# Oxidation in Sea Water.

By

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With 3 Figures in the Text.

Positions of stations referred to in the tables and text.

L1, in Plymouth Sound close to Mallard Buoy.
L2, 200 yards off western end of Plymouth Breakwater.
L3, 50° 18′ N., 4° 11′ W.
L4, 50° 15′ N., 4° 13′ W.
L5, off Eddystone Rocks, 50° 11′ N., 4° 18′ W.
L6, 50° 06′ N., 4° 20′ W.
E1, 10 miles south of Eddystone, 50° 02′ N., 4° 22′ W.
E2, 49° 27′ N., 4° 42′ W.
E3, off Ushant, 48° 34′ N., 5° 13′ W.

During the course of an investigation of the factors which affect the life of marine organisms *in vitro*, attention was directed to the oxidation of organic matter in sea-water. When a typical, open sea, free-swimming organism such as *Calanus finmarchicus* was kept in a limited quantity of water, its length of life was shortened if bacterial action (putrefaction) was apparent in the water. This is the experience of many others.

The very potent effect of the bacteria is due, in part at all events, to the soluble products of putrefaction set free. If the sea-water to which a trace of putrefiable organic matter has been added, is allowed to stand until putrefaction is complete and the bacteria begin to die down, and then filtered through a porcelain candle, the filtrate retains some of the toxicity of the bacteria laden water. Part at least of the toxic products remain some days in air-saturated water without being oxidised to non-toxic substances. Sea-water, to which a little sugar has been added and which shows a faint cloudiness due to bacteria, is very decidedly less toxic to Calanus than sea-water rendered very faintly cloudy by the development of bacteria on added traces of peptone or other nitrogenous organic matter. The specific toxic action of traces of peptone itself, if any, cannot readily be determined, owing to inevitable contamination with bacteria on the Calanus. This suggests that the toxicity is largely due to nitrogenous products of putrefaction, some of which with-

stand oxidation for a considerable time. The growth of green algæ in a toxic water appears to destroy most of the toxic substances.

Preliminary experiments showed that the length of life of Calanus in vitro was not markedly affected by variations in hydrogen ion concentration, by pH between 8·2 and 7·4 (due to carbon dioxide), nor by variations in salinity between 34·2 and 37·6 parts per mille.

It is of interest to note that the smaller the volume of water, the greater is the rate of multiplication of bacteria in it (Whipple, G. C., 1901, *Technology Quarterly*, XIV, 21 ; and Prescott and Winslow, 1908, *Elements of Water Bacteriology*, Wiley and Sons, New York).

At this stage it was considered of interest to investigate the power possessed by sea-water to oxidise organic substances in solution, and to investigate the oxidation of the organic matter in sea-water by added hydrogen peroxide. The addition of peroxide is a recognised method of " purifying " sea-water for small aquaria and for sterilising sea-water for culturing diatoms (E. J. Allen and E. W. Nelson, 1910, " On the Artificial Culture of Marine Plankton Organisms," *Quart. Journ. Micro. Science*, Vol. 55, Part 2, p. 378).

## Decomposition of Hydrogen Peroxide in Sea-water.

It was found that water from the aquarium tanks, and from a position close to the rocks in Plymouth Sound, allowed added Hydrogen peroxide to break down only very slowly for an initial period of several days, after which the reaction proceeded more rapidly (Fig. 1). Both these waters were polluted to some extent. The aquarium water was contaminated, owing to intense colonisation with fish and invertebrates. The Sound water, which was obtained at low tide, was contaminated with sewage and other decomposing organic matter.

With surface water from offshore stations across the mouth of the Channel, the reaction proceeds slowly, in the same manner as with aquarium tank water, but not so slowly. With water taken from a depth the reaction was more or less rapid. Where vertical mixing occurs, owing to tidal movement of the water over an uneven bottom, as at Station E3, off Ushant, the difference is less marked (see Table I), and the surface water more active, owing to admixture with deeper water.

