

remains of fruits and seeds, which is supposed to be more certainly determinable than leaf impressions. It has been laboriously compared with recent material in the Kew herbarium and from other sources and is illustrated by enlarged photographs often showing the recent seed by the side of the fossil. One is impressed with the care with which the work has been done and the authors certainly merit the gratitude of their confrères. I venture to hope that they will feel called upon to give us the benefit of their experience in instituting a comparison, confessedly difficult, between their Pliocene fruit and seed floras of Reuver, Tegelen, Cromer, etc., and the abundant Pliocene floras represented by leaves in France, Italy and throughout southeastern Europe.

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#### SPECIAL ARTICLES

##### THE MEASUREMENT OF OXIDATION IN THE SEA-URCHIN EGG

BECAUSE of its accuracy and convenience, Winkler's method of determining the amount of oxygen in solution has been almost exclusively used in the various studies of oxygen consumption of sea-urchin eggs. This method, as described in various texts of quantitative analysis, depends upon a chain of reactions, which result finally in the liberation of two atoms of iodine for each atom of oxygen originally present in solution. The investigator of egg oxidations measures the oxygen content of some sea-water before and after eggs have been contained in it. The usual procedure appears to be about as follows: The eggs are enclosed in a 300 c.c. bottle filled with sea-water and tightly sealed. At the conclusion of a certain time interval (usually an hour), the supernatant sea-water is siphoned off into a 250 c.c. bottle and tested for oxygen. From the value thus obtained, the oxygen concentration in the 300 c.c. bottle at the conclusion of the experiment can be computed, and thus if the original oxygen content of the sea-water is known, the amount of oxygen consumption is readily obtained by subtraction.

It is obvious that the ordinary Winkler method of determining oxygen loses its efficiency in the presence of any substance which takes up iodine. Now it is a fact that iodine absorbing substances are actually present in sea-water which has stood over sea-urchin eggs. This can best be shown by actual measurement of the iodine absorption of such "egg sea-water."<sup>1</sup> These measurements have been made a number of times. They show a small but quite constant value.

Of course after the eggs have been treated with any cytolytic agent, they give off to the sea-water very much larger quantities of iodine-absorbing substances.

Analytical chemists have suggested at least two methods of making Winkler determinations in the presence of organic substances. Perhaps the Rideal and Stewart method is the one most often used.<sup>2</sup> In this method the organic substances are oxidized by potassium permanganate in the presence of sulphuric acid. This method may do very well for most organic substances, but in order to oxidize proteins completely, hot concentrated permanganate solutions are necessary, and the dilute solutions recommended by Rideal and Stewart can accomplish but very little in the way of oxidations. The extensive literature on the oxidation of proteins by permanganate solutions can not be referred to here; the reader will find many references in Oppenheimer's "Handbuch der Biochemie."<sup>3</sup> In actual practise the Rideal and Stewart method has not proved satisfactory.

Another method is to determine the iodine-absorbing powers of the water which contains organic matter.<sup>4</sup> In this way a correction is obtained which is added to the value determined by the ordinary Winkler method. In measuring egg oxidations, this method is open to the objection that the sample chosen for the correction may not be truly representative of

<sup>1</sup> I. e., sea-water which has stood over eggs.

<sup>2</sup> Rideal and Stewart, *Analyst*, XXVI., 141, 1901.

<sup>3</sup> Vol. 1, pp. 489-495.

<sup>4</sup> Cf. Lunge, "Technical Methods of Chemical Analysis," New York, 1908, Vol. 1, Part II., p. 783.

the entire volume of "egg sea-water." The presence of eggs at the bottom of the bottle makes impossible the thorough mixing which should precede the taking of a sample.

Sea-water which has stood over eggs in shallow beakers exposed to the air always gives much lower values for oxygen content than ordinary sea-water at the same temperature. The difference is of the same order of magnitude as the amount of oxygen used by the eggs in an hour. But it is not due to oxygen consumption, for the "egg sea-water" may be siphoned off and allowed to remain several hours in contact with air, so that equilibrium is certainly established. The difference is of course in part due to the iodine absorption of "egg sea-water," but not wholly so. For if we test a representative sample and obtain the necessary correction for iodine absorption, a difference still remains. If we assume that our method is accurate, we are led to the conclusion that the solubility of oxygen in sea-water is lowered by some substance or substances secreted by, or dissolved away from the eggs. This is not at all unusual, if we remember that Findlay and his collaborators have shown that many colloidal substances exert a well-marked influence on the solubility of gases.<sup>5</sup> Granted that our conclusion is correct, no method of measuring oxidations that depends on a change of oxygen tension (*e. g.*, the Warburg-Siebeck method<sup>6</sup>) is accurate. For any such method assumes that the oxygen solubility of the sea-water remains constant.

Another method of making determinations was devised in the summer of 1914. It was found that the iodine-absorbing substances normally given off by *Arbacia* eggs are colloidal. They do not diffuse through celloidin or parchment membranes. In the measurement of egg oxidations, therefore, the eggs may be enclosed in celloidin tubes instead of being allowed to lie free in the sea-water. Tubes of about 10 c.c. capacity and of narrow bore fit nicely into 300 c.c. bottles. At the conclusion

<sup>5</sup> *Jour. Chem. Soc. Trans.*, XCVII., 536, 1910; CI., 1,459, 1912; CIII., 636, 1913; CV., 291, 1914.

<sup>6</sup> O. Warburg, *Zeit. f. physiol. Chemie*, XCII., 231, 1914.

of an experiment, the tube containing eggs is taken out, the bottle is filled to the top with sea-water of known oxygen content and is tested for oxygen by the Winkler method. The use of the celloidin tube has another advantage, in that oxygen determinations may be made in the same bottle in which the eggs were kept. Thus, siphoning is unnecessary and there is no error from this source. The tube method is, however, open to the objection that in the case of sea-urchin eggs at least, development can not take place if the eggs are too closely packed. Without modification it can therefore not be used for the measurement of oxidations during cleavage.

Determinations have been made both by this tube method and by adding corrections for iodine absorption. The results gained so far are not sufficiently accurate to warrant publication. They do show, however, that partial or complete cytolysis produced by dilute sea-water causes not an increase, but a decrease of oxidations.<sup>7</sup>

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July 26, 1915

A BACTERIAL DISEASE OF WESTERN WHEAT-GRASS.  
FIRST ACCOUNT OF THE OCCURRENCE OF A  
NEW TYPE OF BACTERIAL DISEASE  
IN AMERICA

A VERY unusual type of bacterial disease has been found occurring on western wheat-grass, *Agropyron smithii* Rydb., in the Salt Lake Valley, Utah, and has been given considerable study by the writer during the current season. Although affected plants are usually somewhat dwarfed, the most striking characteristic of the disease is the presence of enormous masses of surface bacteria which form a lemon-yellow ooze or slime. Sometimes this bacterial slime appears in small droplets, but very often it is spread over the surface of the upper portion of the plant including the sheath, upper internode and inflorescence. The glumes which are badly attacked reveal bacterial layers of slime

<sup>7</sup> The tube method can not be used for completely cytolized eggs, as the egg pigment wanders through the walls of the celloidin tubes.