chemical properties. They calculated equilibrium constants for the exchanges

$$H_2S^{34}(g) + S^{32}O_4^= (sol'n) \rightleftharpoons H_2S^{32}(g) + S^{34}O_4^= (sol'n) \quad K = 1.074 \text{ at } 25^\circ C$$

$$H_2S^{34} + \tfrac{1}{2}S_2^{32} \rightleftharpoons H_2S^{32} + \tfrac{1}{2}S_2^{34} \quad K = 1.000$$

which indicates that variations up to 7 or 8 per cent can be expected in chemical exchanges involving the sulfur isotopes. The calculations also

indicate an enrichment in S^{34} in sulfate and a depletion in hydrogen sulfide or sulfur, thus accounting qualitatively for the distribution found.

However, it was necessary to find some mechanism whereby this exchange might take place in nature, since otherwise, conditions would be required both to reduce the sulfates and oxidize hydrogen sulfide. Further it is unlikely that sulfates could be reduced in nature by ordinary chemical agents. Thus Tudge and Thode [71] suggested that the "sulfur cycle" in nature provides the mechanism whereby these exchange processes occur. A diagram of the sulfur cycle, outlining the main processes involved, may be seen in Figure V.

It is known that sulfates are reduced to hydrogen sulfide by anaerobic bacteria in the muds at the bottoms of lakes and seas. Some of this hydrogen sulfide reacts with iron silicate to form pyrite and some escapes into the atmosphere to be oxidized to free sulfur and sulfuric acid. The pyrite sulfur may be converted into hydrogen sulfide by acids, or changed to sulfur dioxide by the heat and oxidizing conditions of volcanoes. The hydrogen sulfide can react with the sulfur dioxide at volcanic vents to produce free sulfur. Sulfur is also recycled through organic compounds by photosynthesis which uses sulfate or hydrogen sulfide, and by decomposition of organic compounds. In each of the reactions fractionation can occur over long periods of time. Furthermore as the sulfur is recycled among the various compounds considerable differences in isotopic abundances may be built up. Thus by means of the natural sulfur cycle it is possible to produce the variations in S^{34} that have been reported.

Because of this interchange of sulfur, it is suggested that many reactions might be unidirectional rather than equilibrium processes. This would explain why the variations reported do not agree quantitatively with

the calculated equilibrium constants for the above reactions. The slight fractionation for unidirectional processes could be additive, since the products of one reaction become the reactants of another reaction, resulting in widespread variations in isotopic distribution.

It would seem likely that the reduction of sulfate to hydrogen sulfide by anaerobic bacteria would be a unidirectional process. One would not expect that the oxidation of sulfide to sulfate would occur under the anaerobic conditions under which these bacteria grow. That is, these anaerobic bacteria would not have a functioning equilibrium system. There are other sulfur bacteria, some of which oxidize sulfide to free sulfur, and others which oxidize free sulfur to sulfate, and thus sulfur would be recycled in this part of the sulfur cycle. While investigating the possible mechanism for the bacterial reduction of sulfate to sulfide, Thode, Kleerekoper and McElcheran [68] reported that the hydrogen sulfide produced by the bacterial strain, Vibrio desulfuricans, was depleted in S^{34} by about one per cent. Although this is in the expected direction, the extent is not as great as expected from the variation between sulfate and hydrogen sulfide in sulfur wells. However, Macnamara and Thode [69] recently reported a 3.2 per cent fractionation in the same direction between sulfate and free sulfur in an African lake where the hydrogen sulfide produced by Vibrio desulfuricans is subsequently oxidized to free sulfur. The latter reaction involves no isotope fractionation (see above.)

A highly significant discovery has been made recently by Macnamara, Fleming and Thode [72] in the investigation of widespread variations in the isotopic content of sulfur in sedimentary sulfides. They found a definite correlation between the S^{34} content of the sedimentary sulfide and its geological age, with the lowest S^{34} content occurring in the most recent deposition. This was graphically illustrated as in Figure II [72]. Sedimentary