Samples were taken throughout more than one year in the Channel, and, in every case except in April and May, a very marked difference was found between the surface and the " deep " water near the bottom.

At the various stations in the North Sea from which samples were examined (three weeks after being obtained), there was no very marked difference between surface and " deep " water.

## Velocity of Reaction.

The rate at which hydrogen peroxide decomposes in the deeper and more active water was found to be approximately proportional to the amount of hydrogen peroxide present at any moment, that is

$$\frac{1}{t} \log \frac{a}{a-x} = K$$

Where $t$ is the time the reaction has been proceeding, $a$ the initial concentration of $H_2O_2$, $x$ the amount of $H_2O_2$ decomposed in time $t$ and K the velocity constant.

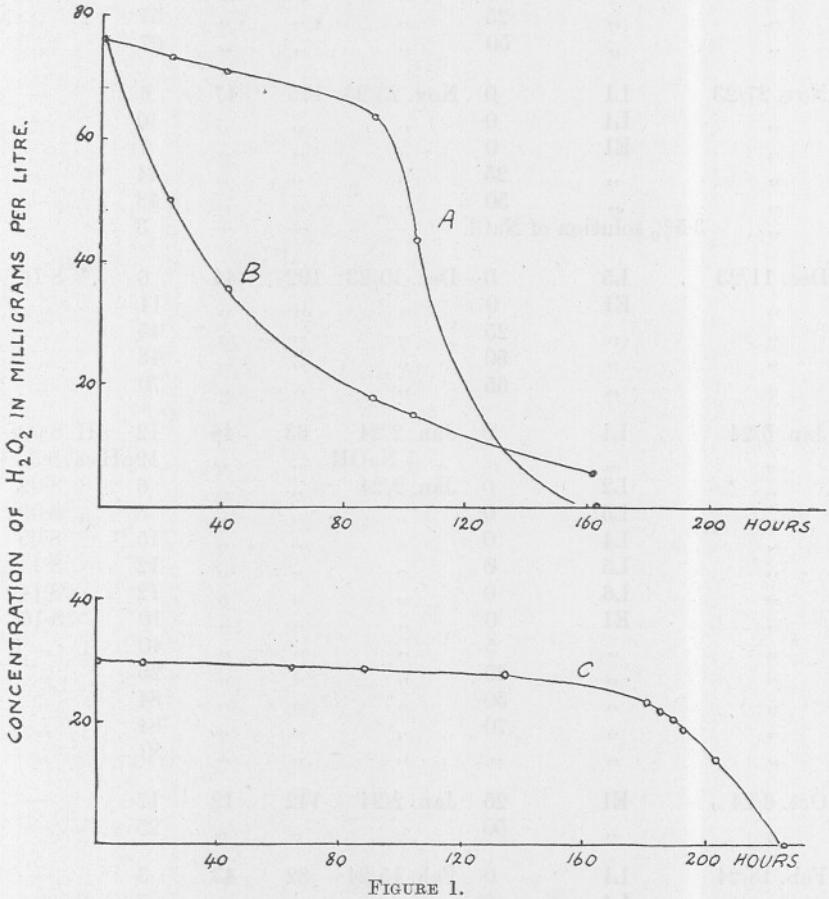


Figure 1.

Curve A. Decomposition of Hydrogen Peroxide in sea-water collected from Plymouth Sound close to Tinside Rocks at low water.

Curve B. Ditto in sea-water collected from Station E1, 22 miles S.W. of Plymouth, at a depth of 50 meters.

Curve C. Ditto in Aquarium tank water.

All at Room temperature.

