CARBON MONOXIDE A RESPIRATION PRODUCT.

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In a paper presented by one of us[1] it was shown that there is present an average of 4% (by volume) of free carbon monoxide in the pneumatocyst (*i. e.*, the floater) of the giant Pacific Coast kelp *Nereocystis leutkeana*.

This unique occurrence of free carbon monoxide within a living plant at once raised the question as to its origin. The intimate chemical relation of carbon monoxide to formaldehyde and formic acid had long ago suggested its possible relation to photosynthetic processes. On the basis of the physiology and structure of the plant there were grounds for the consideration that the carbon monoxide might be a product of respiration.

The possibility of its formation due to the action of enzymes or to processes of decay was the first point investigated. Finely ground kelp was allowed to undergo autolysis in contact with sea water and the gases evolved were examined. No carbon monoxide was formed, but the gas consisted almost entirely of carbon dioxide and hydrogen.

The next step was to determine how rapidly carbon monoxide was formed within the living plant. The method of work and the subsequent discussion will be made more clear if preceded by a brief description of the plant.

Fig. 1 shows the plant as it rests almost submerged in the sea water, but anchored to the rock bottom and supporting the streaming fronds from the top of the hollow gas-filled stipe. The plants vary greatly in size; individuals are often 80 to 100 feet in length and contain several liters of gas, usually at reduced pressures.[2] The inside of the gas cavity is relatively quite dry and is lined with a delicate web-like

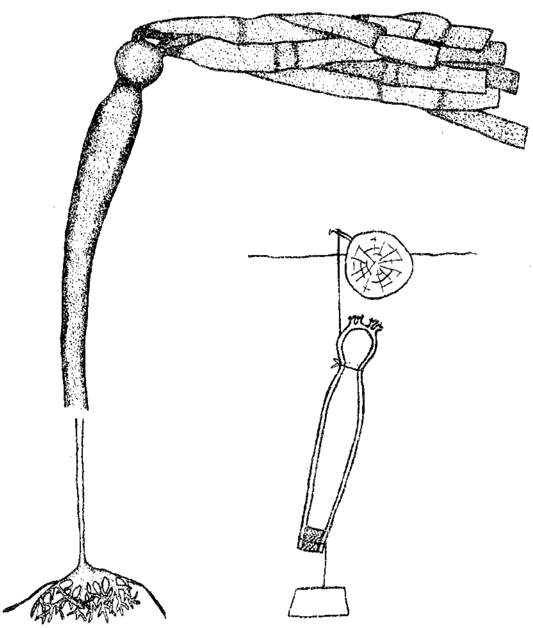

Fig. 1. Fig. 2.

[1] Langdon, This Journal, **39**, 149 (1917).

[2] Frye, *Puget Sound Marine Sta. Pub.*, **1**, 85 (1916).

structure, known as sieve tubes. The plant will withstand a great deal of mutilation and still continue to live and grow if kept in sea water.[1]

It was found practicable to cut off the lower part of the stipe and in the upper part substitute a gas of known composition for that normally present in the pneumatocyst. The cut end was closed by a cork and the weighted plant submerged in the sea tied to a support as shown in Fig. 2. After a suitable interval and changes in the composition of the gas were determined by analysis.

In the first experiments, made primarily to determine the rate of formation of carbon monoxide, air was substituted for the kelp gas. This was accomplished by filling the cut stipe with sea water and then emptying. This process, repeated 3 or 4 times, removed the small bubbles of the original kelp gas that tended to adhere to the delicate sieve tube lining of the interior. The cut end of the now air-filled stipe was corked and the prepared plant anchored near the surface of the sea as previously described.

Analyses of the gases from a series of these cut and air-filled plants were made after various intervals of time. The typical data given in Table I show clearly the gradual formation of carbon monoxide. This is accompanied by a decrease in oxygen content and the appearance of carbon dioxide. The latter is undoubtedly due to processes of decay, since carbon dioxide is not present within the normal plant. In general, the cut and corked section of stipe remained sound enough to be tight for a week or ten days, although evidence of local decomposition was apparent.

The production of carbon monoxide when the stipe was filled with air was confirmed by a large number of determinations with different specimens. In most cases it appeared in quantities greater than 1%. Carbon monoxide was formed by sections cut from any part of the hollow stipe if these were filled with air, corked and similarly suspended in the sea. The leaves, more properly called fronds, seemed to bear no relation to the formation of the carbon monoxide for it was produced just as readily in specimens from which the fronds had been removed.

TABLE I.

Analyses of Gases in Air-Filled Stipes.