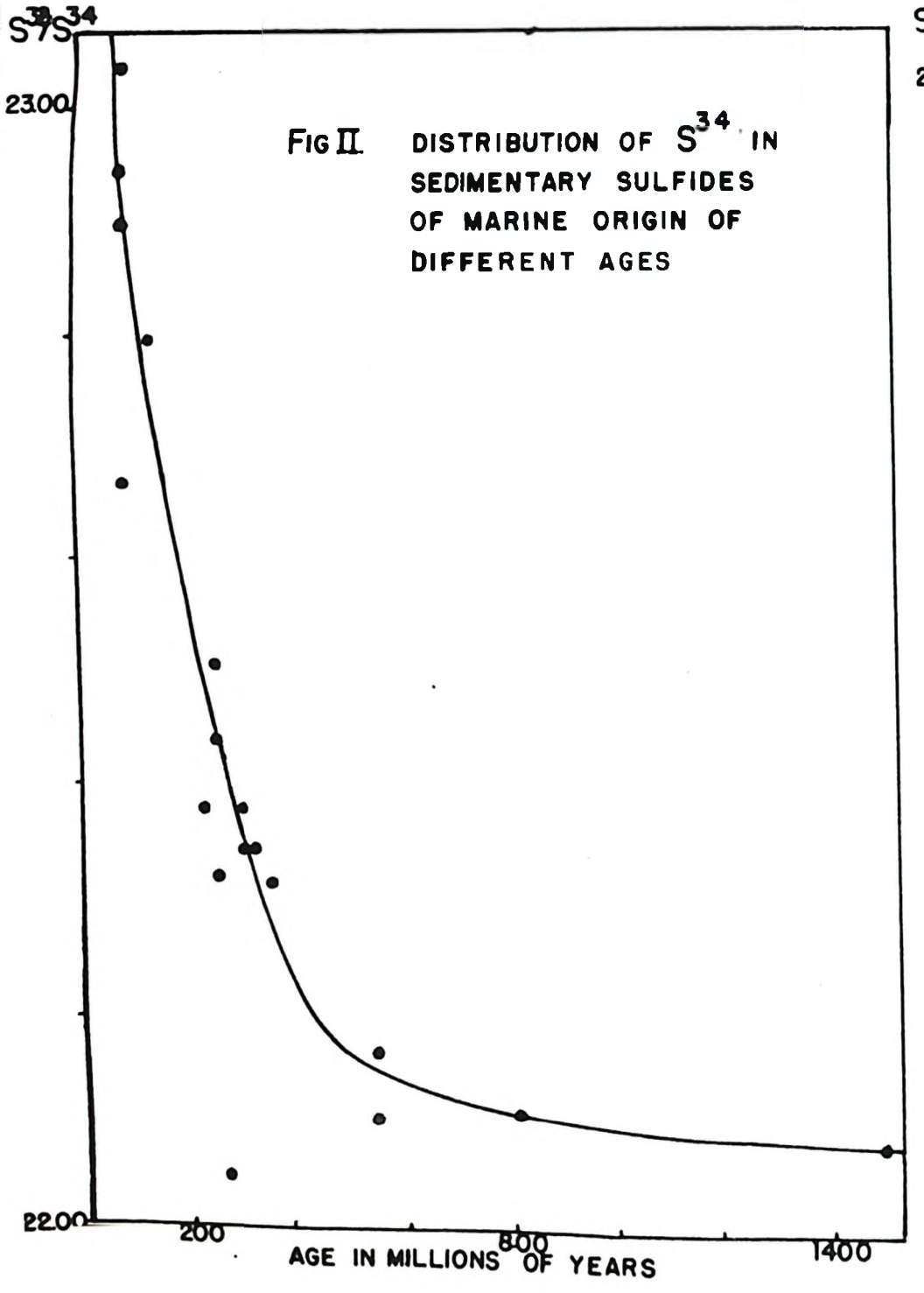

FIG II. DISTRIBUTION OF S^{34} IN SEDIMENTARY SULFIDES OF MARINE ORIGIN OF DIFFERENT AGES

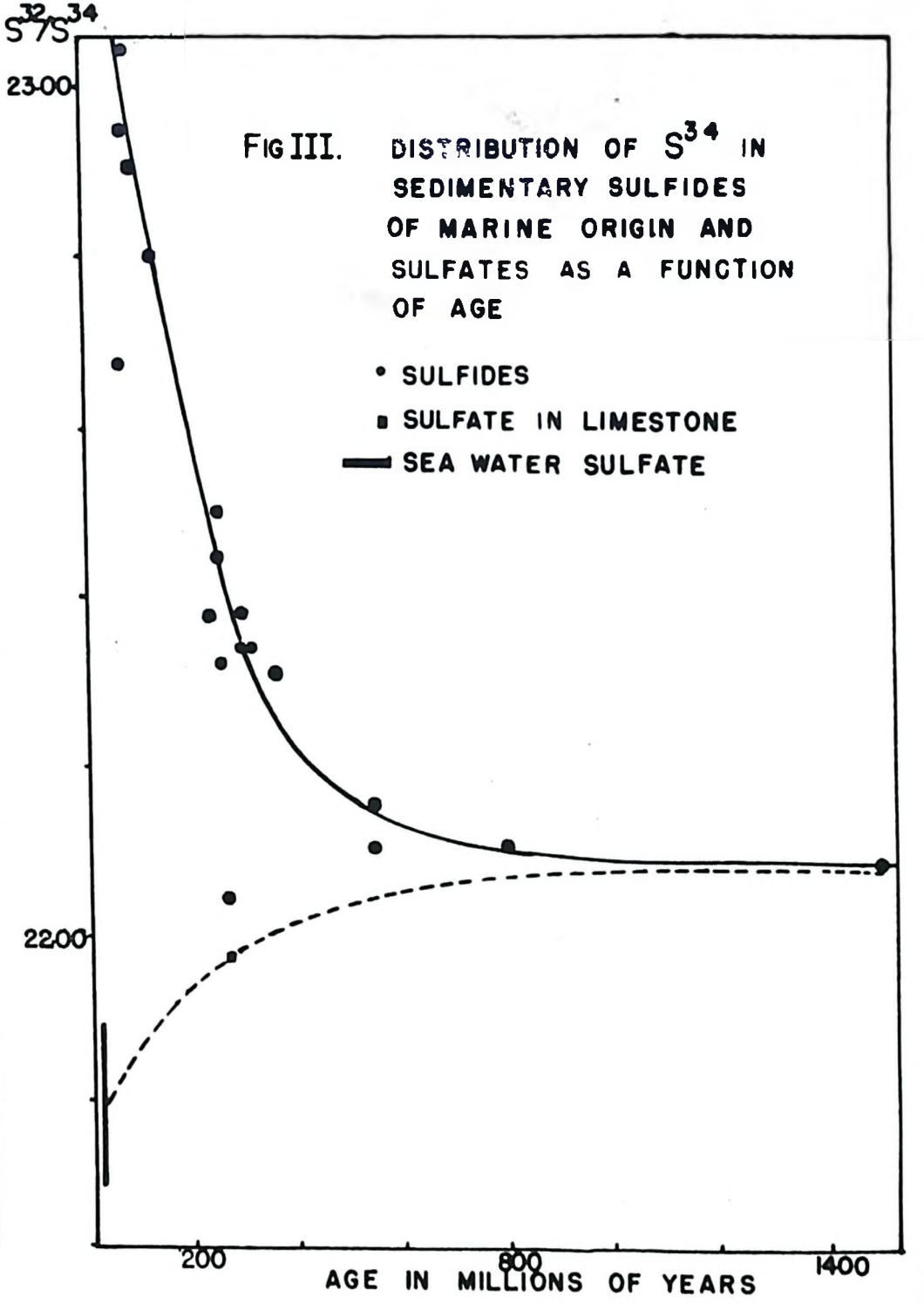

FIG III. DISTRIBUTION OF S^{34} IN SEDIMENTARY SULFIDES OF MARINE ORIGIN AND SULFATES AS A FUNCTION OF AGE

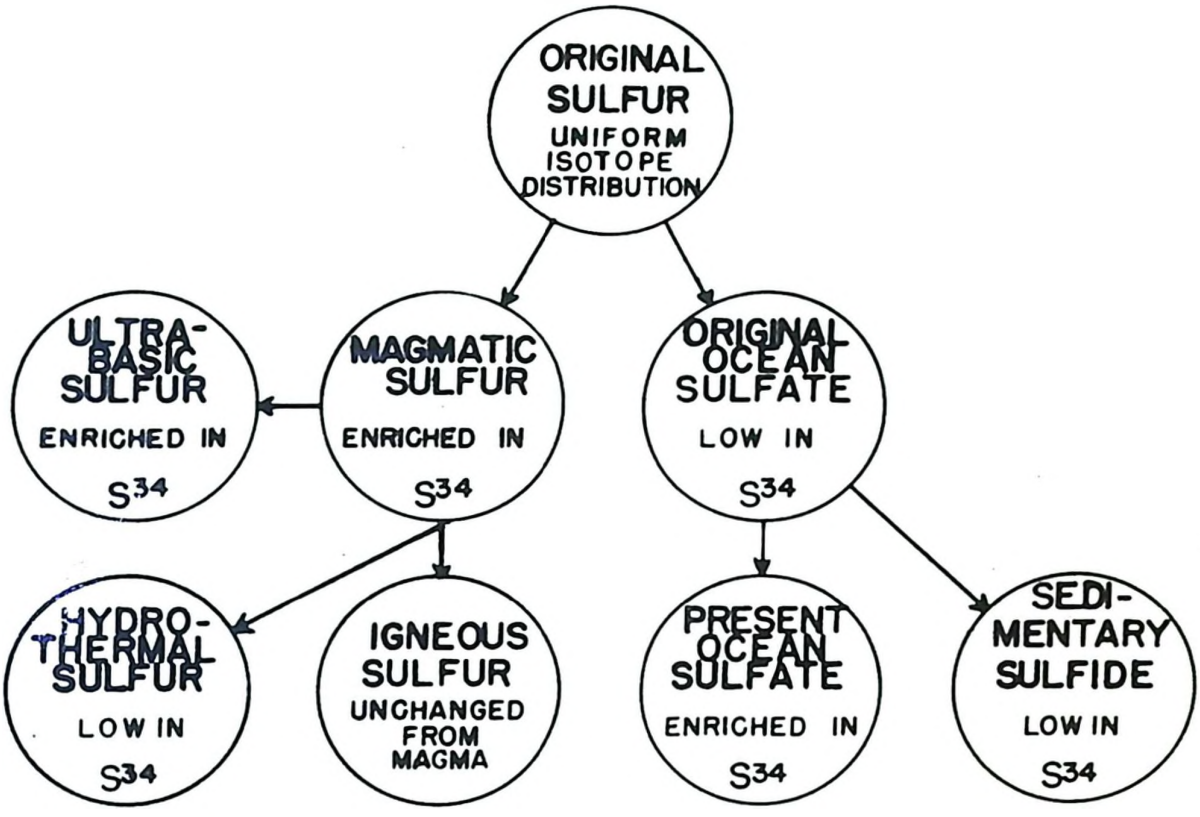

FIG. IV. NATURAL FRACTIONATION OF SULFUR

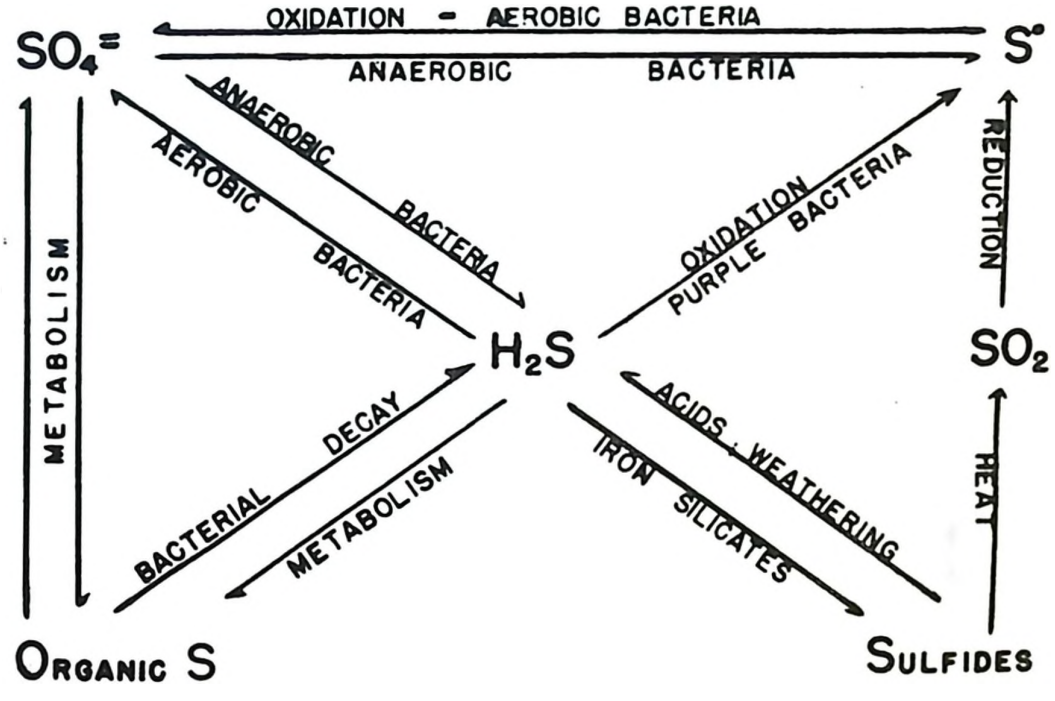

FIG. V. SULFUR CYCLE

sulfides are considered to be of marine origin, resulting from the reaction of iron silicates and the hydrogen sulfide produced in the bacterial reduction of sulfate. A relation between the sea water sulfates and the sulfides was graphically illustrated as in Figure III [72]. The graphs indicate that sulfur isotope fractionation in sedimentary sulfides dates from 600 - 700 million years. ago. If this fractionation has occurred through the sulfur cycle, then the results suggest that life processes began from this date.