TABLE 1.

| Date experiment commenced. | Position from which water sample was taken. | Depth in meters. | Date water sample was taken. | Approximate initial concentration of $H_2O_2$ mg. per litre. | Reaction time in hours. | Percentage of $H_2O_2$ broken down. | Hydrogen ion concentration when collected. |
|---|---|---|---|---|---|---|---|
| Nov. 5/23 | L2 | 0 | Oct. 15/23 | 19 | 90 | 29% | — |
| ,, | E1 | 50 | ,, | ,, | ,, | 76 | — |
| | Aquarium tank | | | ,, | ,, | 15 | — |
| Nov. 14/23 | L1 | 0 | Nov. 7/23 | 27·5 | 42 | 9 | 8·11 |
| ,, | E1 | 0 | ,, | ,, | ,, | 8 | 8·18 |
| ,, | ,, | 25 | ,, | ,, | ,, | 57 | ,, |
| ,, | ,, | 50 | ,, | ,, | ,, | 67 | ,, |
| Nov. 27/23 | L1 | 0 | Nov. 23/23 | 175 | 47 | 6 | — |
| ,, | L4 | 0 | ,, | ,, | ,, | 10 | — |
| ,, | E1 | 0 | ,, | ,, | ,, | 7 | — |
| ,, | ,, | 25 | ,, | ,, | ,, | 14 | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 48 | — |
| ,, | 3·5% solution of NaCl. | | — | — | — | 3 | — |
| Dec. 11/23 | L5 | 0 | Dec. 10/23 | 192 | 44 | 6 | 8·16 |
| ,, | E1 | 0 | ,, | ,, | ,, | 14 | ,, |
| ,, | ,, | 25 | ,, | ,, | ,, | 45 | ,, |
| ,, | ,, | 50 | ,, | ,, | ,, | 43 | ,, |
| ,, | ,, | 65 | ,, | ,, | ,, | 70 | ,, |
| Jan. 5/24 | L1 | 0 | Jan. 2/24 | 63 | 46 | 12 | pH 8·00 |
| ,, | ,, | ,, | ,, +NaOH | ,, | ,, | 12 | pH ca. 8·3 |
| ,, | L2 | 0 | Jan. 2/24 | ,, | ,, | 6 | 8·02 |
| ,, | L3 | 0 | ,, | ,, | ,, | 8 | 8·09 |
| ,, | L4 | 0 | ,, | ,, | ,, | 15 | 8·09 |
| ,, | L5 | 0 | ,, | ,, | ,, | 12 | 8·14 |
| ,, | L6 | 0 | ,, | ,, | ,, | 12 | 8·14 |
| ,, | E1 | 0 | ,, | ,, | ,, | 10 | 8·16 |
| ,, | ,, | 5 | ,, | ,, | ,, | 40 | ,, |
| ,, | ,, | 25 | ,, | ,, | ,, | 25 | ,, |
| ,, | ,, | 50 | ,, | ,, | ,, | 84 | ,, |
| ,, | ,, | 70 | ,, | ,, | ,, | 84 | ,, |
| ,, | ,, | ,, | ,, | ,, | ,, | 81 | ,, |
| Oct. 6/24 | E1 | 25 | Jan. 2/24 | 142 | 19 | 17 | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 25 | — |
| Feb. 18/24 | L1 | 0 | Feb. 15/24 | 82 | 43 | 3 | — |
| ,, | L4 | 0 | ,, | ,, | ,, | 7 | — |
| ,, | E1 | 0 | ,, | ,, | ,, | 14 | — |
| ,, | ,, | 5 | ,, | ,, | ,, | 31 | — |
| ,, | ,, | 10 | ,, | ,, | ,, | 7 | — |
| ,, | ,, | 15 | ,, | ,, | ,, | 14 | — |
| ,, | ,, | 20 | ,, | ,, | ,, | 22 | — |

