

tion to an enzyme. The data which led him to these conclusions were the fact that he could dry leaves for a few days at temperatures not above 30 degrees Centigrade and they would still evolve oxygen when moistened and illuminated.

In repeating Molisch's work it was found that the results could be confirmed readily with some plants but not with others. It was also found that if the plant leaves were ground with the proper buffers and then dried there was loss of the power to evolve oxygen. Luminous bacteria were used by both Molisch and the author to test for the release of free oxygen.

In undertaking further experiments along this line several methods of inhibiting the evolution of oxygen by irradiated organisms containing chlorophyll have been tried.

*Nitella* sp. cells were cut and the contents squeezed out and immediately tested for the evolution of oxygen. The results indicate that a positive test can be found if there is only a few minutes' delay between the time the cell contents are extruded and the test for the evolution of oxygen. The cell contents of *Valonia macrophysa*,<sup>2</sup> mixed with luminous bacteria, remained capable of evolving oxygen for two hours after cell disorganization. These cells were about one centimeter in diameter and even the evolution of oxygen from a single cell could be easily detected.

Press juice obtained by placing lawn clover (*Trifolium repens*) under high pressure evolved oxygen readily. Microscopic examination showed that no cells were present.

The reactions of *Euglena viridis* to low temperatures were the basis for the most of the present work. The *Euglena* cultures were grown in diffuse or artificial light at room temperatures.

When *Euglena* is kept at -4 degrees Centigrade for four hours and then brought back to room temperature (about 20 degrees Centigrade) the microscopic appearance indicates some plasmolysis and a tendency for the cells to become spherical. The evolution of oxygen is very weak and sometimes negative, apparently depending on the state of the culture used. At -40 degrees Centigrade for 20 minutes, with about one hour at room temperature to recover, there is generally evolution of oxygen when tests are made immediately. In fact, some cultures were frozen eight hours at -40 degrees Centigrade and tests for the evolution of oxygen made at once were positive. In all cases where freezing was at -40 degrees Centigrade for six hours the evolution of oxygen was only a temporary matter and the cultures would no longer grow in nutrient media. Tests for the absorption of carbon dioxide in a closed tube using phenolsulfonephthalein as an indicator were negative after four hours' freezing at -4

degrees Centigrade or one hour at -40 degrees Centigrade. Many experiments demonstrated that when both the absorption of carbon dioxide and the evolution of oxygen were negative the cultures would no longer grow. In many cases there was evidence that no carbon dioxide absorption was taking place in light, even though there was a temporary evolution of oxygen by the culture.

*Euglena*, *Spirogyra* and moss leaves showed much variability to freezing, and subsequent tests for the evolution of oxygen seemed to show that in some organisms there was a storage of some substance which would evolve oxygen for a short period of time, even though cells were absent or were so injured that they did not recover. One is reminded of the storage of luciferin-luciferase by *Cypridina* so that the protein enzyme complex can be extracted and perform its reaction of evolving light *in vitro*, but the inability to do the same with luminous bacteria. The explanation usually given for this observation is that the luminous bacteria do not store the luciferin-luciferase system, while *Cypridina* does.

These experiments tend to support the assumption that the evolution of oxygen is an enzyme reaction depending on radiation and not necessarily on the living cell except for the formation of the substance. This is further confirmed by the fact that hundreds of tests made on cell contents have shown that once the power to evolve oxygen is depleted in cell-free material, there is never any recovery. This would probably mean that there is a good chance that the complex responsible for the evolution of oxygen upon irradiation may be isolated, but it does not help much in looking forward to the understanding of how the living cell builds the oxygen-evolving substance.

It seems clear that we may conclude that the cells of some green plants may be disorganized and killed and yet retain for a short time some of their power to evolve oxygen upon irradiation. It is, however, very doubtful whether the absorption of carbon dioxide takes place so that a regular photosynthetic cycle is set up in such triturates. This points to the fact that there is, after all, a close relationship existing between the whole mechanism of photosynthesis and the organized living green plant cell.

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#### A SULFOXIDE OF METHIONINE

OWING to the biological interest attached to the sulfur-containing amino acid methionine any of its oxidation products are of potential significance. When a solution of dl-methionine perchlorate in isopropyl alcohol is allowed to react with an excess of hydrogen peroxide the amount of oxygen consumed upon com-

<sup>2</sup> These *Valonia* cells were supplied by Dr. W. J. V. Osterhout, of the Rockefeller Institute, New York City.

pletion of the reaction corresponds to one atom per molecule of methionine. Neutralization of the perchloric acid with amylamine causes a precipitate that consists, according to analysis and properties, of practically pure methionine sulfoxide in a yield of over 90 per cent. The amorphous precipitate can be converted into the form of microcrystalline aggregates by careful precipitation by acetone from aqueous or aqueous-methanolic solution. The product decomposes at 220–230° and appears to be more soluble than any of the natural amino acids: 1 gram dissolves in about 1.5 cc of water, while about 30 cc are required by the parent substance dl-methionine. The solubility in methanol seems similarly increased. The acidity of an aqueous solution is that of a typical neutral amino acid (pH about 4.5). The compound does not oxidize iodide under the conditions which cause complete deoxygenation of cystine disulfoxide,<sup>1</sup> but concentrated (57 per cent.) hydriodic acid liberates almost instantaneously the expected amount of iodine.

