solutions, as shown in Expts. 1, 2, 5 and 10. Or, before estimation the lactic acid can be separated from acetic acid, as shown in Expt. 3. Or, it can be separated from formic and acetic acids, as shown in Expt. 6; or, from citric acid, as shown in Expt. 9; or, from propionic acid, as shown in Expt. 4; or, from butyric acid, as shown in Expt. 7; or, from formic, acetic, propionic and butyric acids, as shown in Expt. 8.

Summary.

It has been shown that lactic acid may be estimated as guanidine lactate, identified by its melting point after separation by esterification from citric and tartaric acids and by fractional distillation from formic and acetic acids. Further, it has been shown that lactic acid may be separated from mixtures containing formic, acetic, propionic, butyric and citric acids, and accurately estimated by weighing as quinine lactate, which may be identified by determination of its melting point.

The separation from citric acid and other acids whose ethyl esters also have high boiling points is effected by esterification with the vapor of alcohol, containing dry hydrochloric acid gas in solution passed through the mixture suspended in vaseline at a temperature of 100–110°, using zinc chloride as a second catalyzer; the ethyl lactate passes quantitatively into the distillate, while the ethyl citrate remains in the flask. By fractional distillation of the distillate through a Hempel fractionating column filled with glass beads the ethyl formate and ethyl acetate together with a large part of the ethyl propionate and ethyl butyrate are removed. The residue in the flask, containing the ethyl lactate, is hydrolyzed and converted to the quinine salts and the quinine lactate is separated from the propionate and butyrate by the solubility of the quinine salts of the latter in carbon tetrachloride. The quinine lactate may then be weighed and identified by its melting point.

WASHINGTON, D. C.

CARBON MONOXIDE, OCCURRENCE FREE IN KELP.[1]

(*Nereocystis luetkeana.*)

BY SETH C. LANGDON.

Received October 9, 1916.

Studies have been made by Rosanoff,[2] Willie,[3] and Lucas,[4] on the gases contained in the floaters which buoy up certain marine plants. Their results show an oxygen-nitrogen mixture, the percentage composition of which varies with different plants and conditions. During the summer of

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[1] A more detailed account of this work with extension to other gas-bearing algae is to be printed in the publications of the Puget Sound Marine Station.

[2] *Mem. Soc. Imp. Sci, Nat. Cherbourg*, **13**, 143–240 (1868).

[3] Just's *Bot. Jahresber.*, **17**, 226 (1899); *Chem. Zentr.*, **1890**, I, 1006.

[4] *Proc. Linn Soc., New South Wales*, **36**, 626–631 (1911).

1915 Zeller and Neikirk[1] examined the gas contained in the floater of the large Pacific coast kelp, *Nereocystis luetkeana.* They reported carbon dioxide as well as oxygen and nitrogen. Their figures show an average of 2.503% of CO_2 for gas collected between 3 and 4 A.M. and for gas collected between 3 and 4 P.M. the average was 0.293%. They found that the oxygen content exhibited a corresponding variation with the hour of collection. The nocturnal average for oxygen was 10.906 and the diurnal 12.304. On the basis of this work they arrived at the conclusion that the gas contained in the floater, in addition to buoying the plant up, also served as a reservoir in the gas exchange of the metabolic processes.

At the suggestion of Dr. T. C. Frye, of the University of Washington and Director of the Puget Sound Marine Station, the author undertook to check these results. However, numerous measurements made with more refined methods of work failed to confirm the work of Zeller and Neikirk. This is brought out clearly in the quantitative data given in the last part of this paper, which shows that carbon dioxide was present only occasionally and then in minute traces; also that the oxygen content, average about 18%, runs higher than reported by Zeller and Neikirk, and shows so much variation between individual specimens that generalizations with reference to daily changes with light scarcely seem justified.

Zeller and Neikirk assumed that after the absorption of carbon dioxide and oxygen the gas remaining was nitrogen. On subjecting the gas to more complete analysis the author found carbon monoxide in quantities ranging from 1 to 12%, with an average of about 4%. Since *carbon monoxide has never before been found in the free state in a living plant,* the following qualitative tests were made, to demonstrate its presence, and in every case the reactions were pronounced in their positive character.

Qualitative Determinations.—White paper moistened with palladium chloride turned black in the gas from the kelp but was unchanged in the air. A check with known carbon monoxide also turned the palladium chloride paper black.

Ordinary blood diluted with 200 times its volume of distilled water gave a yellowish red solution which changed to a pink on contact with the gas.