| Date experiment commenced. | Position from which water sample was taken. | Depth in meters. | Date water sample was taken. | Approximate initial concentration of $H_2O_2$ mg. per litre. | Reaction time in hours. | Percentage of $H_2O_2$ broken down. | Hydrogen ion concentration when collected. |
|---|---|---|---|---|---|---|---|
| Feb. 18/24 | E1 | 25 | Feb. 15/24 | 82 | 43 | 22 | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 28 | — |
| ,, | ,, | 70 | ,, | ,, | ,, | 41 | — |
| ,, | E2 | 0 | ,, | ,, | ,, | 19 | — |
| ,, | ,, | 80 | ,, | ,, | ,, | 70 | — |
| ,, | E3 | 0 | ,, | ,, | ,, | 20 | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 26 | — |
| ,, | ,, | 105 | ,, | ,, | ,, | 31 | — |
| Mar. 12/24 | L4 | 0 | Mar. 10/24 | 73 | 46 | 20·5<br>17·8 | — |
| ,, | ,, | 45 | ,, | ,, | ,, | 48<br>48 | — |
| ,, | L6 | 0 | ,, | ,, | ,, | 15<br>15 | — |
| ,, | ,, | 25 | ,, | ,, | ,, | 35·6<br>37 | — |
| ,, | ,, | 60 | ,, | ,, | ,, | 48<br>48 | |
| Mar. 18/24 | L4 | 0 | Mar. 17/24 | 96 | 22 | 33<br>23 | |
| ,, | ,, | 48 | ,, | ,, | ,, | 40<br>44 | |
| April 11/24 | E1 | 0 | April 8/24 | 58 | 24 | 14<br>17 | |
| ,, | E1 | 68 | ,, | ,, | ,, | 10<br>17<br>14<br>17 | — |
| May 22/24 | E1 | 0 | May 20/24 | 110 | 24 | 23<br>12 | — |
| ,, | E1 | 65 | ,, | ,, | ,, | 14<br>12 | — |
| Aug. 25/24 | E1 | 0 | ,, | 88 | 22 | 4%* | |
| ,, | E1 | 65 | ,, | 88 | — | 34%* | |
| Aug. 9/24 | E1 | 0 | Aug. 7/24 | 86 | 19 | 9<br>11<br>9 | — |
| ,, | E1 | 67 | ,, | ,, | ,, | 46<br>53<br>44 | — |

* Note effect of keeping.

| Date experiment commenced. | Position from which water sample was taken. | Depth in meters | Date water sample was taken. | Approximate initial concentration of $H_2O_2$ mg. per litre. | Reaction time in hours. | Percentage of $H_2O_2$ broken down. | Hydrogen ion concentration when collected. |
|---|---|---|---|---|---|---|---|
| Aug. 25/24 | E1 | 0 | Aug. 7/24 | 88 | 22 | 14<br>0 | — |
| ,, | E1 | 67 | ,, | ,, | ,, | 31 | |
| ,, | E1 | 70 | July 9/24 | ,, | ,, | 31 | |
| Aug. 26/24 | L6 | 0 | Aug. 25/24 | 88 | 17 | 13 | — |
| ,, | L6 | 60 | ,, | ,, | ,, | 67 | |
| Sept. 4/24 | E1 | 0 | Sept. 3/24 | 80 | 14 | 18 | — |
| ,, | E1 | 68 | ,, | ,, | ,, | 65 | |
| ,, | L1 | 0 | ,, | ,, | ,, | 6 | — |
| Sept. 19/24 | L1 | 0 | Sept. 3/24 | 130 | 20 | 15 | — |
| ,, | L4 | 50 | ,, | ,, | ,, | 72 | — |
| ,, | Lat 57° 51′ N. Long. 6° 39′ E. | 0 | Aug. 30/24 | ,, | ,, | 12 | — |
| ,, | ,, | 200 | ,, | ,, | ,, | 26 | — |
| ,, | ,, | 250 | ,, | ,, | ,, | 23 | — |
| Sept. 22/24 | Lat. 56° 28′ N. Long. 2° 32′ E. | 0 | Aug. 29/24 | 122 | 24 | 16½ | — |
| ,, | ,, | 60 | ,, | ,, | ,, | 23 | — |
| ,, | Lat. 56° 05′ N. Long. 1° 32′ E. | 0 | ,, | ,, | ,, | 18 | — |
| ,, | ,, | 70 | ,, | ,, | ,, | 18 | — |
| ,, | Lat. 57° 10′ N. Log. 4° 33′ E. | 0 | ,, | ,, | ,, | 18 | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 18 | — |
| ,, | Lat. 56° 49′ N. Long. 3° 33′ E. | 0 | ,, | ,, | ,, | 16½ | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 15 | — |
| Oct. 3/24 | L2 | 0 | Oct. 1/24 | 139 | 22 | 1½ | pH 8·24 |
| ,, | L3 | 0 | ,, | ,, | ,, | 7 | ,, |
| ,, | L4 | 0 | ,, | ,, | ,, | 8½ | ,, |
| ,, | L4 | 40 | ,, | ,, | ,, | 26 | ,, |
| ,, | L5 | 0 | ,, | ,, | ,, | 8½ | ,, |
| ,, | E1 | 0 | ,, | ,, | ,, | 17½ | ,, |
| ,, | ,, | 5 | ,, | ,, | ,, | 13 | ,, |
| ,, | ,, | 15 | ,, | ,, | ,, | 11 | ,, |
| ,, | ,, | 25 | ,, | ,, | ,, | 14½ | ,, |
| ,, | ,, | 40 | ,, | ,, | ,, | 29 | ,, |
| ,, | ,, | 65 | ,, | ,, | ,, | 70 | 8·24 |
| Oct. 10/24 | E1 | 0 | Oct. 1/24 | 139 | 20 | 1½ | — |
| ,, | ,, | 50 | ,, | ,, | ,, | 10 | — |
| ,, | ,, | 65 | ,, | ,, | ,, | 17 | — |

