A Survey of the Occurrence

of Oxygen 18 in Natural Sources.

A Thesis

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by

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Abstract.

An investigation of the concentration of the heavy isotope
of oxygen, O^{18}, in various samples of water was carried out. Natural
variations as high as 2.9% were found - glacier water being 2.3%
light and Dead Sea water 2.0% heavy, as compared to Lake Ontario water
as standard. Other values - water from tank oxygen+ 2.9%, atmospheric
water vapor -0.9%, Atlantic Ocean water+ 0.4%, Pacific Ocean water
-0.8%, and atmospheric carbon dioxide -0.5%. The water samples were
equilibrated with tank carbon dioxide, which was then analyzed using
the mass spectrometer. Some work was also done on photosynthesis.
The free water, water of crystallization, and tissue oxygen (as water),
in a normal leaf which had been photosynthesizing for 8 hours, were
analyzed by equilibration with carbon dioxide as above. It was found
that all of these were more enriched in oxygen 18 than the water with
which they were fed. The free water averaged about 1.3% heavy, water
of crystallization as high as 8.7% heavy, and tissue oxygen varied from
0.5% light to 5.5% heavy. This work only just touches the edges of this
field - many more interesting experiments remain to be carried out later.

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Natural Sources.

Introduction.

Originally atomic weights were all based on hydrogen (H = 1.0000), but it was found that if oxygen was taken as the standard (i.e. O = 16.0000) the atomic weights of most of the other elements came out much nearer whole numbers than for H = 1.0000. Even after very careful determinations of the atomic weights by Stas and others, however, it was found that many atomic weights were still not whole numbers. The discovery that most elements consist of various isotopes (i.e. atoms of different atomic weight and same atomic number) helped to explain this fact.

In 1927, Aston, by means of the mass spectrograph, determined that the ratio of hydrogen to oxygen was 1.00778 to 16.0000, and this was in complete agreement with the chemical combining ratios of hydrogen and oxygen. But in 1929, Giauque and Johnston [1] discovered that ordinary oxygen contains not only O^{16} but O^{17} and O^{18} as well - (O^{16} - 99.76%, O^{17} - 0.04%, O^{18} - 0.20% [2]). Chemists continued to take the mixture of isotopes as the standard (O = 16.0000) but physicists took O^{16} = 16.0000 as their standard.

Whereas O^{16} is an excellent standard from a physical point of view, it is quite useless to the chemist since it is not possible nor practical to obtain it pure and in large quantities. Thus the natural mixture of oxygen isotopes has remained the chemical standard and there would be no objection to this if it could be shown that the proportions of the isotopes in the mixture remained constant. Should this be true, then a simple conversion factor would be all that was needed to relate the chemical and physical atomic weight scales.

If it was found that the proportions in the mixture change, (as is actually the case), then the accuracy of the chemical atomic weights is immediately limited by the extent of this variation. This would apply to all atomic weights since each experimenter used as a standard the most convenient source of oxygen. Unless the relative abundance of the oxygen isotopes in each of these sources is known, no comparison can be made of the atomic weights beyond a certain point which is determined by the variation in the oxygen in the sources.

Considerable work has been done in this field already. At first the aim of the research was to determine the actual amount of oxygen 18 in the mixture. Early values of the ratio $O^{16}:O^{18}$ ranged from 1250:1 down to 536:1 (3,4,5,6,7,8,9,10). Smythe in 1934 (11) found a value of $503 \pm 10/1$ and this value is now accepted (13,12) (rounded off to 500:1). From this time on, measurements were accurate enough to allow small variations to be detected and a great deal of research was carried out along this line. Manian, Urey, and Bleakney (14) used the mass spectrograph to compare the O^{18} content of stone meteorites with that of terrestrial stones but found no variation within their experimental error of 2.5%. However, in 1935, Urey and Greiff (15), using spectroscopic data, calculated the equilibrium constants and the enrichment factors for a large number of isotopic exchange reactions of the lighter elements, including oxygen. Since the various enrichment factors are not unity, there is a tendency for O^{18} to concentrate in certain molecules more than in others. As a result there should be detectable variations in the O^{18}/O^{16} ratio, depending on the source of the oxygen. The ratio is determined by burning the oxygen to water and analyzing the water by density methods. The calculated value (15) of the variation is approximately 10 p.p.m. Hence, atomic weights given more

precisely than this variation are meaningless unless the isotopic composition is specified. Dole was one of the first to confirm these predictions when he found (16) that atmospheric oxygen water was heavier than fresh water of the same deuterium content by 4.6 p.p.m., using density methods of analysis.

At this point a word about the various methods of analysis might be helpful. Earlier workers (4,5) studied the absorption spectra of gases. This method depends on the fact that in band spectra isotopic atoms in the molecule give rise to bands identical to those originating from the ordinary molecule but slightly displaced. Another method used for the isotopic analysis of water is the interferometric method (18,17) in which the refractive index is measured. As the isotopic composition changes, the refractive index changes also. Very careful purification of the sample is required but the analysis itself is "rapid, very precise and requires only very small samples" (18). It is not possible to distinguish between a change due to oxygen isotopes and one due to hydrogen isotopes by this method however.

The two most important ways of determining the ratio at present are by the mass spectrometer and by density measurements. Various investigators have used the density of water as a basis for the determination of the ratio O^{16}/O^{18} - the most important work being that done by Dole (16,20,24,19,21,22,23). He used the totally immersed float method (21,25) first developed by Richards and Shepley (26) which consists of adjusting the density of the water to that of the float by altering the temperature of the water. In this way density measurements were obtained with a precision of $\pm$ 0.2 p.p.m. (21) ($\pm$ 0.1%) and the limiting factor then became the purity of the water samples. These density measurements are difficult to perform and require the development of technique to a high degree. The time required for each determination is considerable

and the preparation of the samples requires a great deal of painstaking labor.
Each sample must be carefully purified by numerous redistillations, and then
to be sure that the difference in density is due only to oxygen isotopes and
not hydrogen, it is necessary in most cases to electrolyse the water and recombine
the oxygen with hydrogen from a specific source. All of these processes are liable
to result in fractionation of the isotopes and even when all precautions are
taken, there is the possibility of small amounts of impurities being present which
would change the value of the densities and yet be analytically undetectable.