Vogel's spectroscopic method of analysis for carbon monoxide hemoglobin, which is the final toxicologic test, gave positive results, as is clearly shown by the series of photographs given in Fig. I. The characteristic absorption spectrum of dilute normal blood, that is, oxyhemoglobin, shows two dark bands in the yellow and green region. (See E of Fig. I.) D of the same figure shows the absorption spectrum of the same blood after it has been treated with ammonium sulfide and the oxyhemoglobin reduced; the two dark bands which have disappeared are replaced by a

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[1] *Puget Sound Marine Sta. Pub.,* 1, 25–30 (1915).

single, less clearly marked, band. As shown in A, the absorption spectrum of blood which has been treated with known carbon monoxide is almost identical with that of normal oxyhemoglobin (E), but on treatment with ammonium sulfide the carbon monoxide hemoglobin is not reduced and there is no change in the absorption spectrum (see B). Samples of the dilute blood treated with kelp gas gave the absorption spectrum shown in F, which is similar to A and B and was not changed (compare C) by attempted reduction with ammonium sulfide. Hempel ("Methods of Gas Analysis," Dennis' translation of 3rd edition, page 211) says: "This reaction is of especial significance, because the carbon monoxide cannot be confounded with another gas."

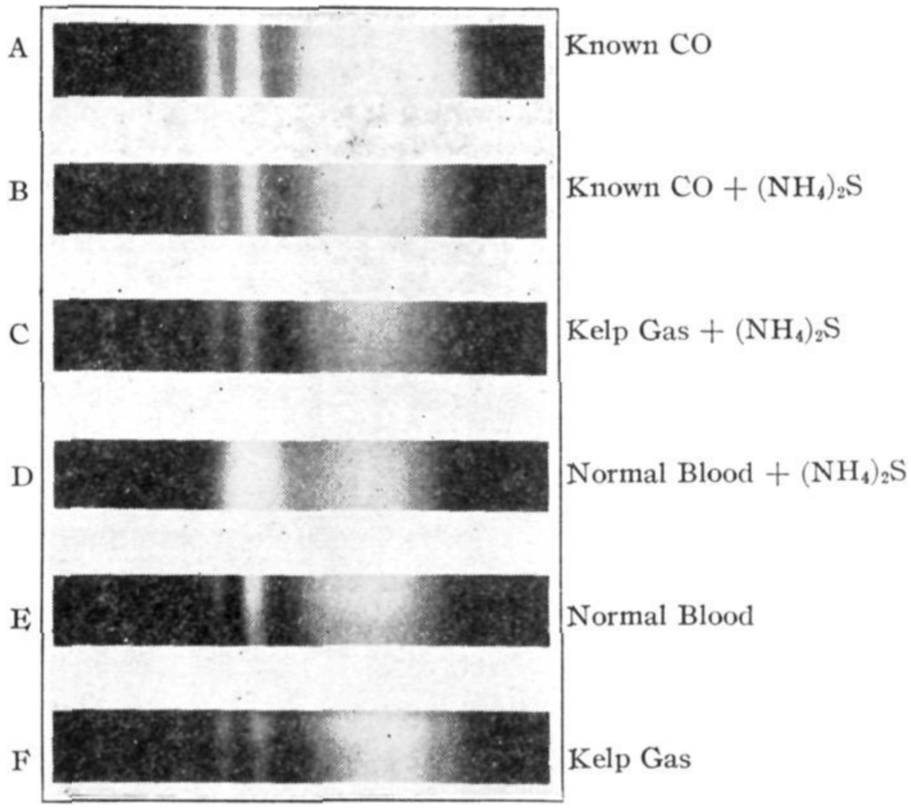

Fig. I.

A guinea pig placed in a vessel through which kelp gas was passing died in less than ten minutes. Death was not due to the absence of oxygen, for the gas contains about 18% of oxygen, and fresh gas was being continuously forced through the vessel. Post-mortem examination of the body showed the characteristic appearances associated with carbon monoxide poisoning. The nose, lips and conjunctivae had assumed a red color. The blood was of a bright scarlet color which seemed permanent, for it was still unchanged after three weeks' exposure in an open evaporating dish. The diluted blood subjected to spectroscopic examination showed the two dark absorption bands, which were not changed on warming the blood with ammonium sulfide. Ferrous sulfate did not cause the blood to darken, as is the case with normal blood. Two cubic centimeters of the "carbon monoxide" blood from the guinea pig, mixed with an equal quantity of water and three drops of a one-third saturated solution of copper sulfate, gave a brick-red precipitate, while normal blood gives a greenish brown precipitate.