On the other hand, with aquarium tank water, with water close to the shore and with surface water, the velocity increases with the time the reaction has been proceeding. Hence, the greater the initial concentration of hydrogen peroxide, the greater is the proportion $\frac{x}{a}$ decomposed after the lapse of a definite period of time. The experimental evidence is given later.

The rate increases with the temperature and with the alkalinity (OH concentration) of the water.

Changes take place in the water on storage. In no case has water from the aquarium tanks become similar to water from the surface offshore, and in no case has surface water from offshore become similar in activity to " deep " water. In all cases, except one, where a change has taken place during storage, " deep " water has lost activity. The one exception occurred in May, 1924, when water from close to the bottom at Station E1, initially only slightly active, became more active after a month's storage. This one instance might well have been an experimental error, since a minute trace of organic matter in the tube in which the experiment was carried out in May would have been sufficient to decrease the activity of the water. The influence of minute traces of organic substances on the reaction, which is discussed later, was not realised at the time, and no *extra* ordinary precautions were taken in the earlier experiments.

### The Catalyst Action upon Hydrogen Peroxide.

When a " deep " water is heated to 100° C., its activity in decomposing Hydrogen peroxide is not decreased, unless it is heated for long enough for a precipitate of magnesium hydroxide etc. to settle out. Then it completely loses its activity. If the precipitate is redissolved in the least quantity of dilute hydrochloric acid, its addition to a surface water increases the activity of that towards hydrogen peroxide, the hydroxide concentration having been adjusted to the original value.

The addition of a trace of ferric or ferrous salt (3 mg. $FeCl_3$ per litre) causes a precipitate to be formed in sea-water, and when this occurs in the case of a deep water, the activity is lost, the catalyst being carried down with the precipitate.

The activity of a deep water is not affected by mercuric chloride (14 mg. $Hg\,Cl_2$ per litre), but it is destroyed by the addition of a trace of soluble cyanide (6 mg. KCN per litre).

Although ferric ions do not occur in detectable quantity in sea-water, a minute trace of iron in solution can be detected after boiling with nitric acid. This ferric ion is more probably produced by the breakdown of organic iron compounds than by the oxidation of free ferrous ions, which

would not occur unoxidised in an alkaline air saturated solution. An analysis of water from the surface and from 68 meters at Station E1, collected in September, 1924, gave a value between 0·003 and 0·006 milligrams per litre of iron in solution.

