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.

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

BY A. W. HOMBERGER AND C. S. MARVEL.

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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

[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).

with tin will show turbidity, due to albumin precipitation. The turbidity is due to the action of the acid content of the beer. These men have shown[1] that the acid content of beers affects their stability, at ordinary temperature. A reduction of acidity causes turbidity in a few days. A slight increase in acid content prevents changes. Further increase in acidity causes a turbidity, which increases in intensity in proportion to the acidity. This precipitate is hop resins and albuminous material.

Emslander[2] says that the separation of albumin from beer is caused by chemical changes brought about by electrolytes, which result in the coagulation of the albuminous material.

In order to determine more fully the causes of the appearance of turbidity in beverages apt to contain small quantities of maltose, glucose, or both, a series of experiments were undertaken, in which solutions of known composition were used. The solutions contained different combinations of glucose, maltose, alcohol, carbon dioxide, and water. Precautions were observed to prevent contamination from microörganisms which might cause abnormal results.

The solutions were made up in three sets. The first set contained varying proportions of glucose, alcohol, carbon dioxide, and water. In the second set maltose was used instead of glucose. In the third set, equal parts of glucose and maltose were used.

The solutions were made up by dissolving the sugar in a known volume of boiling distilled water and then adding the alcohol. The solutions were put in bottles of thick green glass, like the ordinary beer bottle. The bottles were first sterilized by boiling in water for about an hour. The stoppers used were corks which had been saturated with paraffin to render them air-tight. All solutions, except those saturated with carbon dioxide, were sealed hot, by means of sealing wax over the paraffin corks. The solutions which were saturated with carbon dioxide were bottled and cooled. They were then placed in an ice-salt freezing mixture and saturated with carbon dioxide which was generated by dropping hydrochloric acid on marble. The bottles were then sealed in the manner described above.

The solutions were allowed to stand several weeks and were then examined to see what changes they had undergone. The analyses for glucose and maltose, when separate, were made according to the methods adopted by the Association of Official Agricultural Chemists, as given in Bulletin 107 (revised) of the Bureau of Chemistry. The methods were first carefully tried on solutions of known composition in order to insure they were suited to this work. The gravimetric method, using

[1] Schoenfeld and Hirt, *Wochschr. Brau.*, 28, 649–657 (1911).

[2] Emslander, *Kolloid Chem. Beihefte*, 374–384; through *J. Chem. Soc.*, 100, I, 935 (1912).

TABLE I.

Sugar.	Amount alcohol.	Amt. CO_2.	No. days allowed to stand.	Sugar used. %.	Sugar found. %.	Sugar loss. %.	Appearance.	No. days until change.
Glucose.....	None	None	74	1.25	0.9745	0.2755	Mouldy growth	2 days
Glucose.....	None	None	53	2.5	2.138	0.3627	Mouldy growth	2 days
Glucose.....	3%	None	45	1.25	1.145	0.105	Sediment	2 days
Glucose.....	2%	None	46	1.25	1.115	0.135	Sediment	2 days
Glucose.....	1%	None	47	1.25	1.148	0.102	Sediment	2 days
Glucose.....	0.5%	None	47	1.25	1.1635	0.096	Sediment	2 days
Glucose.....	3%	Saturated	46	1.25	1.143	0.107	Sediment	2 days
Glucose.....	2%	Saturated	47	1.25	1.145	0.105	Sediment	2 days
Glucose.....	1%	Saturated	42	1.25	1.1125	0.1375	Sediment	1 day
Glucose.....	0.5%	Saturated	54	1.25	1.114	0.136	Sediment	1 day
Glucose.....	None	Saturated	41	1.25	1.1035	0.1465	Sediment	17 days
Glucose.....	None	Saturated	41	1.25	1.1025	0.1475	Feathery growth	17 days
Maltose.....	3%	None	41	1.25	1.125	0.1245	Clear	
Maltose.....	2%	None	41	1.25	1.1075	0.1425	Clear	
Maltose.....	1%	None	41	1.25	1.1095	0.1405	Clear	
Maltose.....	0.5%	None	41	1.25	1.097	0.141	Clear	
Maltose.....	None	None	41	1.25	1.09	0.141	Clear	
Maltose.....	None	Saturated	41	1.25	1.165	0.085	Clear	
Maltose.....	3%	Saturated	41	1.25	1.1305	0.1195	Clear	
Maltose.....	2%	Saturated	41	1.25	1.1805	0.069	Clear	