Time, start. Hrs.	CO_2. %.	CO. %.	O_2. %.
	0.0	0.0	20.8
24	0.3	0.0	16.5
48	0.0	0.4	13.0
73	0.6	1.0	7.0
97	1.0	3.2	6.2
110	1.1	4.5	5.0

[1] Fallis, *Puget Sound Marine Sta. Pub.*, 1, 1 (1916).

In all of the experiments detailed above, the test specimens, while anchored in the bay, were exposed to the light during the long summer day. The next step was to determine if the carbon monoxide would be formed in the dark.

For this purpose boxes were constructed which were light-tight but which would allow a ready flow of water through them. These boxes were one foot square and 18 feet long. The ends were closed by light traps, the baffle boards of which were inclined in the direction of flow of the water as shown in Fig. 3. The lids were light-tight. All holes and cracks were closed with pitch and the whole interior was painted a dead black.

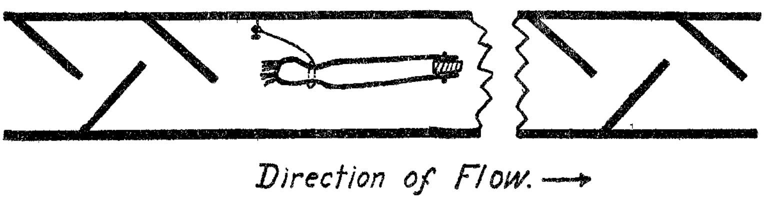

Fig. 3.

The boxes were weighted so as just to float; the waves washed entirely over them except when the water was perfectly quiet. They were anchored in the bay (Friday Harbor, Wash.), where the tidal currents are heavy so that at almost all hours of the day there was a flow of water through them. They were large enough to hold several specimens without materially impeding the flow of water.

In the first experiment the top foot and a half of stipe from the kelp was filled with air by displacement, as previously described, then corked and placed in sea water in the dark boxes, the fronds being removed from half of them. After 5 days in the dark the gas was analyzed. All specimens showed carbon monoxide. The range was from 0.4 to 1.7% with an average of 0.7% of carbon monoxide. The 20.9% of oxygen in the air with which they had been originally filled had practically disappeared and there was about 4% of carbon dioxide. The oxygen was, no doubt, used by respiration and decay processes.

This data was checked by repeated similar series of experiments and it was made certain that in the dark as well as in the light carbon monoxide was formed regularly in the air-filled sections of the stipe, and that there was no relation between its appearance and the presence or absence of the fronds.

An analogous appearance of carbon dioxide and lowering of the oxygen content was shown when unmutilated plants were kept for some time in the dark. The experiment and results are as follows: Twelve whole plants were carefully collected from the same bed. Precautions were taken to avoid in any way disrupting the gas cavity. The gas from 6 of them was analyzed at once and showed an average of 15% of oxygen, 3.2%, of carbon monoxide and no carbon dioxide, the unabsorbed residue being nitrogen. The 6 other plants were placed intact in the dark boxes and after being anchored out in the tidal currents for 6 days showed the following average gas composition: Oxygen 4.7%, carbon monoxide 2.9%, and carbon dioxide 0.5%. There was thus a marked decrease in the oxygen content, an appearance of carbon dioxide, which is not present in the plants when freshly collected, while the carbon monoxide content was practically unaltered. These changes in the oxygen and carbon dioxide content produced by stopping photosynthetic action for a prolonged period throws interesting side-lights on the gas exchange equilibria within the living plant. The formation of the carbon dioxide on protracted standing in the dark shows that gaseous respiration products certainly do find their way into the interior cavity of the plant. Whether or not these gases bear only an incidental relation to the metabolic processes of the kelp has not been determined but should prove a fruitful field for research.

The substitution of gases other than air for those normally present was next undertaken.

As a result of more than 40 carefully executed experiments, in which nitrogen was substituted for the kelp gas, it can be confidently stated that *no carbon monoxide was formed*, either in the light or in the dark, either when the fronds were present or when they had been removed, or at any intermediate time between the initial filling with nitrogen and the 8 to 10 days that elapsed before decay had become so pronounced that observation could no longer be made. It should be remarked that carbon dioxide was formed to the extent of several per cent., even though there was no oxygen present.

Nitrogen prepared by 3 different methods was used. First, by heating ammonium chloride and potassium nitrite, then washing through alkali and then conc. sulfuric acid. Second, from air by absorbing the oxygen in alkaline pyrogallol. Third, the commercial product obtained from the distillation of liquid air. This last contained a little more than a half of one per cent. of oxygen. The character of results was the same for the nitrogen, irrespective of the source.

Similar experiments were carried out in which hydrogen was substituted for kelp gas. The 15 determinations made showed no formation of carbon monoxide within 5 to 7 days, either in the light or in the dark. Here as in the case of the nitrogen-filled kelp several per cent. (1% to 9%) of carbon dioxide was formed. It should be remarked that there was always a marked reduction in pressure for hydrogen filled kelp This amounts to an absorption or an outward diffusion of the hydrogen. The whole relation of hydrogen in this connection deserves a more exhaustive study.