A canary bird lived less than fifteen seconds in gas from the kelp bulb. Removal of the feathers from the breast showed that the skin was a mottled red. The internal organs were a bright red and the blood a cherry-red.

A young chicken died in the kelp gas in about 1 minute. Another chick of the same size and age was killed in the same time by a mixture of 1 part illuminating gas (CO, 10%) with 5 parts of air. The blood, subjected to spectroscopic examination and compared with blood from a similar chick killed by decapitation, showed results analogous to those previously discussed. The blood from the chick killed by the kelp gas was not reduced by ammonium sulfide.

The above qualitative tests were quite conclusive, and are preliminary to the discussion of quantitative results, which, however, will be preceded by a brief description of the plant from which this very interesting gas was obtained.

Description of Nereocystis luetkeana.—The kelp *Nereocystis luetkeana* is a brown alga thriving only on the Pacific Coast. Its general appearance is indicated in B of Fig. II. The stipe, or stem, is hollow from the bulb-like top to within a short distance of the holdfast. The walls of the stipe are from a quarter to three-quarters of an inch in thickness and are lined on the inside with a delicate weblike structure which is relatively quite dry. The hollow space, in large specimens, has a capacity of from three to four liters. The gas is almost always under less than atmospheric pressure. Frye reports an average of 77 mm. of mercury below normal air pressure. The full-grown kelp vary greatly in length. The author has worked with specimens as large as 85 feet in length, but much longer ones have been reported. This enormous growth takes place in the short period of 10–15 weeks, the largest and healthiest kelp growing where the current flows the strongest and where there is consequently the most chance for the great surfaces of the fronds to come in contact with fresh sea water, charged with carbon dioxide. Another interesting and perhaps significant feature of this plant is that while it is about 92% water, more than one-fourth of the remaining 8% is potassium chloride.[1]

These facts, coupled with the unprecedented presence of carbon monoxide, make this plant of especial interest from a chemical point of view. Also as a subject for photochemical research marine algae offer an ex-

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[1] Rigg, *J. Ind. Eng. Chem.*, **8**, 568 (1916).

ceptional field, since by reason of their environment they are subject to relatively constant conditions. In addition they are among the simplest of plants.

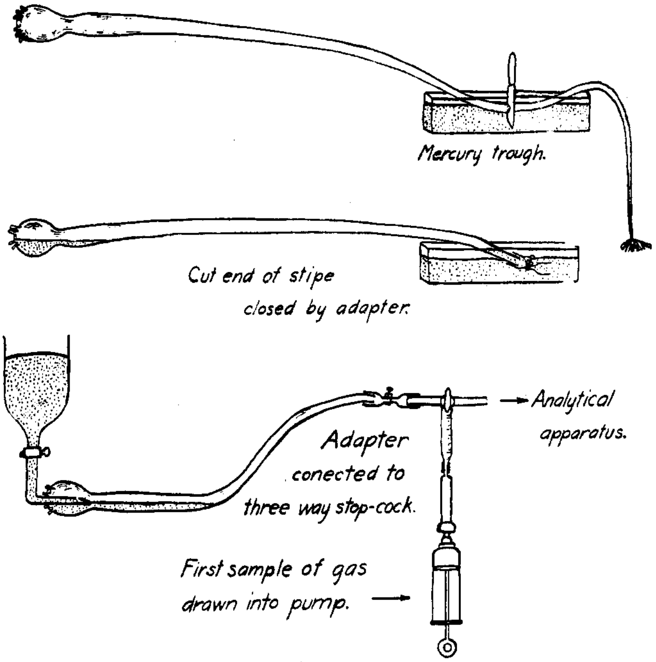

Fig. II.

Quantitative Determinations.—In the early part of the work precautions were taken to prevent loss of carbon dioxide. The method of collecting the gas for examination is illustrated by Fig. II, the gas being forced out of the cavity and into the analytical apparatus by mercury. Thus the gas came in contact only with mercury before being transferred to the absorption apparatus.

The analytical device was a Williams modified Orsat apparatus specially designed for flue gas work, and which gave results that checked within 2 to 3 parts per thousand. Throughout the wörk fresh, carefully prepared, reagents were used and the apparatus was tested daily by analysis of known gases and air.

TABLE I.