It is to be expected that isotope fractionation of sulfur would take place in the geological processes occurring since the earth was formed. The kinds of sulfur produced in earth processes and the isotopic distribution of each kind, have been outlined in Figure IV [73]. This outline was based on the generally accepted hypothesis of the earth's origin and history. It was suggested [73] that as the gas mass condensed, volatile sulfur compounds, enriched in the lighter, more volatile isotopes, would remain in the atmosphere and the liquid magma would be enriched in S^{34}. The ultrabasic deposits, partly immiscible sulfides, in crystallizing from the cooling magma, would be somewhat enriched in heavier isotopes. Since the hydrothermal deposits are volatiles which were forced out of the magma by increased pressure during cooling, it is to be expected that they would be low in heavier isotopes.

Referring to Table VI [74], which lists the terrestrial distribution of sulfur it is seen at once that the weathered igneous rock could not have supplied the sulfur content of the oceans and the sedimentary rock. Thus it was suggested that this sulfur must have originated in the volatile sulfur compounds which remained in the earth's atmosphere and were carried down by condensing water vapour as the earth cooled. Although this indicates that originally the ocean sulfate was depleted in S^{34}, it is easy to suppose that through the sulfur cycle, sulfides were produced which were depleted in S^{34}

and consequently the sulfate became enriched in this isotope. The table also shows that the highly enriched source of sulfur, pyrite, salt deposits and native sulfur, contribute a negligible amount to the overall variation in isotopic abundance of terrestrial sulfur, and indicates that biological processes have played a predominant role in producing these variations.

Table VI

Terrestrial Distribution of Sulfur[≅]

Source	Av. Mass (kg/cm^2)	Av. S. Content (per cent)	Abundance of S (gm/cm^2)
Oceans	278.1	0.0884	246.0
Weathered Igneous Rock	160.0	0.052	83.0
Brosphere Salt Deposits	0.003	1.0	0.03
Sedmimentary Rock	155.0	0.26	420.0
Sulfide Deposits Mineral Water	negligible	40.0	negligible
Native Sulfur	negligible	90.0	negligible

≅ Only orders of magnitude certain

More recently Urey has suggested that life began on this planet under reducing conditions, that is, carbon in organic form, nitrogen as ammonia, and sulfur as sulfide. In other words, sulfate would not be present in the sea when life processes began on earth. This suggestion of course conflicts with the above explanation.

In view of the many variables in naturally occurring processes, it is important that each step in the sulfur cycle be studied under controlled laboratory conditions to determine the isotope fractionation that occurs under

different conditions. In this connection, the reduction of sulfate to hydrogen sulfide by anaerobic sulfur bacteria is being investigated. The metabolism of sulfur compounds in organisms discussed in the following section suggests a path for the plant metabolism of sulfate. The isotope fractionation in the synthesis of organic sulfur from sulfates in plants has been investigated and the results are reported in this thesis.

The Metabolism of Sulfur Compounds

Numerous investigations of the metabolism of sulfur in animal have been carried out. The subject has been excellently reviewed by Fromageot (75) and an outline of the metabolic path elucidated to date is shown in Figure VI. Very little has been reported on the plant metabolism of sulfur. The main path of plant metabolism is a process of reduction of the sulfur as compared to the oxidative path of animal metabolism. Biochemists are generally agreed that the main reactions of metabolism are the same for all life although exactly the same reactions will not necessarily be found in all organisms. In the evolution of more complex organisms the ability to perform some reactions may be lost. Thus the outline in Figure VI represents in reverse the metabolic path of sulfur in plants.

The important metabolic path of sulfur is:

Methionine ⟶ homocysteine ⟶ cystathionine ⟶ cysteine ⟶ cystine

This has been definitely established, and the enzyme systems have been isolated. The path is not completely reversible in every step in mammals but most bacteria have been found to reduce sulfur as easily as animals oxidise it. Although a probable path has been suggested, very little is known of the final oxidative steps. Steinberg (76) has shown that the assimilation of sulfur by plants, unless the compound is a metabolic product, occurs only

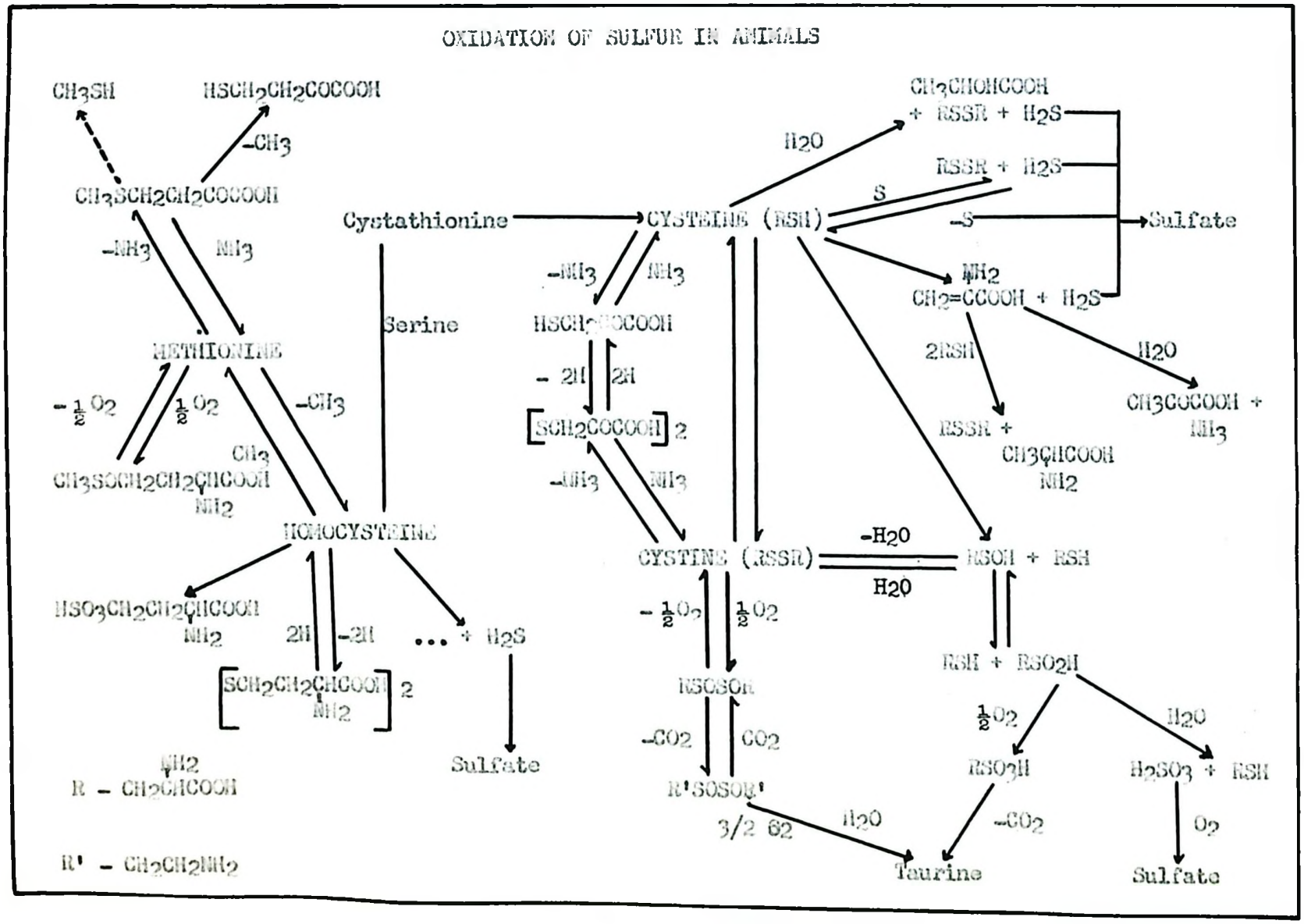
OXIDATION OF SULFUR IN ANIMALS
CH3SH
HSCH2CH2COCOOH
-CH3
CH3SCH2CH2COCOOH
-NH3
NH3
METHIONINE
-½O2
½O2
CH3SCH2CH2CHCOOH
NH2
-CH3
CH3
HOMOCYSTEINE
Cystathionine
Serine
HSO3CH2CH2CHCOOH
NH2
2H
-2H
SCH2CH2CHCOOH 2
NH2
... + H2S
Sulfate
NH2
R - CH2CHCOOH
R' - CH2CH2NH2
CH3CHOHCOOH
+ RSSR + H2S
H2O
RSSR + H2S
S
-S
Sulfate
CYSTEINE (RSH)
-NH3
NH3
HSCH2COCOOH
- 2H
2H
SCH2COCOOH 2
-NH3
NH3
NH2
CH2=CCOOH + H2S
2RSH
H2O
RSSR +
CH3CHCOOH
NH2
CH3COCOOH +
NH3
CYSTINE (RSSR)
-H2O
H2O
RSOH + RSH
- ½O2
½O2
RSOSOR
-CO2
CO2
R'SOSOR'
3/2 O2
H2O
Taurine
RSH + RSO2H
½O2
H2O
RSO3H
H2SO3 + RSH
-CO2
O2
Sulfate