Hæmatin can be added to sea-water without precipitation. It was

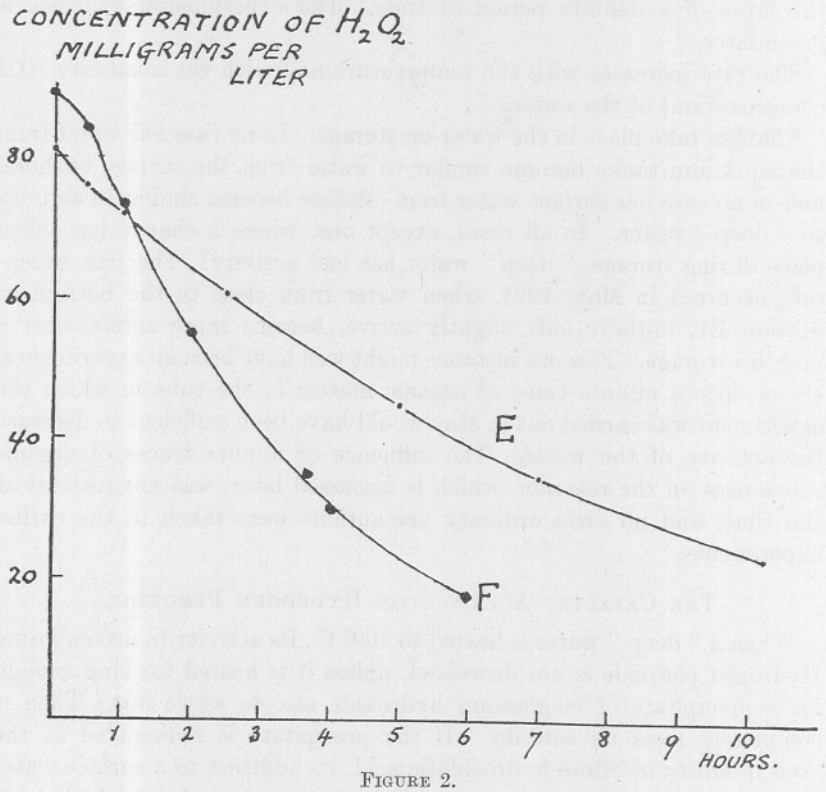


FIGURE 2.

Curve E. Decomposition of hydrogen peroxide in water collected from Station E1, 22 miles S.W. of Plymouth, at a depth of 70 meters, at a constant temperature of 20° C.

Curve F. Ditto in surface water collected at Station L4, 3½ miles off Rame Head, at a constant temperature of 30° C.

found that the addition of a trace of hæmatin increased the activity of a surface water towards hydrogen peroxide.

Cytochrome, occurring almost universally in animals and containing iron-pyrrol groups, behaves as a heat stable oxidase and catalase. (Keilin, *Proc. Roy. Soc.*, B. Vol. 98, p. 312, 1925.)

The inhibition by cyanide and the above considerations together point to the active catalytic agent being an organic compound of iron in solution in the sea-water.

It should be mentioned that no actual *proof* of the catalyst being an iron compound is afforded by its inhibition by cyanide. Among other heavy metals, copper salts catalyse the decomposition of hydrogen peroxide, and are known to be present in sea-water in very minute traces from the occurrence of copper in the hæmocyanin blood of many marine invertebrates; they form a complex cyanide which is not precipitated, like other copper salts, on the addition of a ferrocyanide. (Meyerhof, *Chemical Dynamics of Life Phenomena*. Philadelphia, 1925, p. 39.)

### Inhibition of the Catalyst in Surface and Inshore Waters.

Plymouth aquarium water, inshore and surface waters from the Channel contain iron in solution; there is no reason to suppose that such waters show little activity towards hydrogen peroxide on account of lack of iron.