Many of the difficulties encountered above are either eliminated or
minimized by the use of the mass spectrometer for direct isotopic analysis. The
time required for the determination of the isotopic ratio is cut from a period
of several weeks to a matter of hours. The necessity of electrolyzing samples and
of recombining the oxygen with "normal" hydrogen is eliminated since the mass
spectrometer differentiates between differences due to oxygen isotopes and those
due to hydrogen. Impurities (if present in small concentrations) have little
effect on the results from the mass spectrometer and water samples can be
purified by simple means (distillation with alkaline permanganate). The objection
to the mass spectrometer has been that it is not as sensitive as the density
method. It is now possible to obtain a precision of $\pm$ 0.1% on our instrument
which is quite comparable to results obtained by the density method.

Dole's first determination of the excess density of water from
atmospheric oxygen (16) (4.6 p.p.m.) was revised to 6.0 p.p.m. and this agreed
with values determined by later investigators (29,27,28,22). The oxygen in our
present atmosphere must be of photosynthetic origin since the present rate of
photosynthesis is sufficient to produce the quantity of oxygen in the atmosphere
in a few thousand years (30). The oxygen of photosynthesis is produced according

to the equation $nCO_2 + nH_2O \rightarrow n(CH_2O) + nO_2$. Thus the O^{18} content of atmospheric oxygen must bear a definite relation to that of the CO_2 and water entering into the reaction. Ruben et al (31) performed experiments which indicated that the O_2 of photosynthesis came from the water and not CO_2. Dole and Jenks (23) confirmed this result later. Thus it would be reasonable to expect that the atmospheric oxygen would be of the same O^{18} content as the water instead of 6.0 p.p.m. heavier as is found. By assuming that the oxygen of the air is in equilibrium with sea water rather than with fresh water, about 2.0 p.p.m. can be accounted for since the ocean water is heavier by that amount (34,32,33). This still leaves a discrepancy of 4.0 p.p.m. between sea water and atmospheric oxygen. According to the enrichment factors of Urey and Greiff (15) oxygen in equilibrium with water should be enriched by 1.2 p.p.m. at $25^\circ C$. However, there still remains a discrepancy of almost 3 p.p.m. (often called the "Dole effect") which has not been explained.

One of the first theories brought forth to explain the high O^{18} content of atmospheric oxygen was that of Greene and Voskuyl (29). CO_2 in equilibrium with water is enriched by approximately 10 p.p.m. (15,35) in O^{18} and since this equilibrium is rapidly established, it is almost certain that atmospheric CO_2 is in equilibrium with the water of the earth. Greene and Voskuyl assumed that at least part of the heavy oxygen from the CO_2 was given off as oxygen of photosynthesis and this would then be heavy also. However, both Ruben (31) and Dole (23) have experimental evidence which indicates that the oxygen comes from water. Ruben's experiments were carried out using oxygen 18 as a tracer and there is a possibility of exchange here. He found the O^{18} content of photosynthetic oxygen to be the same as that of the water. Dole's experiments, however, used the natural enrichment in O^{18} of CO_2, thus eliminating exchange

reactions. It was found in this case that the O^{18} content of the oxygen is 1.2 p.p.m. greater than of the water - close to the value predicted by Urey and Greiff (15) for the equation $2H_2O^{18} + O_2^{16} \longrightarrow 2H_2O^{16} + O_2^{18}$. Greene and Voskuyl's theory in the light of this evidence appears very doubtful and Dole suggests (19) that there may be an equilibrium between oxygen and water in the lower regions of the stratosphere. At this level where the temperature would be $-50°C$. the enrichment factor for the reaction is 1.0338 (24) and this would account for the difference of 6.0 p.p.m. which is found. There is as yet no direct evidence supporting this theory. A good summary of the relation of photo-synthesis to the O^{18} in the atmosphere is given by Kamen and Barker (2).

As was mentioned earlier, ocean water has been found to be heavier than fresh water. Gilfillan (34) found a difference of 2.3 p.p.m. in Atlantic coast water (both deep sea and surface). Greene and Voskuyl found that water from the Atlantic ocean near the equator was 2.4 p.p.m. heavy and from the North Atlantic only 1.7 p.p.m. (standard - Lake Michigan water). They suggest that the difference is due to the greater amount of evaporation in the tropics (H_2O^{18} having the lower vapour pressure (36)-would concentrate in the residual liquid and H_2O^{16} in the vapor). A survey of sea water was carried out by Wirth, Thomson and Utterbeck (33) in which the average increase above fresh water was 1.4 p.p.m.; in the Mediterranean Sea, Indian Ocean and Red Sea the difference was 1.3 p.p.m.; in Antarctic, North Pacific Oceans and Bering Sea 1.2 p.p.m. In the Pacific near the equator a difference of 1.5 p.p.m. was found and in the Baltic Sea there was only 0.3 p.p.m. difference. This low value was attributed to dilution by fresh water. The density was also found to vary with the depth. Considering only the North Atlantic the excess density over Lake Michigan water is accepted as 1.7 - 1.8 p.p.m. and this is explained by the fact

that fresh water is mainly formed by evaporation of sea water and later precipitation. In the process of evaporation, fractionation of the isotopes takes place and the heavy isotope concentrates in the "residual" ocean water while the lighter liquid is carried away to form fresh water.

As stated earlier the mass spectrometer was originally a less sensitive method than the density method. Now that greater precision is possible with better instruments, certain of the measurements done by density methods have been repeated and the field extended to include a larger number of samples. Water samples have been obtained from various sources to be analysed as O_2 (electrolysed) and as CO_2 (by equilibration).