Fig. VI.

when oxygen is present on an adjacent molecule or bound to the sulfur. Further, he found that sulfides and disulfides and mercaptans could not be assimilated, but that sulfonates and sulfinates were easily assimilated. This appears to be a confirmation of the above path, and it is expected that the plant reduction of sulfates will be reversal of the oxidative path.

The cleavage of thioethers, such as homocysteine and cystine, to produce H_2S hasrecently been established, which indicates a probable path for the reduction of sulfate to hydrogen sulfide by bacteria. Sulfate is successively reduced through the sulfonic and sulfinic acids to cysteine which may be further metabolized to other sulfur compounds or cleaved to produce hydrogen sulfide and alanine.

EXPERIMENTAL

A. AQUEOUS CULTURE OF MUSTARD PLANTS

Apparatus for Plant Culture:-

The apparatus used in the aqueous plant culture was designed to keep the nutrient solution sterile at all times. A diagram of the apparatus is presented in Figure VII. The section of the diagram in the upper half of Figure VII represents the purification assembly for compressed air. (A) was a mercury-filled pressure regulator and safety valve. The air passed through a bubbler (B) containing concentrated sulfuric acid, through a safety trap (C), and through a bubbler (D) containing sterile distilled water. The bubblers were equipped with fritted-glass disc which ensured small air bubbles with consequent efficient washing. From the bubblers the air passed to X and Y. At X the air, entering below the constriction in the U-tube, forced the solution up into the reservoir (E). In this way the nutrient solution was circulated through the apparatus, and the speed was easily controlled by the number of bubbles that were allowed to enter per unit time. Air was also admitted to the apparatus through Y and a fritted-glass disc. The air escaped from the apparatus through a side-arm in the reservoir. This side-arm had a sterile filter (F) consisting of a layer of cotton wool between two layers of glass wool.

The plants were grown in (G) which was made from Pyrex glass tubing, 75 mm. I.D., and had two longitudinal rows of holes, 1 cm in diameter. The tubing was filled with 3 mm. glass helixes to support the plant roots. A separate plant was placed in each hole. One end of the tubing was drawn down to a test-tube end and a U-tube joined at the lower edge. The other

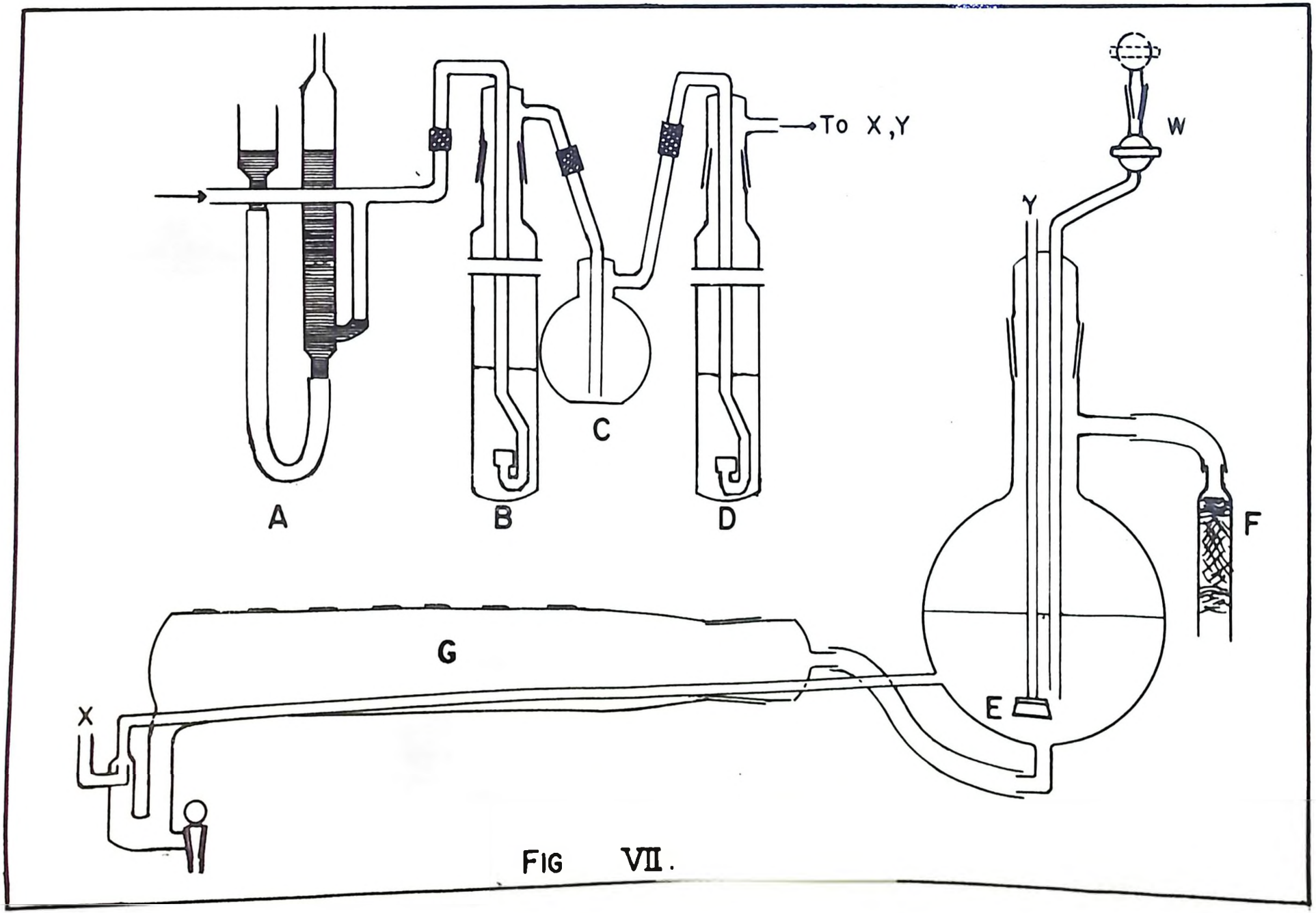

FIG VII.

end was joined to a ground-glass joint. The male section of this joint had a short length of 8 mm. tubing joined concentrically to it. This was connected by a piece of gum-rubber tubing (which is free of sulfur) to a similar piece of glass tubing joined to the bottom of the reservoir. The U-tube, which was constricted above the air entry to form the circulating pump, was incorporated to provide the necessary back pressure to prevent air from pushing back into C instead of lifting the water to the reservoir. At the bottom of the U-tube a stopcock was added to drain off the old nutrient, when fresh solution was added to the reservoir through glass tubing, W, which had a stopcock and a ground-glass joint.