Maltose.....	1%	Saturated	41	1.25	1.166	0.084	Clear	
Maltose.....	0.5%	Saturated	41	1.25	1.1565	0.0935	Clear	
Maltose.....	None	None	40	0.625	0.6006	0.0144	Sediment	Not noticed
Glucose.....				0.625	0.5593	0.0657		
Maltose.....	3%	None	41	0.625	0.6175	0.0075	Sediment	Not noticed
Glucose.....				0.625	0.5868	0.0382		
Maltose.....	2%	None	42	0.625	0.5594	0.0656	Feathery growth	Not noticed
Glucose.....				0.625	0.6214	0.0036		
Maltose.....	1%	None	40	0.625	0.572	0.043	Growth and sediment	31 days
Glucose.....				0.625	0.588	0.037		
Maltose.....	0.5%	None	40	0.625	0.6209	0.0041	Sediment	31 days
Glucose.....				0.625	0.527	0.0971		
Maltose.....	None	Saturated	40	0.625	0.585	0.0383	Sediment	31 days
Glucose.....				0.625	0.594	0.031		
Maltose.....	3%	Saturated		0.625	0.526	0.091	Sediment	31 days
Glucose.....				0.625	0.614	0.0104		
Maltose.....	2%	Saturated	40	0.625	0.569	0.056	Sediment	31 days
Glucose.....				0.625	0.594	0.031		
Maltose.....	1%	Saturated	40	0.625	0.6132	0.0118	Sediment	31 days
Glucose.....				0.625	0.5455	0.0745		
Maltose.....	0.5%	Saturated	40	0.625	0.6117	0.0133	Sediment	31 days
Glucose.....				0.625	0.5535	0.0715		

Soxhlet's modification of Fehling's solution and weighing the cuprous oxide directly, gave the best results.

When both maltose and glucose occurred in the same solution they were determined by the method adopted by the Association of Official Agricultural Chemists as given in Bulletin No. 132 of the Bureau of Chemistry under the report on beer. This method makes use of the optical rotation and the weight of the cuprous oxide obtained in the usual manner. The formula used to calculate glucose is

$$D = \frac{6.23\ C\text{-}p}{10.6}$$

where D equals the grams of glucose per 100 cc.; p the polarization due to the sugars and C the cuprous oxide reduced by 100 cc. of beer. The formula for maltose is

$$M = \frac{p - 1.38\ C}{6.3}$$

where M equals the number of grams of maltose per 100 cc. and the other letters have the same value as in the preceding formula. This method was carefully tested on solutions of known concentration before it was applied to the analysis of the unknown sugar solution. The results showed the method to be sufficiently accurate to use in these experiments.

The results of these analyses and observations are summarized in Table I.

The loss in the sugar content of the solutions is very slight in most cases and might be due, easily, to experimental error. In the first two solutions some sugar seems to have been used up in some manner.

In order to determine the cause of the turbidity and sediment in the glucose solutions, some of the sediment was taken from the solutions and examined with the microscope. The sediment showed an organized structure similar to the mycelium of a mould. When a sterile nutrient solution was inoculated with the sediment, growth appeared in a few days. This growth was shown to be the common green mould, *Penicillium glaucum*.

Another set of experiments was then undertaken to show what effect *Penicillium glaucum* would have in solutions of maltose and glucose in nutrient media under aerobic conditions. Laurent's yeast medium, which contains the necessary inorganic ions for the growth of the mould, was used as the basis for the nutrient solution. To 25 cc. of this solution were added 1.25 g. of glucose. The solution was then thoroughly sterilized by boiling. Two such solutions were inoculated with spores from *Penicillium glaucum* and allowed to stand at room temperature. The mould appeared in about two days and thereafter grew rapidly. The solutions became very turbid. After standing fifteen days the solutions were fil-

tered and made up to 100 cc. and analyzed. In the first solution it was found that the mould had used up 0.772 g. of glucose and in the second solution 0.618 g. of glucose to form alcohol, lactic acid, fat and protein.