Considerable work on photosynthesis has been done at McMaster by Bennett (37), Haines (38) and Joyce (39). These experimenters did all their work using either H_2O or CO_2 enriched with O^{18} and as a result of this there was always the possibility of exchange of the O^{18} between the various water levels in the leaf and the CO_2, as well as any interchange due to the photo-synthetic reactions. In nearly all of the earlier experiments using O^{18} enriched materials, results were found to be erratic and in general not reproduc-ible. Now by eliminating the use of enriched material the possibility of exchange is removed. Ordinary CO_2 in the atmosphere, which would be in equilibrium with the water in Lake Ontario (our standard) is enriched approximately 3.5% (at 25°C.) in O^{18} (15,35). Thus it should be possible to use the O^{18} from this natural source as a tracer - a very elegant method in which the probability of exchange is reduced to a minimum because the CO_2 and H_2O used in the experiments are already in equilibrium. Experiments of this type have also been investigated in this paper.

Experimental.

(a) O^{18} content of water.

Water samples were purified for these experiments by distillation of the entire sample with alkaline potassium permanganate in an all-glass system. The distillation was then repeated without use of alkaline potassium permanganate. The first third is discarded and the middle third is collected in a clean, dry flask. These purified samples are then prepared for analysis in two ways.

(i) Electrolytic Apparatus.

The first method is the electrolysis of the water sample producing hydrogen and oxygen, of which only the oxygen is collected. The water sample is made 1.0N in sodium hydroxide (the oxygen in sodium hydroxide does not affect the results within the limits of our precision - see Appendix A). The cell used in the first run, El, is shown in Figure 1, and the cell used in succeeding runs in Figure 2. Both these cells consist simply of two electrodes of thin platinum sheet, each spot welded to a nickel sleeve which covers the tungsten wire of a pyrex press seal. The surface area of each electrode is approximately 1.2 - 1.4 sq. cm. and the electrodes are separated by a sintered glass disc. Rectified alternating current (25 cycle) of from 2 to 40 volts is passed through the cell until the gases come off at the desired rate. The nickel sleeves are essential because the sodium hydroxide solution will otherwise destroy the tungsten wire of the press seals. The sintered glass discs were made at McMaster by the method outlined by A. S. Tulk (40) and are necessary to ensure that the oxygen and hydrogen do not mix. The oxygen from the cell passes through a trap immersed in dry ice, where any water vapor is frozen out, and then through a sample tube into the atmosphere. The cells hold about 15 ml. and 10 ml. respectively and