This product, which has not been isolated previously, although it has been discussed,<sup>2</sup> is of interest (a) chemically, because it should consist of a mixture of equal parts of four stereoisomeric structures as the molecule contains, in addition to the "asymmetric" carbon atom, an "asymmetric" sulfur atom; (b) technically, on account of its high solubility which might be utilized in the isolation of the natural l-methionine; and (c) biologically, in connection with Hammett's<sup>3</sup> theory<sup>4</sup> on the role of intermediate oxides of sulfur in the control of cell division. Separation of the four isomers seems desirable and should also be of interest in relation with the problems of the metabolic decomposition of methionine.

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## COMMENTS ON THE SHAPE, GROWTH AND QUALITY OF THE AMERICAN OYSTER

LUNZ<sup>1</sup> gave data showing that oyster shells from Indian shell heaps are larger than present-day oysters from South Carolina commercial beds and stated that small size of the latter is probably due to intensive commercial oystering, which prevents maximum growth. He stated that the evident quality of pre-colonial oysters far surpassed those of to-day. The

situation is more complex than the short treatment indicated.

Unfortunately, there was a misstatement about the percentages by which the pre-colonial oysters exceeded present oysters in size. Ordinary pre-colonial oysters were said to be 62.23 per cent. longer and 76.89 per cent. wider than modern oysters. The correct figures are 60.67 and 42.61, respectively, according to the data.

Oysters take various shapes imposed on them by contact with their fellows or other solid materials, but there are two general shapes. On hard bottom with sufficient room oysters grow almost as wide as long and in rare instances wider. The long axis of growth curves to the right. Illustrative of this type are wild oysters of Karankawa Bay, Texas, mentioned by Galtsoff.<sup>2</sup> Round oysters have heavy shells with both valves cupped. They have a large adductor muscle, are usually fat, have a high gallon yield per barrel of shell stock and shrink little due to loss of fluid after being opened, so that they are always in demand by the trade.

Where oysters are crowded or growing on soft bottom and slowly sinking the shell grows straight, upright and very long. The shell is thin, much longer than wide, the bottom (left) valve alone is troughed, the adductor muscle grows rapidly forward and smaller in proportion to the size of the animal than in round oysters. These oysters are known as snappers or coons. Galtsoff and Luce<sup>3</sup> discussed conditions which produce them and stated that in "old times" coon oysters existed in Long Island Sound and Connecticut, with the unstated inference that these have given way to the rounder cultivated type.

Coon oysters from Coon Island, Matagorda Bay, culled for market size by an oysterman, were selected for straightness and the top valve length and width measured with a vernier caliper. The small culls were also measured. Oysters from Wells Point, Matagorda Bay, selected for market size and roundness, were measured. Measurements of unattached seed oysters from the same locality, unselected, although they were of the round type, were on hand. These data were compared with those of Lunz (changed to centimeters for comparison) in the table. Measurements of the lower valve from hinge to bill, as made by Lunz, are slightly shorter than the upper valve.

Examination of the length/width ratios show that selected oysters from Indian shell heaps were "coons," as were selected oysters from South Carolina commercial beds. Likewise, average oysters from shell piles were nearer the coon type than average oysters from

<sup>1</sup> *Jour. Biol. Chem.*, 113: 583, 1936.

<sup>2</sup> *Biochem. Jour.*, 22: 1417, 1928, and 26: 2041, 1932.

<sup>3</sup> *Protoplasma*, 11: 382, 1930.

<sup>4</sup> In its application to neoplasia a new confirmation of this theory, by sulfur oxides of the sulfanilamide type (*Biochem. Jour.*, 32, 1207, 1938, has just been added to an earlier confirmation by cystine disulfoxide (*Am. Jour. Cancer*, 26: 554, 1936).

<sup>1</sup> G. Robert Lunz, Jr., *SCIENCE*, 87: 367, 1938.

<sup>2</sup> P. S. Galtsoff, *Bur. Fish. Invest. Rep. No. 6*, 1–30, 1931.

<sup>3</sup> P. S. Galtsoff and R. H. Luce, *Bur. Fish. Doc. 1077*: 61–100, 1930.