This addition of fresh solution was accomplished without contamination by using the apparatus illustrated in Figure X. A 500-ml. Erlenmeyer was joined to a 24/40 ground glass joint. The corresponding female joint had a stopcock and another 7/25 ground-glass joint joined to it as shown. When the apparatus was assembled, air could enter through the side arm and long glass tube. This air vent had a filter of sterile absorbent cotton to decrease contamination by influxing air. Nutrient solution was autoclaved in the flask and after cooling the flask was inverted so that the small ground glass joint fitted into the joint at W. The surfaces above both stopcocks were thoroughly flamed before joining.

Before the final assembly of the shell (C), to the reservoir, the sections, with all holes containing cotton wool plugs, were autoclaved for two successive periods of two hours at a temperature of 105°C . The autoclave was allowed to cool slowly to minimize any contamination due to influxing air. All joints were flamed as the apparatus was assembled. Immediately before the apparatus was used, steam which had passed through a water trap

and a glass-wool filter, was passed through the assembled apparatus for several hours.

Illumination was furnished by four 150-watt "Daylight" and three 100-watt "Frosted" lamps. These were placed 18 inches from the apparatus. Lesser illumination was used at first which resulted in thin, long stems with a retarded development of leaves and branches. These symptoms are characteristic of plants receiving inadequate illumination. The illumination finally used was considered to be the minimum. Extra illumination from three 150-watt flood lamps was used for short intervals only, since sustained illumination from this source burned the plants.

Preparation of the Culture Solution:-

The water used in the culture solutions was prepared by passing distilled water through a bed of cation-exchange resin, converted to the hydrogen cycle by the action of dilute sulfuric acid on the sodium salt. The resin was Amberlite IR-120, manufactured by Rohm and Haas Company, Philadelphia, U.S.A., and supplied by Fisher Scientific Company, Toronto, Canada. Details of this operation - flow rates, concentration of the acid, column size, etc., - closely followed the suggestions of the manufacturer[77,78].

The nutrient solution used in the aqueous culture of the mustard plants, species Brassica rapa, was New Jersey No. 1 [79] with the following composition reported in Table VII. It has been recommended [79] that for physiological balance in the nutrient solution, trace elements should be supplied as H_3BO_4, $CuSO_4$, $ZnSO_4$ and $Fe_2(SO_4)_3$. Since experiments with seedlings seemed to indicate a better growth of the plants without these trace elements, they were omitted from the solution. In the actual culture of

Table VII

Chemical	Concentration in the Nutrient Sol'n.
$Ca(NO_3)_2$	0.736 gm. / 1.
$(NH_4)_2SO_4$	0.095 gm. / 1.
KH_2PO_4	0.312 gm. / 1.
$MgSO_4$	0.276 gm. / 1.

the plants, however, the absence of iron was injurious to plant growth. Iron was therefore included in the solution at a concentration of 0.5 mgm $Fe_2(SO_4)_3$ / litre of solution.

The nutrient solution was prepared by the dilution of a stock solution whose concentration of chemical was forty times that of the nutrient solution. A separate stock solution of $Ca(NO_3)_2$ was necessary because precipitation of calcium phosphates occurred in the stock solution when $Ca(NO_3)_2$ was included with the other chemicals. In preparation, stock solution of $Ca(NO_3)_2$ was added after the other stock solution had been diluted at least 30:1, followed by dilution to the concentration required for the nutrient solution. In this manner precipitation of calcium phosphates did not occur. After dilution the solution was adjusted to pH5.5 with dilute KOH or HNO_3 solution. It has been reported (80) that for optimum growth of mustard plants the pH should be 6. Too much precipitation in the solution occurred at this value but a solution of pH5.5 gave satisfactory results. The solution was autoclaved for 40 minutes at 105°C, and the autoclave allowed to cool gradually to prevent a sudden influx of air with resulting

contamination.

The nutrient solution was completely renewed every five days. The 'spent' solution was drained from the apparatus, and fresh solution introduced in the manner mentioned above. Additional solution was added daily to replenish the loss through evaporation, because a constant volume of liquid in the apparatus was desirable. The amount of additional solution was about 10 ml. per day.

Germination, Transfer and Growth of the Plants:-

Mustard seeds of the Brassica rapa species, kindly supplied by the R.T. French Co. Rochester, N.Y., were found to have a high percentage of germinations, and were used in this experiment. Disinfection of the seeds before germination was carried out as follows: The seeds were placed in 70 per cent alcohol (aq.) and gently agitated for fifteen minutes. This was followed by a similar washing with $\frac{1}{2}$ per cent aqueous solution of Hg_2Cl_2. The seeds were rinsed at least three times with sterilized distilled water, with suitable precautions to prevent contamination during the washings. The seeds were transferred with a spatula to Petri dishes containing a few layers of filter paper moistened with sterilized distilled water. The Petri dishes and filter paper were previously sterilized by heating in an oven at a temperature just below the charring point of paper. The dishes containing the seeds were placed in a warm moist atmosphere. Usually after one week the seeds had germinated and were ready to be transferred to the apparatus.

A cotton plug, which had prevented contamination after the apparatus had been autoclaved, was removed from one of the holes. The surrounding area was sterilized by swabbing with alcohol which was then ignited. The seedling

was wrapped in sterile absorbent cotton and tamped into the hole, with the root extending into the solution. In this way the cotton acted as a support for the seedling and prevented any contamination from the air. The absorbent cotton was fixed to the glass by a ring of Apiezon wax, a putty-like material that is chemically inert. The cotton plug was dampened with sterile water and surmounted by a small beaker during the first two or three days to minimize evaporation. Careful work resulted in "planting" without contamination.

In the course of the investigation it was found that the presence of consumer-gas was apparently very injurious to mustard plant growth, since, all other conditions being apparently identical, the plants grew only in a locale where there was no source of the gas. The plants reached a height of 15 inches in six weeks but no flowering occurred. The reason is not known although perhaps more illumination is required for the plants to produce flowers.

When the plants were harvested the tissue was very "wet", which is to be expected for this method of growing plants. There was a lack of mustard oil in these plants, since upon analysis the volatile sulfur component was very low in comparison with that of plants of the same species growing under field conditions. Whether the non-flowing of the plants was due to this, of this was due to the non-flowering, is not known.

B. ANALYSIS OF GREEN PLANT MATERIAL

The Volatile Sulfur Component

As early as 1914 Peterson [81] noted that some loss of volatile sulfur compounds occurred during oven-drying of green plant material. His method of drying plant material and determining the volatile sulfur component was used in this investigation. In this method, warm air is passed over the plant material and into an oxidizing furnace where the volatile sulfur compounds are oxidized over hot copper oxide to SO_2 and $CuSO_4$, and the SO_2 is trapped in a concentrated KOH solution.

A diagram of the apparatus for drying the plant material is illustrated in Figure VIII. All connections between pieces of the apparatus were made with Tygon tubing. Where rubber stoppers were used, their surfaces were covered with two or three coats of silicone resin, DC-804, supplied by Dow Corning Co., through Fiberglass Canada Limited. This resin was used to coat all connections to make them airtight. Air was drawn through the apparatus by suction from a water pump applied to the bubbler (F). The air was purified of any possible sulfur content by passage through the bubbler (A), containing 30 per cent aqueous KOH solution. The air was then dried by passage through concentrated sulfuric acid contained in bubbler (B). A fritted-glass dispenser was used in the caustic wash but it caused excessive foaming in the acid wash; glass tubing drawn down to a fine orifice was however satisfactory. A glass-wool filter (C) was placed between the acid wash and the drying-tube (D) to trap any acid spray.