To Atmosphere

Press Seal

To Line

Nickel Sleeve

Platinum Electrode

CAPACITY = 15 ml

Sintered Glass Disc

FIG. 1   CELL FOR ELECTROLYSIS   OF WATER

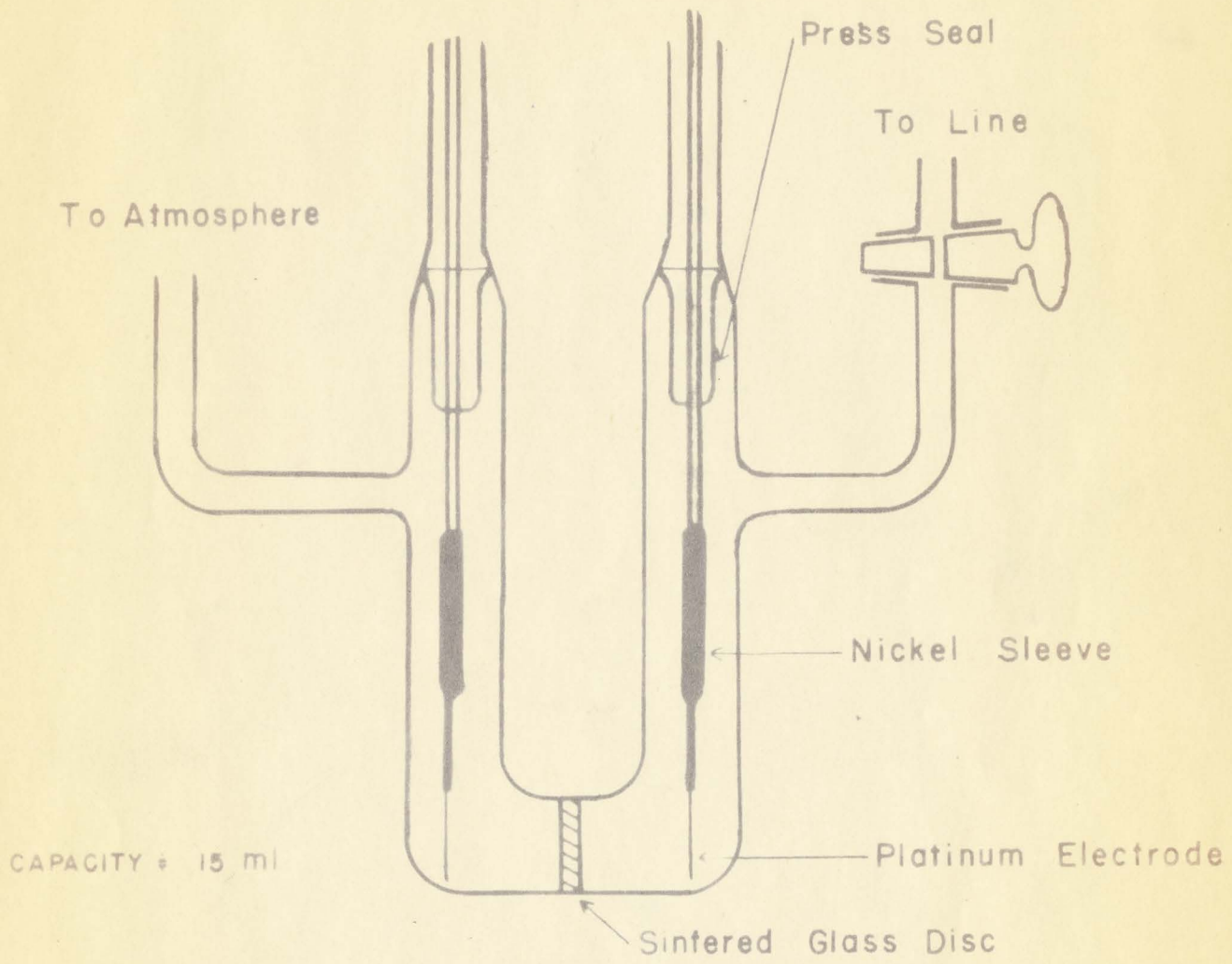Press Seal

To Atmosphere

To Line

Nickel Sleeve

Platinum Electrode
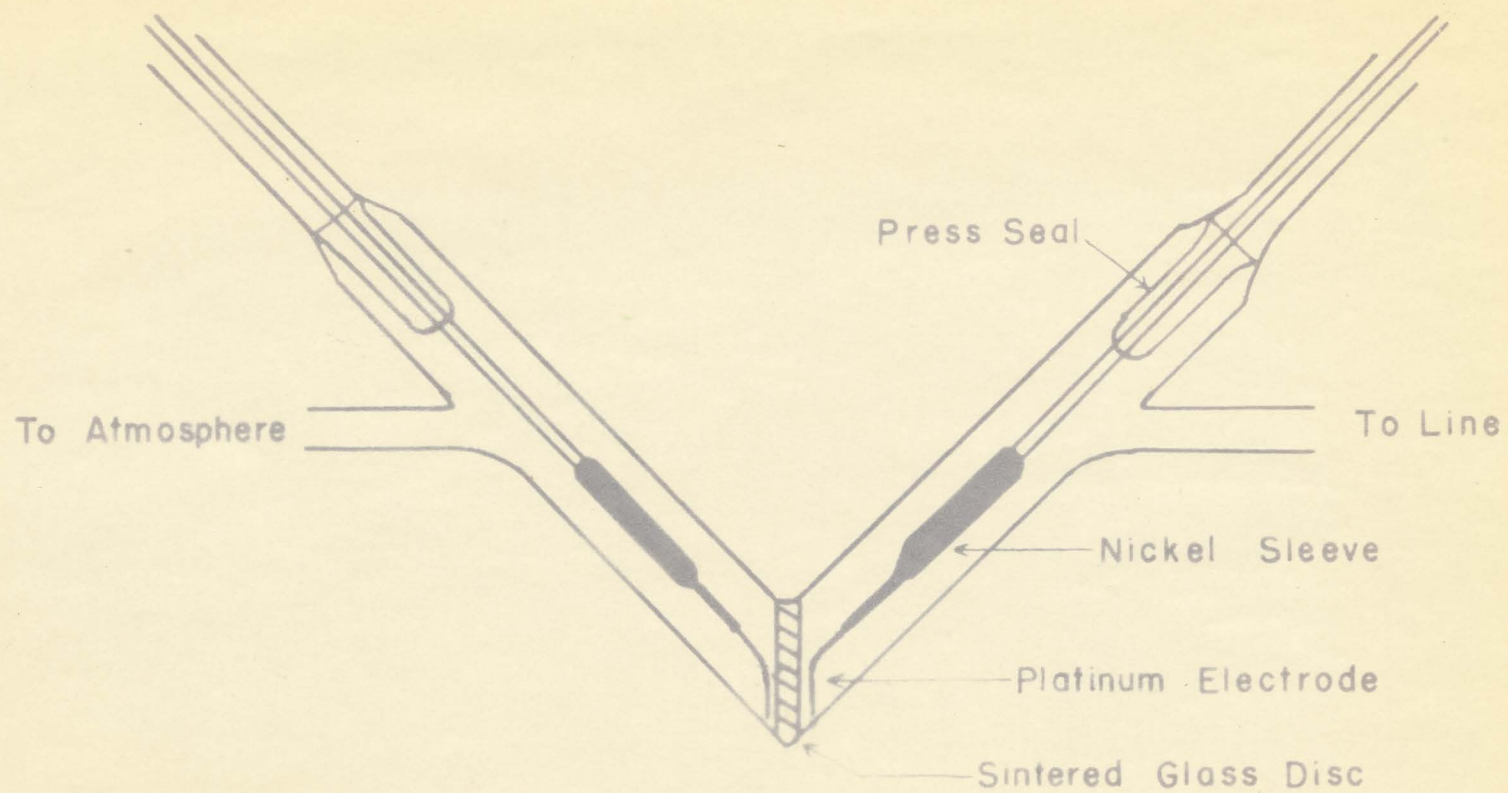
Sintered Glass Disc

FIG. 2   CELL FOR ELECTROLYSIS OF WATER

electrolysis is carried out until the water is two-thirds gone. For cell #2 this leaves 3.3 ml. residue after approximately 4 litres of oxygen have been given off. The total volume of the line is 450 ml. and thus the line has been flushed with nine times its own volume of oxygen. The sample tube is then sealed off - it now contains the oxygen sample, dry and at atmospheric pressure.

A current of about 1.0 to 1.4 amps. gives the best rate of electrolysis - the oxygen comes off slowly enough that the effervescence is not too violent and yet fast enough that a sample is obtained in a reasonable time. During the electrolysis the cell must be immersed in a water bath containing a cooling coil, to prevent the cell from heating up. If it heats, the resistance decreases, the current then increases, and the cell heats up more. The temperature of the cooling bath must be regulated about $5^{\circ}C$. below the temperature of the cell and this is found to give a relatively constant current.

Samples of oxygen from the following places have been prepared:- Lake Ontario water (standard), Tank Oxygen, Atmospheric water vapor (i), Pacific Ocean water, Atlantic Ocean water, Greenhouse Oxygen, Atmospheric Oxygen, and Lake Louise Water. These samples have not yet been analysed, but as soon as the results are known, they will be included.

(ii) Equilibration with Carbon Dioxide.

The second method of treatment is to take the purified samples and equilibrate them according to the method of Cohn and Urey (41), with normal CO_2 carefully dried and purified. In this case the carbon dioxide is analysed.

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(i) collected by passing air through a trap immersed in dry ice and collecting the water vapor frozen down.