The hydrogen used was from two sources: first, the action of dil. sulfuric acid on the so-called arsenic-free zinc, and, second, electrolytic hydrogen.

A number of sections of kelp stipe were filled with a mixture of nitrogen and oxygen. The gas was 15.2% oxygen and the remainder nitrogen. After 6 days' anchoring out in the sea water carbon monoxide had formed in all cases, the quantities ranging from 0.8% to 2.1%. The oxygen content had decreased and some carbon dioxide was formed just as in the case of the kelp that had been filled with air. Similar results were obtained when a mixture of hydrogen and oxygen was substituted for the gas originally present in the kelp.

The kelp withstands exposure very well and can remain hours or even days out of water and will resume normal activity when returned to the sea, that is, if not too severely sun burned or desiccated. Tightly corked air-filled sections of stipe were found to produce carbon monoxide, either in the light or in the dark, when simply exposed to the air. These plants were still alive, although local decay soon set in. When the substituted gas was nitrogen or hydrogen no carbon monoxide was formed. It appeared only when free oxygen was within the gas cavity.

That the formation of carbon monoxide takes place only within the living plant was shown by its complete failure to be formed in the air-filled stipes of kelp that had been killed. Some of the plants were killed by immersion for 10 minutes in sea water at 50°; others by being placed in 0.02 *N* copper sulfate solution for 18 hours. These filled with air or other gas mixtures containing oxygen failed to produce carbon monoxide whether in the sea water or exposed dry to the air.

Conclusions.

The several per cent. of free carbon monoxide which occurs in the floater of the giant Pacific Coast kelp, *Nereocystis leutkeana*, is considered to be a respiration product for the following reasons: It forms only when oxygen is present within the floater; it forms as readily in the dark as in the light; is not formed by enzyme or fermentation process when the substance of the plant undergoes autolysis and decay, and is not formed in killed plants.

The kelp, *Nereocystis leutkeana*, seemed to be remarkably well adapted to research on the gas exchange of living cells. By the use of the very refined methods of gas analysis some very interesting and valuable information might be gained as to the mechanism of plant processes. It is possible that traces of hydrogen, carbon monoxide, or other gases, not revealed by the technical analytical methods used in this work may be playing unsuspected and perhaps important roles in plant processes.

Conditions have now arisen which make it highly improbable that either of the present authors will pursue this investigation farther, and it is with some regret that we leave this field to other workers.

This research was carried out during the summer of 1917 at the Puget Sound Marine Station, at which time the authors were associated with the University of Washington.

Summary.

1. The existence of several per cent. of carbon monoxide in the gas contained in the Pacific Coast kelp, *Nereocystis luetkeana*, is confirmed.

2. The substance of the kelp, when ground and allowed to undergo autolysis and decay does not form carbon monoxide by enzyme action or fermentation process.

3. Kelp plants, in which the gas normally present within the floater is replaced by air, form several per cent. of carbon monoxide within a few days.

4. The formation of carbon monoxide takes place only when oxygen is present as one of the gases within the floater. No carbon monoxide is formed when the floater is filled with hydrogen or nitrogen.

5. Light does not affect the rate of formation of carbon monoxide.

6. It is concluded that the carbon monoxide is formed as a product of respiration rather than as a intermediate step in photosynthesis.

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A COMPARISON OF THE ACTIVITY OF CERTAIN UNSATURATED GROUPS WITH THE ACTIVITY OF THE ALLYL GROUP IN CERTAIN ETHERS.[1]

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It has long been an established fact that the allyl group is bound only very loosely to oxygen, nitrogen, sulfur, or halogen. As examples of the mobility of the allyl group may be mentioned the ease of rearrangement of allyl thiocyanate to allyl isothiocyanate,[2] the great reactivity of allyl halides,[3] the readiness with which the allyl group is eliminated from certain nitrogen compounds[4] and the rearrangement of O-allyl acetoacetic ester or O-allyl acetylacetone[5] into the corresponding C-substituted compounds. The most striking example, however, is the quantitative

[1] This communication is an abstract of work done by S. G. Powell in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at the University of Illinois.

[2] *Ber.*, **8**, 464 (1875); *Ann.*, **178**, 89 (1875).

[3] *J. Chem. Soc.*, **97**, 416 (1910).

[4] *Ibid.*, **57**, 767 (1890); **104**, 39 (1913); *Ann.*, **382**, 1 (1911); *Ber.*, **33**, 1438, 2728 (1900).

[5] *Ber.*, **45**, 3157 (1912).