The drying tube (D) was an elongated U-tube made from Pyrex glass tubing, 26 mm. I.D. It was immersed in a corn-oil bath which was heated by a blade-type heater and thermostatically controlled to the appropriate temperature. An electric stirrer kept the oil in motion and the temperature uniform to $\pm$ 1°C. The drying tube was connected by a piece of large-diameter glass tubing to the oxidizing furnace (E). From the furnace the air passed into the absorption bubbler (F), containing 30 per cent aqueous KOH solution. I n

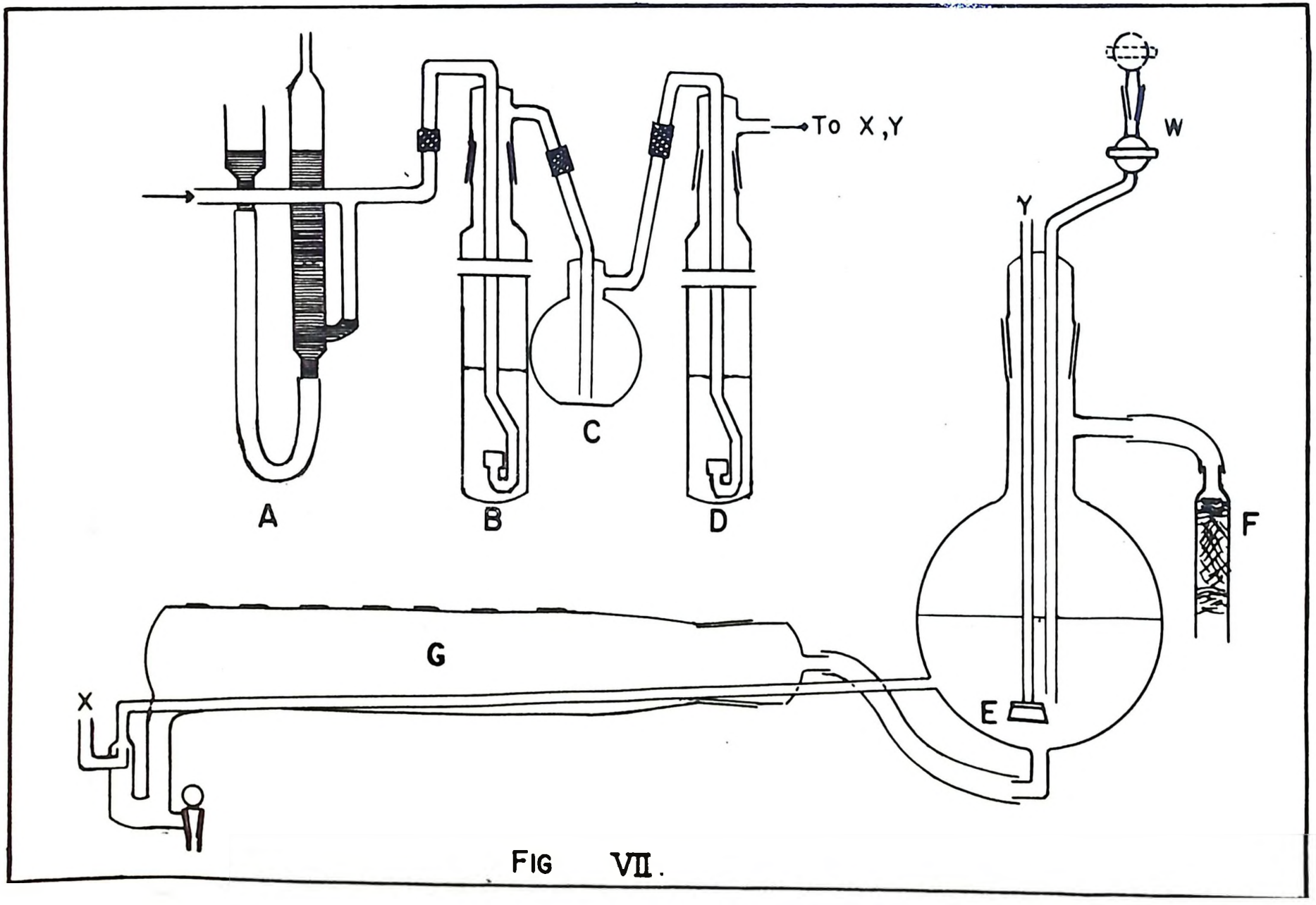

FIG VII.

this part of the apparatus it was advisable to have the ends of the glass tubing close together because the hot air from the furnace promoted the collapse of the Tygon tubing. The vapours from the furnace were condensed in the absorption bubbler (F) by surrounding it with ice. The non-condensable SO_2 reacted with the KOH to produce $KHSO_3$.

The furnace was made of a length of copper tubing with a Kovar seal (copper to glass) silver-soldered to each end. The copper tube was heated with Nichrome resistance wire wrapped evenly around the tube and insulated from the tube by a layer of asbestos paper. The wire was covered with several layers of asbestos paper and a final coat of 'asbestos mud' - a thick slurry of asbestos powder - which formed a hard surface after drying and heating. The heating element was connected to an auto-transformer (Variac), and a calibration of Variac readings versus temperature was made. Finely-divided copper oxide was placed in the copper tube.

The analysis: About 50 grams of finely-cut material were quickly macerated and transferred to the drying tube. The apparatus was assembled and tested for leaks. Leaks were indicated if bubbles appeared in (F) with suction applied and the air intake at (A) closed. After the leaks were eliminated, a flow of air was started which did not result in excessive frothing in the bubblers. The temperature of the furnace was raised to 690°C, and the temperature of the oil bath was kept at 30°C for two hours. It has been reported [82] that in one hour at 30°C the majority of allyl isothiocyanate (mustard oil) is cleaved from the glucoside sinigrin, by the enzyme myrosinase The temperature was then raised to 95-100°C until drying was complete (5 - 6 hours).

After the furnace had cooled the copper oxide was removed, and the

furnace and oxide rinsed two or three times with distilled water, combining the washings with the solution in the absorption tube (F). Contrary to Peterson's observations [81], no copper sulfate was ever detected. The resulting solution was filtered and evaporated, then carefully adjusted to pH 7 with hydrochloric acid. This solution was poured into saturated bromine water, and the excess Br_2 expelled by boiling. An excess of $BaCl_2$ solution was added to the hot solution with stirring and the precipitate of $BaSO_4$ allowed to digest overnight. The $BaSO_4$ was filtered from the hot mixture onto Whatman No. 42 filter paper.

The Separation of Sulfate and Protein Sulfur Compounds:-

The method of Balks and Wehrmann [83] was used to separate the sulfate from the protein sulfur component in the plant material. The protein sulfur was converted to sulfate according to the method of Bertrand and Silberstein [84]. Plant material was extracted with hot hydrochloric acid, and the sulfate already present in the plant material was precipitated with $BaCl_2$ solution. The filtrate from this precipitation and the residue from the extraction were digested with fuming nitric acid. After digestion the solution was evaporated to dryness, the mixture was fused with Na_2CO_3; the melt was taken up with hydrochloric acid and sulfur determined as $BaSO_4$. Although the procedures were more easily carried out with four-gram samples, larger samples could be analysed with the appropriate increase in quantities of reagents. In the analysis of any material which had a volatile sulfur content too low to permit the SO_2 prepared from it to be analysed mass spectrometrically, the total dry material was analysed and the $BaSO_4$ from the volatile sulfur was combined with that from the protein sulfur of the total dried material.

A mixture of 4 grams of dried material, 200 ml. of water and 10 ml. of 20 per cent hydrochloric acid was evaporated to 50 ml., then 200 ml. of 20 per cent hydrochloric acid and the evaporation to 50 ml. repeated. The mixture was filtered through an extraction thimble, and the filtrate diluted to 250 ml. The thimble was placed in a Sohxlet apparatus and extracted for 2½ - 3 hours with the filtrate. After extraction the solution was made distinctly acid with hydrochloric acid and 15 ml. of $BaCl_2$ solution (10 per cent $BaCl_2\ 2H_2O$) was added slowly with stirring to the hot solution. The precipitate stood overnight and then was filtered from the hot solution onto Whatman No. 42 filterpaper. This $BaSO_4$ came from the sulfate originally present in the plant.

The filtrate from the above $BaSO_4$ precipitation was made alkaline with 20 per cent NaOH solution. Solid Na_2CO_3 (anhydrous), in an amount sufficient to react with all the $BaCl_2$ which had been added, was added to the solution to precipitate the excess Ba as $BaCO_3$ (0.044 gm. Na_2CO_3 will precipitate the Ba^{++} in one millilitre of the $BaCl_2$ solution). The gelatinous and voluminous precipitate was filtered and washed thoroughly. To the filtrate was added 12 ml. of 30 per cent H_2O_2 and 8 ml. of NaOBr solution, and evaporation almost to dryness was carried out. Concentrated hydrochloric acid was very cautiously poured into the mixture until effervescence nearly ceased, and then the solution was evaporated to dryness. The plant residue from the hydrochloric acid extraction was added to this residue, and 25 ml. of fuming nitric acid cautiously added. After the initial foaming had ceased the mixture was heated gently over a Bunsen flame and 10 ml. of fuming nitric acid added. The solution was partly evaporated, then transferred to a porcelain casserole and evaporated to a pasty consistency. With the addition

of 15 ml. of 10 per cent Na_2CO_3 solution in 1 ml. increments effervescence had subsided, and 15 ml. more of the Na_2CO_3 solution were added.