A sample of atmospheric CO_2 was also collected by freezing the CO_2 out of dried air in a liquid air trap.

Analysis of Samples.

All analyses were done on a 180° mass spectrometer. The sample of carbon dioxide in equilibrium with water is first frozen down with liquid air and any slight gaseous impurities are pumped off. The liquid air is then put on a sample tube attached to the mass spectrometer and the carbon dioxide sample is transferred to this tube. A standard sample of carbon dioxide is at all times kept on another sample tube attached to the mass spectrometer line. When either of these is introduced into the mass spectrometer tube, the carbon dioxide is taken off dry ice and passed through a spiral trap also immersed in dry ice before entering the tube. In every analysis any drift in the mass spectrometer or recorder is followed by taking a series of runs on the standard, then doing the sample, and immediately repeating the standard. If the standards do not agree within the precision of our measurements then the entire run is rejected. In our analyses a "set" consists of four peaks - 44, 46, 46, 44 - run in that order (see spectrogram Figure 4). At least five sets are run on each sample - a total of fifteen (or more) for a given run of standard, sample, and standard. For every run the instrument is checked to make sure that all values are constant. Case current, trap current, magnet current and acceleration potential are all kept as close as possible to the value for previous runs. The pressure is adjusted so that the peak heights for all samples are approximately the same. Before the analysis, the tube was baked and pumped down so that the residuals were negligible. The main line pumping trap on the mass spectrometer tube must be cooled by dry ice rather than liquid air. When this is the case a sample
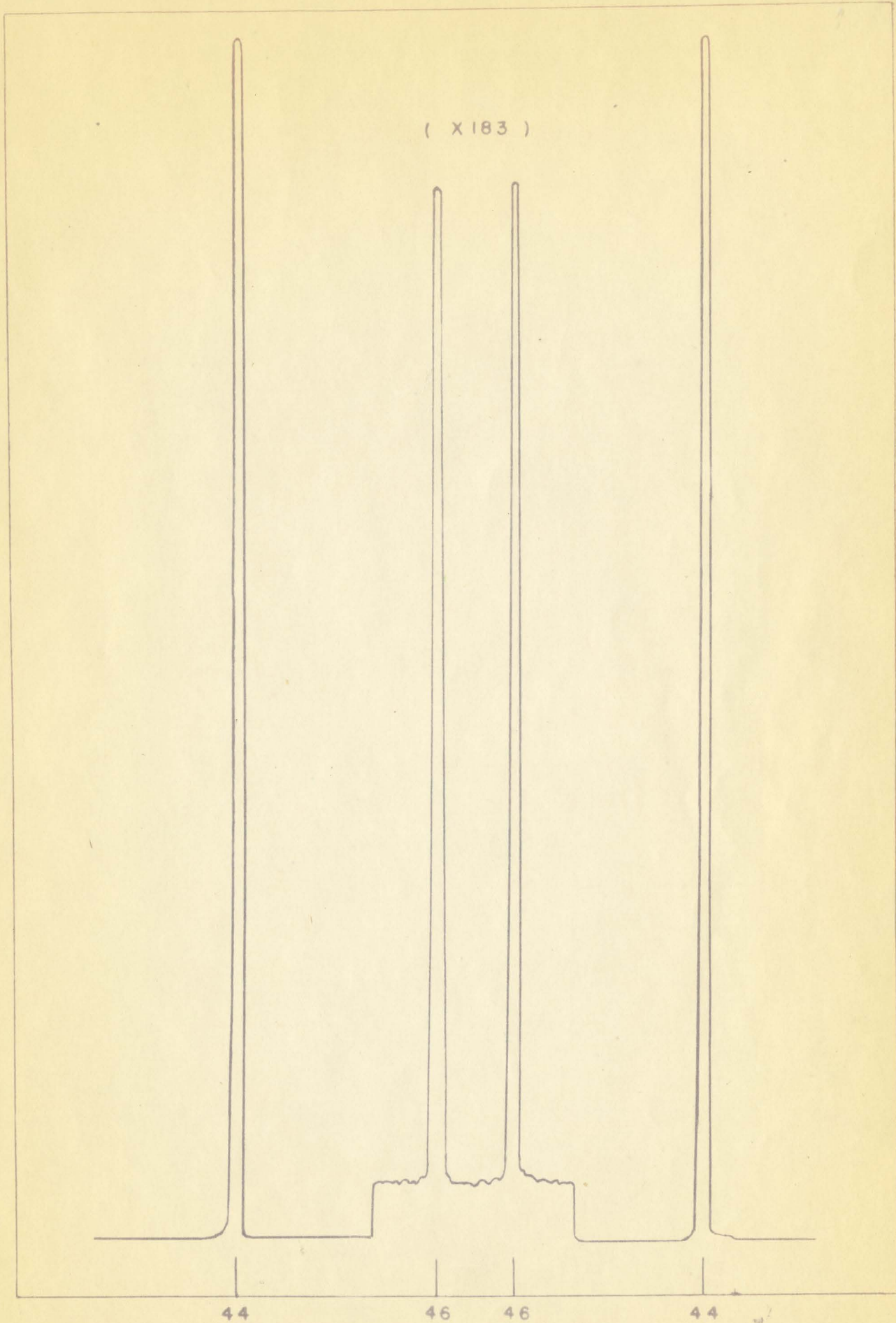
(X 183)

ION CURRENT

44   46   46   44

MASS   NUMBER

can be pumped down to 0.1% of its original pressure in one or two minutes, and
a new sample can then be introduced. Everything is done to ensure that any
and all variations are reduced to a minimum.

Table 1

A typical set (P8#2 May 13, 1948).
Water sample at 110°C. from 90 normal geranium leaves.

Shunt	Mass	Peak Height (cm.)	Average	Ratio
6	44	22.80	44 - 22.77 (x 182.95)	
1	46	18.91		220.29 ± 0.07
1	46	18.90	46 - 18.91 (x 1)	
6	44	22.73		

These are the values for the peaks shown in Figure 4.