A similar experiment was carried out using maltose. The growth appeared in two days and the solution became turbid. Spores appeared in four days. The solutions were allowed to stand fifteen days and were analyzed in the manner described above. In one solution the mould had used up 0.5207 g. of maltose and in the other it had used up 0.5749 g. maltose. The products found in the maltose solution were the same as those found in the glucose solutions.

The results of these experiments show that the mould used up more glucose than maltose in a given time. The growth on the glucose medium was heavier. Spores were formed more quickly on the maltose medium. These facts tend to show that maltose is a less favorable medium for the growth of *Penicillium glaucum* than is glucose.

Since the sediment from the bottles containing glucose solutions will grow into the common form of the mould *Penicillium glaucum*, when placed under favorable conditions, the sediment must be spores of the mould, which have germinated and grown to a slight extent. The appearance of a growth of this kind in a solution which does not contain nitrogen, potassium or phosphorus is uncommon. Klebs[1] has shown that the spores of *Aspergillus repens* will not germinate in pure water, or in inorganic nutrient solutions or even in peptone solutions, unless some inorganic salt such as saltpeter is added, but in a 0.5% solution of grape sugar germination does take place. The action of the grape sugar must be explained as that of a chemical stimulus.

In our experiments the spores of *Penicillium glaucum* must not have been destroyed even under the precautions used. The glucose acted as a chemical stimulus and thus caused germination of the spores. Since no mineral ingredients were present, continued development of the mould was impossible. The turbidity and sediment which appeared in the glucose solutions seem to have been caused by these germinating spores. Maltose does not act as a stimulus on the spores, as is shown by the absence of turbidity and sediment in the solution containing maltose alone. The difference in the time period before appearance of turbidity can be explained only on the assumption that the glucose acts as a chemical stimulus to the spore. The solutions containing less glucose would develop turbidity less quickly.

Summary.

From the preceding work the following conclusions may be drawn:

First: That turbidity may occur in solutions containing glucose without the presence of albuminous or mineral constituents.

[1] Jörgensen, "Microörganisms and Fermentation," p. 174.

Second: That the turbidity is due to germination of the spores of the mould *Penicillium glaucum*, due to the action of glucose as a chemical stimulus.

Third: That the presence of alcohol and carbon dioxide have no appreciable effect on the appearance of the turbidity in glucose solutions.

Fourth: That maltose does not act as a chemical stimulus on spores of *Penicillium glaucum* and hence maltose solutions do not grow turbid on standing.

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[CONTRIBUTION FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

ON THE ASSUMED DESTRUCTION OF TRYPSIN BY PEPSIN AND ACID. II.[1] OBSERVATIONS ON ANIMALS.

BY J. H. LONG AND MARY HULL.

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In our recent paper with the above title[2] we showed by a long series of experiments *in vitro* that the common proteolytic enzyme of the pancreas, isolated as trypsin, is capable of withstanding a rather long digestion in presence of hydrochloric acid and pepsin, provided sufficient protein of some form is present to combine with all or part of the acid and so bring the hydrogen-ion concentration down to a certain level. This behavior of protein is of great practical importance, and failure to recognize it led to much confusion in the study of the mutual action of certain enzymes. It was further brought out that trypsin must exhibit a certain degree of activity in solutions which have a faintly acid reaction, rather than the usual alkaline reaction. This is contrary to the long accepted notion that this enzyme is active in alkaline medium only, but many phenomena point to this relation.

In the subsequent prosecution of the work we have relied on evidence secured through experiments on animals, partly with dogs and partly with the human subject. Satisfactory methods were not immediately available. In the work with dogs our first attempts were in this direction. Duodenal fistulas were made in a number of animals at a point between the pylorus and the entrance of the main pancreatic duct. A bent glass canula introduced into this opening was provided with an expanded end and so turned that it would collect the chyme flow from the stomach, but would prevent the upward flow of the bile and pancreatic secretion. Working in this way it appeared possible to obtain the unmixed

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[2] THIS JOURNAL, 38, 1620 (1916).