The mixture was evaporated over a Bunsen flame with utmost caution since excessive foaming occurred and a voluminous crust formed. When the reaction had slowed the foaming ceased; the crust melted and the mixture became molten. Fusion was continued over a Meker flame until CO_2 no longer was evolved. The melt was cooled and taken up in 200 ml. of 1:4 hydrochloric acid, filtered, and $BaCl_2$ solution added to the hot filtrate with stirring. The $BaSO_4$ precipitate stood overnight and then was filtered from the hot solution onto Whatman No. 42 filter paper.

The Reduction of Barium Sulfate:-

In the investigation of the variations in the isotopic distribution of sulfur, it has been customary to use carbon (65) or iron (85) in the reduction of $BaSO_4$ to combustible sulfide.

A more efficient reduction of $BaSO_4$ with zinc dust however has been used in this investigation. Conversion of the sulfate to sulfide was found to be 80 per cent. The sulfate was well mixed with excess zinc dust, and the mixture gradually brought to the temperature of a Meker flame. It was heated at this temperature for one-half hour, and then gradually cooled. The mixture was treated with concentrated hydrochloric acid in a generator to produce hydrogen sulfide which was passed into a 1:2 mixture of molar lead acetate solution and saturated sodium acetate solution. This buffered solution prevented the precipitation of small amounts of lead chloride which were produced by HCl present in the evolving gases. The lead sulfide precipitate was washed twice with the saturated sodium acetate solution, then washed at

least three times with distilled water. It was filtered onto glass wool in a suitable length of 6 mm. glass tubing, and over-dried.

The Preparation of Sulfur Dioxide:-

The apparatus used to prepare SO_2 for mass spectrometric analyses is illistrated in Figure IX. The same source of oxygen of known isotopic abundance was used for the combustion of all sulfide samples. The oxygen was purified by passage over bone charcoal in trap (A) which was surrounded by dry ice. It was then dried by passage through concentrated sulfuric acid in trap (B). The purified oxygen passed over the lead sulfide in the tube (C). This tube was heated to the softening point of glass in such a manner that the glass did not collapse. The gases passed through P_2O_5 (D), which removed any water formed in the combustion, into trap (H). This trap was surrounded with liquid air which caused the condensation of sulfur dioxide and carbon dioxide. Non-condensable gases (oxygen) escaped through the mercury manometer (J). Fresh P_2O_5 was used in every combustion. The connections to the glass tubing were made with gum-rubber tubing.

After combustion, stopcock (E) was closed and the system evacuated through stopcock (F) to remove non-condensable gases. The liquid air around trap (H) was replaced with a dry ice - acetone mixture and the system again evacuated. In this manner the carbon dioxide impurity, always present in the sulfur dioxide, was removed, since it is gaseous at the temperature of the dry ice - acetone mixture. The vapour pressure of sulfur dioxide at this temperature was 5 mm. of mercury. Thus to prevent considerable loss of sulfur dioxide, the carbon dioxide was not completely removed. Carbon dioxide, in amounts up to five per cent of the sulfur dioxide, will not interfere, however, in the mass spectrometric analysis.

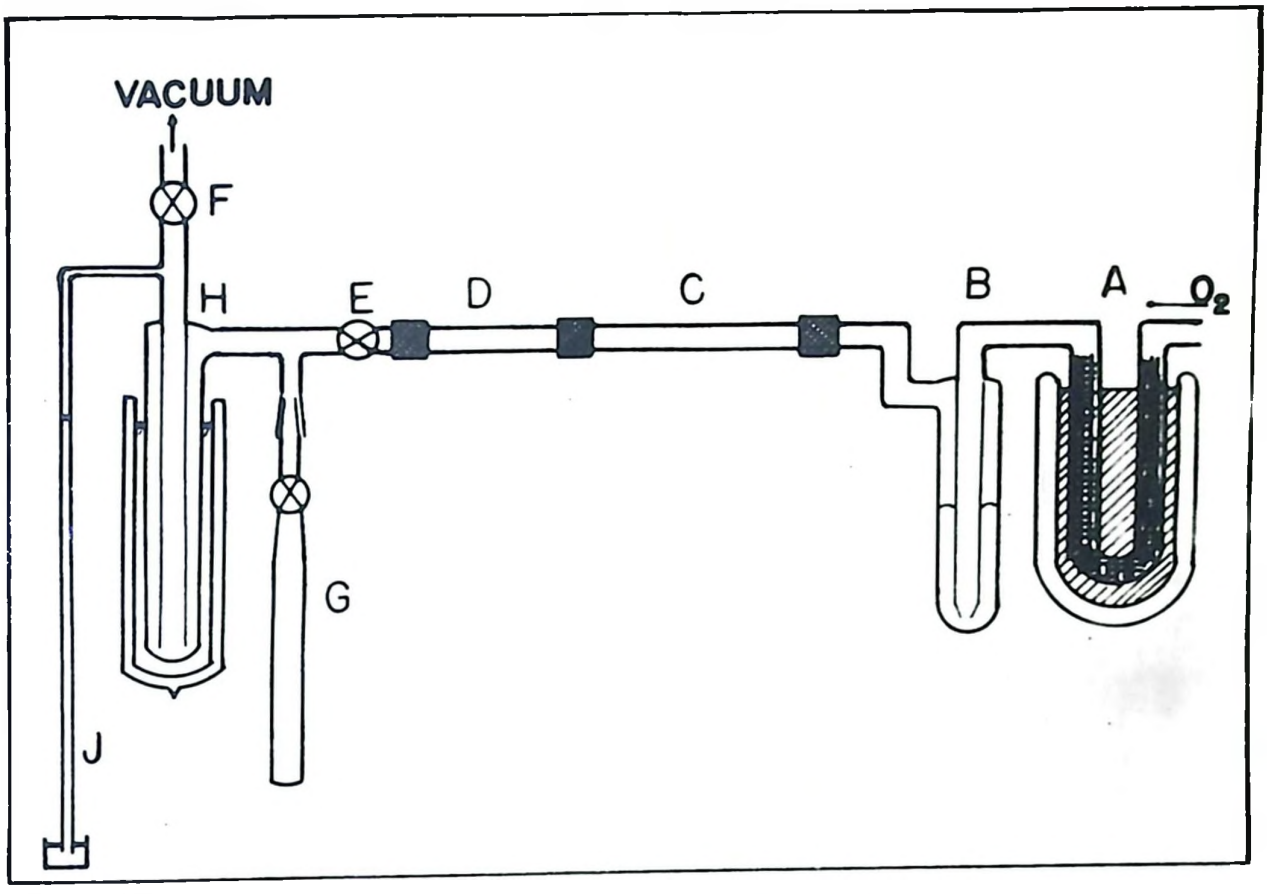

FIG IX. LEAD SULFIDE COMBUSTION TRAIN

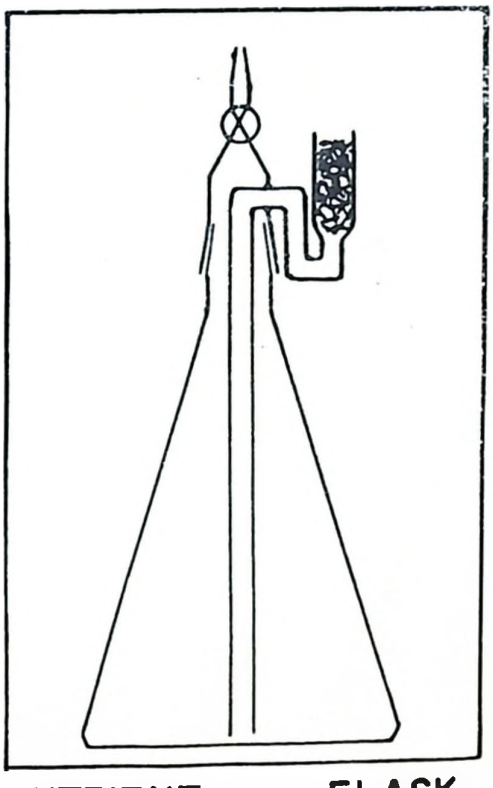

FIG X. NUTRIENT FLASK

In the elimination of carbon dioxide from small samples, the above procedure could not be used because of the vapour pressure of the sulfur dioxide at the dry ice - acetone temperature. A simple fractionation procedure was used: The liquid air was removed from trap (H). When the material warmed to the sublimation temperature of carbon dioxide, the system was evacuated for about five seconds, while the sulfur dioxide still remained a liquid. The material was frozen again by liquid air, and the procedure repeated. Usually three or four repetitions of this procedure sufficiently purified the sulfur dioxide for mass spectrometric analysis.