Table 2

A typical run (March 15, 1948)

Standard Ratio 1st time	Sample P6#1	Standard Ratio 2nd time
233.83 ± 0.08	231.52 ± 0.50	233.29 ± 0.49
233.63 ± 0.12	230.97 ± 0.05	233.72 ± 0.06
233.73 ± 0.02	230.85 ± 0.17	233.86 ± 0.08
234.02 ± 0.27	231.01 ± 0.01	234.12 ± 0.34
233.54 ± 0.21	230.73 ± 0.29	233.92 ± 0.14
Average		
233.75 ± 0.15	231.02 ± 0.20	233.78 ± 0.22

Standard ratio (average)   233.8 ± 0.2

Sample ratio   231.0 ± 0.2

Normality Ratio 233.8/231.0   = 1.012 ± 0.002 N.

(b) Photosynthesis experiments.

Normal bean plants were grown in the greenhouse in soil. After a bright sunny day, during which the plants were photosynthesizing for eight hours (or more), one leaf (Run #P3-4-5) was cut from the plant and cut into small pieces with a razor blade. This was then placed in a glass boat in the leaf analysis apparatus (Figure 3). The system was evacuated at room temperature for 24 hours and the water collected in a sample tube immersed in dry ice. After a further evacuation of at least 24 hours (until no more water was collected) the furnace was turned on and the control set at 110°C. The sample of water at this temperature was collected in the same manner as that at room temperature and the procedure was the same for the sample at 400°C. All samples were equilibrated with carbon dioxide as before (41). It is assumed that at room temperature the free water of the leaf is collected, at 110°C. the water of crystallization comes off, and at 400°C. the oxygen from the tissue burns to form water. Very little water was collected at 110° and 400°C. so another set of experiments (Run #P6 and 7) was carried out using 12 to 15 bean leaves obtained under the same conditions as above. Enough water was obtained in these experiments to form droplets in the tubes, and these were purified by vacuum distillation, while the water at room temperature was purified by distillation over alkaline potassium permanganate and then over chromic acid. It was found that carbon dioxide came off at 400°C. and a sample of this was taken also (collected in a tube immersed in liquid air).

Since larger samples would permit better purification, still another run was carried out (Run #P8) using 90 geranium leaves obtained as before. The leaf analysis apparatus was modified here and instead of a furnace, a 1 litre distilling flask was used. This was placed in a Glascol heater connected to a Varitrans so that the temperature could be varied. (Temperature was determined

110 V. A.C.

TO VACUUM
PUMP

1. RHEOSTAT
2. FURNACE
3. SAMPLE TUBES
4. MANOMETER
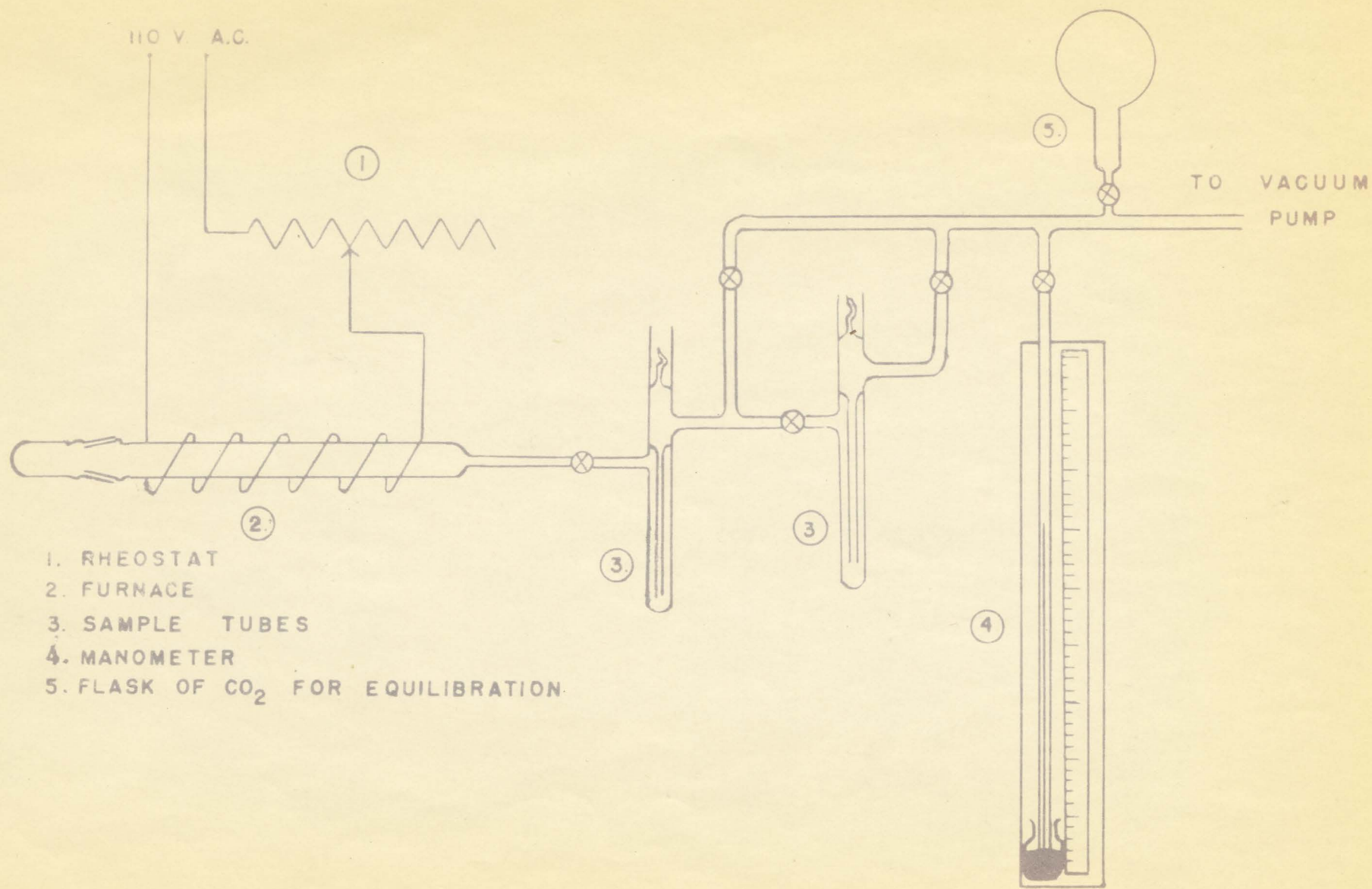5. FLASK OF CO_2 FOR EQUILIBRATION