Per cent. CO_2.	Per cent. O_2.	Per cent. CO.	Per cent. CO_2.	Per cent. O_2.	Per cent. CO.
0.0	18.8	3.8	0.0	18.0	5.0
0.0	21.2	3.0	0.0	17.4	2.6
0.3	19.5	3.4	0.0	23.0	4.2
0.0	20.0	1.1	0.0	23.4	3.2
0.4	16.8	2.6	0.0	18.6	6.5
0.0	24.7	5.6	0.0	18.3	4.3

Table I gives the results of 12 analyses which are typical of those obtained where special precautions were taken to prevent loss of carbon dioxide. These figures make it clear that carbon dioxide is present only very occasionally and then in small, though unmistakable, quantities.

Similar results were obtained by a more extended series of analyses where the gas was displaced by water saturated with gas from other specimens which had been collected at the same place and time. Mention should be made of one exceptional case in which an apparently healthy kelp showed 1.2 % of carbon dioxide.

That the carbon dioxide content did not vary with the time of day is brought out in Table II.

TABLE II.

Hour collected.	Pressure in mm.	Per cent. CO_2.	Per cent. O_2.	Per cent. CO.	Hour collected.	Pressure in mm.	Per cent. CO_2.	Per cent. O_2.	Per cent. CO.
Midnight	585	0.0	21.2	3.4	11 A.M.	575	0.0	17.3	3.7
Midnight	555	0.0	20.8	5.2	11 A.M.	595	0.0	20.3	2.0
Midnight	555	0.5	22.2	7.0	11 A.M.	540	0.0	17.3	3.7
Midnight	555	0.0	20.1	3.4	11 A.M.	565	0.0	20.0	10.3
Midnight	530	0.0	18.9	1.9					
4 A.M.	625	0.0	18.0	1.3					
4 A.M.	570	0.0	17.8	3.2	5 P.M.	670	0.0	22.1	2.6
4 A.M.	520	0.0	19.8	7.0	5 P.M.	625	0.0	17.8	2.0
4 A.M.	530	0.0	22.4	5.0	5 P.M.	590	0.0	14.4	3.2
4 A.M.	585	0.0	21.2	5.2	5 P.M.	635	0.0	20.2	7.2
4 A.M.	535	0.0	17.4	12.2	....	...	...	...	...
8 A.M.	595	0.0	22.9	7.5	9.30 P.M.	...	0.0	17.5	1.5
8 A.M.	580	0.0	19.9	1.2	9.30 P.M.	...	0.0	16.0	5.4
8 A.M.	555	0.0	19.6	1.7	9.30 P.M.	...	0.0	15.2	1.5
8 A.M.	530	0.0	16.4	4.8	9.30 P.M.	...	0.0	16.0	0.4
8 A.M.	565	0.0	20.0	7.0	9.30 P.M.	...	0.0	19.5	2.2
8 A.M.	585	0.0	16.9	8.6					

The above analyses were made on samples collected July 4, 1916. The day was bright except for about an hour in the early afternoon. The tide was high (+8.7 ft.) between 5 and 6 A.M., then low (+2.3 ft.) at noon and high again (+11.2 ft.) between 8 and 9 P.M.

The occurrence of carbon dioxide, while seemingly irregular, is of interest and deserves investigation by methods more refined than those of commercial gas analysis. The presence of carbon monoxide is of even more striking interest due to its possible relation to the mechanism of photosynthesis. Baeyer's theory, published in 1870,[1] suggests carbon monoxide as an intermediate step in the reduction of carbon dioxide in the photosynthetic processes of plants. However, it is not the purpose of this paper to discuss photosynthesis, but to offer what has heretofore been lacking, namely, experimental evidence of the actual *existence* of carbon monoxide in a living plant.

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[1] *Ber.*, 3, 63–78 (1870).

As yet it has not been determined whether or not this gas functions actively in the metabolic processes of the plant, whether it is an accumulated waste product or is formed by processes of decay. Work is now under way which it is hoped will answer these questions.