After the carbon dioxide had been removed, the sulfur dioxide was transferred to the sample tube (G) by surrounding the tube with the liquid air which was removed from around the trap (H). With the stopcock on the tube closed the sample could be removed for analysis.

The sample tube was replaced, and the line was heated while being evacuated. With fresh P_2O_5 and another lead sulfide sample in place, stopcock (E) was carefully opened with stopcock (F) closed. The liquid air was replaced around trap (H) and then another combustion could be started.

C. MASS SPECTROMETRY

The analyses of the sulfur dioxide samples for the isotopic ratios were accomplished with a 180-degree, direction-focussing mass spectrometer. This was a high-precision Nier-type instrument which has been previously described [63,86]. The direct-current amplifier and manual shunt selector were replaced by a vibrating reed electrometer (i). The isotopic ratios were determined from the measurements of the positive ion current in the SO_2^+ mass range.

With the electrometer amplifier set for highest sensitivity, the sulfur dioxide sample was scanned in the SO_2^+ mass range for peaks adjacent to those to be measured. The adjacent peaks would indicate the presence of impurities and these would probably contribute to the ion currents for SO_2^+ and so invalidate the results.

Mass spectrograms were obtained by scanning over the mass range using appropriate sensitivities to achieve suitable peak heights. The peak heights were directly proportional to the ion currents. The ion currents associated with each mass from six double spectrograms were averaged and the isotopic ratio S^{32}/S^{34} determined.

The analysis of a sulfur dioxide sample for its relative isotopic abundance was accomplished by bracketing the sample with a standard sample. As quickly as possible the standard was analysed, then the unknown sample was analysed, and then the standard analysed again. By this procedure relative isotopic abundances could be determined with a precision of 0.1 per cent. The standard sulfur dioxide sample in this work was obtained from Park City pyrite whose isotopic abundance was arbitrarily assigned.

Since the concentration of S^{34} is very much larger than that of S^{33}

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i. Vibrating Reed Electrometer, Model 30. Manf. by Applied Physics Corporation, Pasadena, Cal., U.S.A.

and S^{36}, investigations have been confined to variations in the concentration of S^{34}. Reference to the species contributing to the SO_2^{++} spectrogram will show that the species $S^{32}O^{17}O^{17}$, and $S^{33}O^{16}O^{17}$ are so low in concentration that their contribution to the mass 66 peak is negligible - 0.15 and 6.0, respectively, in a total abundance of 45,806 for mass 66. The species $S^{32}O^{16}O^{18}$ makes a significant contribution however - 3,800 in the same total abundance of 45,806, for mass 66. In relative abundance investigations errors in the assignment of an absolute abundance of O^{18} may be disregarded, providing the same source of oxygen is used, as in this investigation.

The S^{32}/S^{34} ratio is calculated from the mass spectrometric ratio mass 66/ mass 64 as determined from the SO_2^{++} spectrum:

$$\frac{66}{64} = \frac{S^{34}O^{16}O^{16} + S^{32}O^{16}O^{18}}{S^{32}O^{16}O^{16}} = \frac{S^{34}}{S^{32}} + \frac{O^{18}}{O^{16}}$$

when the O^{18} content is low. The value of O^{18}/O^{16} was found to be 0.00208.

$$\text{Hence} \quad \frac{S^{32}}{S^{34}} = \frac{1}{\frac{(66)}{(64)} - 0.00416}$$

The ratio mass 66/mass 64 for the sample was found in the following manner:

$$\frac{66}{64} = \frac{A \times 20.25}{B}$$

where 20.25 is the mass 66/mass 64 ratio assigned to Park City pyrite

A is the average 66/64 ratio of the sample,

B is the average 66/64 ratio of the standard,

both averages being obtained from at least six double spectrograms.

RESULTS AND DISCUSSION

The results of the isotopic investigation of sulfur from three species of mustard plant are shown in Table VI . Values, as S^{32}/S^{34} ratios, are reported for organic sulfur and stored sulfate in the plant and for the sulfate source of two samples. The volatile sulfur and protein sulfur components were analysed together in two samples but the values of the volatile sulfur of one sample is reported. The fractionation is calculated from the difference between stored sulfate and organic sulfur except, as noted, for the sample for which the volatile sulfur and protein sulfur were analysed separately.

In general it has been shown that there is some fractionation in the mustard plant metabolism of sulfate, occurring in the expected direction. That is, there is a depletion of S^{34} in organic sulfur. The average fractionation was 0.7 per cent.

A fractionation of 2.1 per cent was observed between volatile sulfur and the sulfate of Brassica rapa, sample (a). But since the ratio of the amount of volatile sulfur to protein sulfur is about one to four, the average S^{32}/S^{34} ratio for total organic sulfur in this sample is not appreciably altered from the value for protein sulfur.

It is interesting to note the relation between the organic sulfur and the stored sulfate of the species, Tumbling Mustard and the sulfate in the soil in which the plant grew. Apparently the enrichment in S^{34} of the plant sulfate is about equal to the depletion in S^{34} in the organic sulfur.

Although some isotope fractionation has apparently occurred in the plant metabolism of sulfate, none was observed for the Brassica rapa grown in nutrient culture under sterile conditions. However this result was obtained

Table VIII

Isotopic Distribution of Sulfur in Mustard Plants

Species	Growth Conditions	S^{32}/S^{34} ratios for				Fractionation
		Volatile Sulfur	Protein Sulfur	Plant Sulfate	Source Sulfate	
Tumbling Mustard	Field	....	22.19	22.04	22.13 (Soil)	0.7%
Berteroa Incana	Field	22.11 (1)		22.01	....	0.5%
Brassica Rapa (a)	Field	22.48	22.19	22.02	....	Vol.S 2.1% Prot.S 0.9%
Brassica Rapa (b)	Nutrient Culture	22.21 (1)		22.23	22.22 (Nutrient)	0

(1) This result is obtained from the analysis of the combined fractions of volatile sulfur and protein sulfur.

only once and the experiment must be repeated.

Since the fractionation of sulfur isotopes in nature has been found to be as high as seven per cent[69], a larger fractionation than 0.7 per cent would be expected in the plant metabolism of sulfate. Similarly Urey's[56] work on the variation in isotopic content of carbon in photosynthesis showed that the organic carbon was depleted in C^{13} by 2.97 per cent.

An explanation for the higher isotopic fractionation of the volatile sulfur component is suggested on the assumption that the plant metabolism of sulfur compounds is a reversal of the oxidative path for sulfur in animals. The volatile sulfur compounds of plants consist mainly of allyl isothiocyanate - mustard oil - and to a lesser extent of allyl and vinyl sulfides and mercaptans[67]. Although there is no suggested mechanism for isothiocyanate formation, a possible mechanism for mercaptal formation is shown in Figure VI. It is seen that the formation of mercaptans involves the rupture of another C-S bond over methionine and cystine formation. Therefore mercaptans should show a greater isotopic fractionation.

The procedure for separating the volatile sulfur component is not considered to result in a depletion of S^{34} in the component through the decomposition of sulfur proteins. Peterson[81] determined the sulfur in plant material as four components: volatile sulfur, soluble unoxidized sulfur, insoluble unoxidized sulfur, and sulfate components. He compared the total sulfur value, obtained from these component values, with a direct analysis for total sulfur, and concluded that his method of determining volatile sulfur was quantitative.

The leaching of sulfate from plant material with hydrochloric acid

according to the method of Balks and Wehrmann[83] has also been investigated by Stotz[88], and both claim the method to be quantitative.

The method of Bertrand and Silberstein for determining sulfur in plants has been compared by Junge[89] with the Carius method which gives true sulfur content, and found to be in excellent agreement. Since the former method is carried out on the combined soluble and insoluble material after the sulfate has been leached, it is considered that no isotopic fractionation would occur in the preparation of the protein sulfur component.

Although the reduction of $BaSO_4$ with zinc does not go to completion, it is considered that no isotopic fractionation occurs at the temperature of the reduction. Reduction by iron has been carried out at the same temperature in other work and although the reaction did not go to completion, no fractionation of the isotopes could be attributed to preparation of the samples[70].

Summary:-

A procedure for the analysis of green plant material suitable for isotopic investigation on sulfur has been developed. A system has been devised for the aqueous culture of mustard plants under sterile conditions. An average fractionation of 0.7 percent in the S^{34} concentration has been observed in the metabolism of sulfate by mustard plant from under field conditions. The fractionation is in the direction expected, i.e., a depletion in S^{34} for the organic sulfur. The extent of fractionation has been found to be small in comparison with the variations found in nature, and no fractionation was found when the plants were grown under sterile conditions.

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