FIG. 3   LEAF   ANALYSIS   APPARATUS

by means of an iron constantan thermocouple). At room temperature, over a period of five and a half days of evacuation, 100 ml. of water came off and then evacuation was continued for 48 hours more, even though no water came off. The Glascol heater was then set at 110°C. and by chance it was found that some carbon dioxide came off here too. This was collected as well as the water at that temperature (of which there was about 1 ml., purified by vacuum distillation). At 400°C. tremendous quantities of carbon dioxide came off and two samples were taken. About 2 - 3 ml. of water were collected and purified by distillation over chromic acid and again with nothing added. All water samples were equilibrated with carbon dioxide as before (41).

In previous runs no oxygen of photosynthesis was obtained and so one run (Run #P9) was carried out with four normal geranium leaves, following the method outlined by R. S. Joyce (Page 6 of reference 39). Normal carbon dioxide and hydrogen were used.

Treatment of Samples.

Samples were again analysed by means of the mass spectrometer as explained earlier (page 10).

Materials.

Thanks are due to Professor Farkas of the Hebrew University, Palestine, for the sample of water from the Dead Sea, and to Professor Coffin of Dalhousie University for the sample from Halifax Harbour. All other samples were collected by the writer.

Table 3

Results of Survey of Water Samples - analysed by Equilibration with carbon dioxide.

Source of Water	Ratio $CO_2^{16}/CO^{16}O^{18}$	Enrichment in O^{18}
Lake Ontario (Standard)		1.000 ± 0.002
Tank oxygen and hydrogen combined	234.1/227.5	1.029 ± 0.002
Atmospheric water vapor	233.5/235.7	0.991 ± 0.002
Atlantic Ocean (Halifax Harbour)	233.6/232.7	1.004 ± 0.002
Glacier Water (Lake Louise, Alta.)	233.6/238.7	0.977 ± 0.002
Pacific Ocean (English Bay)	233.5/235.5	0.992 ± 0.003
Dead Sea (surface water)		1.020 ± 0.002
*Atmospheric carbon dioxide	234.0/232.9	1.005 ± 0.002 ?

* This sample was not equilibrated with water at all.

Discussion.

Standard.

Lake Ontario water ("normal" water) was taken as the standard throughout. Samples were taken at various times over a nine month period and each ratio agreed, within the limits of precision, with those determined previously.

Tank Oxygen. (+ 2.9%)

Tank oxygen is prepared by liquefaction of air. The air is cooled gradually and the oxygen (having the higher boiling point) liquefies first. From the method of preparation it would be expected that the tank oxygen and atmospheric oxygen would have the same O^{18} content. Experimentally this is found to be the case.

<u>Atmospheric Water Vapor</u> (-0.9%).

As would be expected the amount of O^{18} in atmospheric water vapor is lower than for Lake Ontario water since this water vapor presumably came largely from Lake Ontario. The vapor pressure of H_2O^{18} is less than that of H_2O^{16} and thus when the water from the Lake evaporates the lighter O^{16} concentrates in the vapor.

<u>Atlantic Water</u> (+ 0.4%)

This sample was taken in Halifax Harbour in February 1946. This result is lower than the generally accepted value for Atlantic water (about 0.9% - see Introduction). However, Wirth et al (27) in their survey found northern waters to be only 0.6% heavy. In February there is a current from the North Atlantic near Greenland and Labrador which passes along the Nova Scotia coast line (42), and thus our sample probably consisted largely of northern waters. This same current passes across the mouth of the St. Lawrence River and it is possible that some of the fresh water from this source would also be carried along past Halifax and the Nova Scotia coast. Both of these factors would tend to produce the low value shown.

<u>Glacier Water</u> (-2.3%)

Lake Louise, Alta., fed by the Victoria Glacier, is the source of this sample. Atmospheric vapor (already light by 0.9% in O^{18}) is precipitated on the mountains forming snow and ice. The edges of such glaciers and snow patches then gradually melt. This thawing process also tends to the accumulation of light oxygen (43) and this may account for all or part of the remaining 1.4% deficit in oxygen 18.

Pacific Water (-0.8%)

This cannot really be called Pacific Ocean water, since it actually came from English Bay (in the Strait of Georgia) at the mouth of the Fraser River. Here sea water from the Pacific (heavy by about 0.8%) (33) mixes with Fraser River water. This latter water would hardly be expected to be normal since it has its sources in the mountains and is therefore largely fed by glacier water and snow water, which as we have seen above is 2.3% light. These effects combine to give the resulting decrease of 0.8% in O^{18} content.

Dead Sea (+2.0%)

Water is continually flowing into the Dead Sea, but none flows out. The only method of escape for the water is evaporation and H_2O^{16} (with the higher vapor pressure) would evaporate more rapidly. As a result it would be expected that the Dead Sea water would be heavy in O^{18} and the results verified this supposition.

Atmospheric carbon dioxide (+0.5%)

From this result it would seem that atmospheric carbon dioxide is not in equilibrium with Lake Ontario water or else it would be approximately 3.5% heavy (13,30). It is quite likely, however, that this was not a representative sample of atmospheric carbon dioxide. The sample was collected during the day time when all of the heavy industries in Hamilton were running and it is probable that carbon dioxide produced by these industries formed a large percentage of the atmospheric carbon dioxide. If this is the case, then the result is quite meaningless since the value would not be reproducible.

Table 4.

Results of runs made using normal leaves - normal carbon dioxide and water.