During the period from June 14 to August 20, 1916, the gas from a little less than 1000 specimens of *Nereocystis* was examined and almost without exception carbon monoxide was present. Table II gives a rather extended set of measurements all made within 24 hours. The gas was collected (see Fig. III) over sea water and in glass jars which were sealed and then brought to the laboratory for analysis. The samples were in all cases collected from large specimens of kelp from the same location, and as nearly uniform in appearance and size as possible. Column 2, Table II, gives the pressures of the gas as taken just before collection. The figures show such irregularity that there is no evident relation between pressure and chemical composition of the gas. Also for samples collected at the same hour of the day there is great variation in the percentage composition. For example, in the six samples collected at 8 A.M., the range of carbon monoxide content was from 1.2 to 8.6% and the oxygen range from 16.4 to 22.9%. The existence of such great variation between individuals would necessitate the analysis of an enormous number of samples in order to obtain average values, which would indicate whether the composition of the gas varies with the time of day, that is, with the light intensity. The rate of flow of water past the plant may have some bearing on the composition of the gas and it appears to be true that the highest

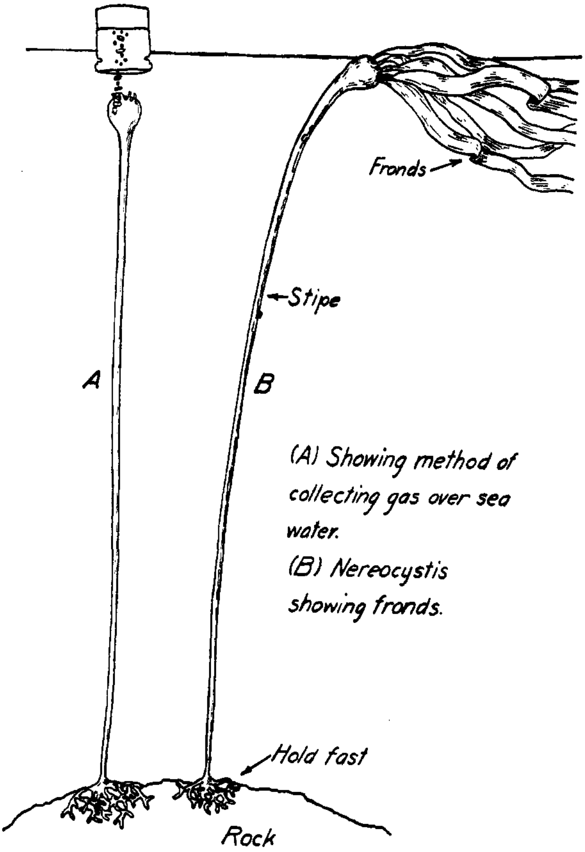

Fig. III.

carbon monoxide content is to be found in those larger and more healthy kelps which grow where the tidal currents are the swiftest. The mature kelps have a higher carbon monoxide content than do the young ones. Repeated analyses were made with the Morehead technical gas apparatus and these failed to show the presence of hydrogen or hydrocarbon gases, either saturated or unsaturated.

Summary.

In this paper the following points have been established:

1. The gas in the floater of the giant kelp, *Nereocystis luetkeana*, contains carbon monoxide, the quantity varying considerably in different individuals.

2. The presence of carbon dioxide is only occasional and the quantity minute.

3. Previous work, which tended to show that the quantities of carbon dioxide and oxygen vary with the time of day, has not been confirmed.

In conclusion the author wishes to express his gratitude to Dr. T. C. Frye, of the Puget Sound Marine Station, for many courtesies extended during the course of this research.

Chemistry Department, University of Washington,
Seattle, Washington.

[Contribution from the Chemical Laboratory of the Illinois Wesleyan University.]

RATE OF TURBIDITY IN BEVERAGES CONTAINING MALTOSE, GLUCOSE, OR MALTOSE AND GLUCOSE.

By A. W. Homberger and C. S. Marvel.

Received November 13, 1916.

Much time has been spent in investigating the causes and rate of turbidity in beverages containing maltose, glucose, or maltose and glucose combined. Since this type applies mostly to beverages on the order of beers, there have been many explanations offered and essentially all of them attribute the turbidity to albuminous materials, which have precipitated out of solution.

Parsons[1] refers to the difficulty of the preserving, for any great length of time, a malt substitute made from glucose as the sole source of alcohol.

Will[2] says that crystals of calcium oxalate are found in small quantities in beer and reports a case where turbidity of beer was caused by an unusually large quantity of these crystals. He suggests that the separation is due to the removal of some protective colloid, which ordinarily would have held it in solution.

Schoenfeld and Hirt[3] have shown that beer which has been in contact

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[1] Parsons, This Journal, **24**, 1170 (1902).

[2] Will, *Z. Ges. Brau.*, **33**, 129–132 (1911).

[3] Schoenfeld and Hirt, *Wochschr. Brau.*, **27**, 633–635 (1911).