Sample	Run P3 1 leaf	Run P6 12 leaves	Run P8 90 leaves
H_2O - room temperature	1.015	1.012	1.013
H_2O - 110°C.	1.087	1.053	1.065
CO_2 - 110°C.	*	*	1.030
H_2O - 400°C.		0.995	1.013
CO_2 - 400°C.	*	0.995	1.055[#]
CO_2 - 400°C. second sample	*	*	1.026[#]

Runs 3 and 6 - bean leaves, 8 - geranium leaves.

Runs 3, 6, and 8 - photosynthesis took place all day - leaves still on plants and in natural surroundings - in sunlight.

* sample not collected.

\# only a small portion of carbon dioxide collected here.

In all of these runs normal water (Lake Ontario) and normal carbon dioxide (Naturally enriched by 3.5%) were used. It is interesting then to note that the free water circulating in the plant is not normal, as is the water which is being fed to the plant, but is enriched by 1.3 ($\pm$ 0.2%). This is probably accounted for by the fact that this water is the source of the water of transpiration of the plants. Since this latter water is continually evaporating off, it is quite conceivable that there should be a certain amount

of fractionation resulting in an increase in the O^{18} content of the plant.
Even more startling is the fact that the water taken off at $110^{\circ}C.$ - possibly
the water of crystallization - is enriched by 6.8 ($\pm 1.9\%$). In run P8 when the
water samples were quite large, approximately 100 ml. of water came off at room
temperature and only about 1 ml. at $110^{\circ}C.$ It would then seem possible that
during the process of pumping off the water at room temperature, some fractionation
may have taken place. If this were true, the last remaining ml. of water at
room temperature would have a high O^{18} content and there might conceivably be
some exchange between this and the water of crystallization. Considering that
the initial concentration of O^{18} is 1.013N and that all conditions are optimum,
the final concentration for a decrease in volume from 100 ml. to 1 ml. calculated
by the Rayleigh distillation formula (36), would be 1.055N. However, in this
case the conditions are far from optimum. Instead of being an equilibrium
process, this is a unidirectional process and the enrichment factor is inversely
proportional to the square root of the masses ($\sqrt{20/18}$ = 1.054). If the last
ml. of the free water is in equilibrium with the water at $110^{\circ}C.$ the enrichment
is explained, but there is no data to show whether or not such is the case. It
would be advisable to repeat the run P8 and collect the first 99 ml. of water
and the last ml. separately. Analysis of this last fraction would immediately
show whether or not there was any fractionation.

One sample of carbon dioxide was taken off at $110^{\circ}C.$ - this very
likely comes from compounds containing loosely bound carboxyl groups. This
sample was found to be 3.0% enriched above carbon dioxide in equilibrium with
normal water, showing that the oxygen in these carboxyl groups is not normal
even in a normal leaf. The carbon dioxide which the plants are taking in is
3.5% enriched over the water in equilibrium with it, and the oxygen of photosynthesis
has been found to be of the same O^{18} content as the water. The extra O^{18} must

go somewhere and it seems logical that some of this would go into the tissue. It should be noted that the carbon dioxide and water at $110^\circ C$. are certainly not in equilibrium, for if they were, the carbon dioxide would be slightly enriched (at $110^\circ C$. the factor would be very close to 1.000) over the water, but actually its O^{18} content is 3.5% below that of the water. In run P8 where ninety leaves were used, it was found that a large amount of carbon dioxide came off below $400^\circ C$. and even as low as $300^\circ C$. This carbon dioxide which is due to the breaking down of the various compounds in the leaves, did not come off evenly, but rather in large gushes with intervals of several degrees between. A small sample taken of the first rush of carbon dioxide was 5.5% heavy, while another taken later was only 2.6% heavy. This would indicate that the oxygen in the various parts of the starches and sugars in the leaf was not enriched to the same extent. Several further experiments are suggested by these results. The leaves could be heated up very slowly to $100^\circ C$. and then samples of carbon dioxide taken off, first at large intervals (50°) and then, when $300^\circ C$. is reached, at 10 to 15° intervals up to $400^\circ C$. This would show just how the O^{18} content varied from one group to another as the compounds in the leaves were degraded by steps. A simpler experiment would be to take a pure starch or sugar and degrade it slowly, collecting the carbon dioxide at different temperatures. This might show how the O^{18} content varies within these compounds.

The samples taken at $400^\circ C$. in runs P6 and P8 do not seem to check. In the first place the carbon dioxide and water have the same O^{18} content in P6, but this is probably due to the fact that some water passed through the first trap and into the second where it equilibrated with the carbon dioxide. In P8 the carbon dioxide samples were both collected before any water came off, and there was no chance for equilibrium to be established. Also it must be borne in mind that in P8 only two small portions of the carbon dioxide were collected

at $400^{\circ}C$. since there was too large a quantity to collect it all. The O^{18} content seems to be decreasing and if all had been collected, an average sample of the whole mixture might have been much closer to normal. The interesting thing about the results from P8 is that the first sample of carbon dioxide (which came off at about $300^{\circ}C$.) has a higher O^{18} content than samples taken off at higher temperatures. Further experiments (outlined earlier) should prove even more interesting. The tissue oxygen can come from two sources:- from carbon dioxide of the atmosphere (3.5% enriched), or from the free plant water which is 1.3% enriched. Since both sources of oxygen are enriched, the final product must also be enriched.

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Appendix A.

Calculation to show that the oxygen in sodium hydroxide does not affect the results within the limits of our precision.

Solution is 1 M in NaOH.

There is 1 mole of NaOH per 55 moles H_2O

i.e. 1 gram-atom of oxygen from NaOH for every 55 gram-atoms from water.

This amounts to a 2% dilution of the water oxygen.

The maximum variation of the O^{18} concentration in normal water is 5 parts per 100 (5%) and the O^{18} in normal NaOH would vary by the same amount.

Therefore, the dilution effect of any O^{18} from the sodium hydroxide is 2% x 5% = 0.1%.

However, this is the same as our precision on the mass spectrometer and thus any variation due to the sodium hydroxide oxygen will not be detected.

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