This indicates that the activity of the catalyst is inhibited in such waters, assuming that the catalyst consists of a compound or compounds of iron in solution. In the case of aquarium water the inhibiting substances are in excess, since several days' action of dilute hydrogen peroxide at room temperature is necessary to decompose them, and to allow the catalyst to act upon the peroxide (Fig. 1). In the case of a surface offshore water the decomposition of added hydrogen peroxide proceeds from the start, the velocity of the reaction increasing as the inhibitor is oxidised and put out of action.

Fig. 2 (curve F) shows the rate of decomposition of hydrogen peroxide added to a surface water from Station L4, 3½ miles off Rame Head. The curve is not a logarithmic curve, as for a mono- or bimolecular reaction, but is "skewed." The initial concentration of Hydrogen peroxide was approximately 90 milligrams per litre, and the values of $K_{30°C.} = \frac{1}{t} \log \frac{a}{a-x}$ found after varying intervals were as follows :—

| Reaction time (at 30° C.) | $\frac{a}{a-x}$ | $K = \frac{1}{t} \log. \frac{a}{a-x}$ |
|---|---|---|
| 0 h. 30 mins. | 1·06 | ·051 |
| 1 h. 0 mins. | 1·21 | ·083 |
| 2 h. 0 mins. | 1·60 | ·102 |
| 3 h. 40 mins. | 2·54 | ·109 |
| 4 h. 0 mins. | 2·86 | ·114 |
| 6 h. 0 mins. | 4·95 | ·116 |

The proportion of peroxide decomposed after a definite time by the same sample of water, was found to be greater, where the initial concentration of added peroxide was greater and more rapidly oxidised the inhibiting substances. Thus in an experiment made at room temperature

with the same water, after 47 hours the value $\log \frac{a}{a-x}$ was found to be as follows :—

| Approximate initial concentration of $H_2O_2$ in mg. per litre. | Value of $\log \frac{a}{a-x}$ after 48 hours. |
|---|---|
| 134 | ·523 |
| 81 | ·389 |
| 54 | ·286 |

During the ensuing six hours the velocity of the reaction in all three cases was more nearly the same, the major portion of the inhibiting substances having been already oxidised.

| Concentration of $H_2O_2$ after 47 hours (a–x) | After 53 hours ($x_2$) | Value $\log \frac{a-x}{x_2}$ |
|---|---|---|
| 41 | 31 | ·12 |
| 33 | 27 | ·09 |
| 29 | 24 | ·08 |

As previously stated, the velocity of the decomposition of peroxide in active deep water approximates to that of a unimolecular reaction. A sample collected at Station E1 on November 15, 1924, to which was added approximately 80 milligrams per litre of hydrogen peroxide, gave the following values for K at 20° C. :—

| Reaction time T in hours. | $\frac{a}{a-x}$ | $K=\frac{1}{T}\log \frac{a}{a-x}$ |
|---|---|---|
| 0·5 | 1·06 | ·051 |
| 1·0 | 1·13 | ·053 |
| 2·5 | 1·34 | ·051 |
| 5·0 | 1·80 | ·051 |
| 7·0 | 2·31 | ·052 |
| 10·23 | 3·45 | ·052 |

The proportion of peroxide decomposed after a definite time by this water was found to be practically the same irrespective of wide differences in the initial concentration of the peroxide. In an experiment conducted at room temperature, the following values of $\frac{a}{a-x}$ were found after the reaction had proceeded for 25 hours.

| Approximate initial concentration of $H_2O_2$. | $\log \frac{a}{a-x}$ |
|---|---|
| 133 mg. per litre | ·24 |
| 66 ,, ,, ,, | ·30 |
| 41 ,, ,, ,, | ·25 |

When a slightly active, that is partially inhibited, surface water is mixed with an active deep water, the velocity of reaction with hydrogen peroxide was found to be rather less than the value proportional to the

relative amounts of the two waters in the mixture. The same quantity of peroxide (80 mg. per litre) was added to mixtures of surface water and water from 70 meters collected on January 2nd, 1924, at E1, and the concentration, a–x, determined after 19½ hours at room temperature.

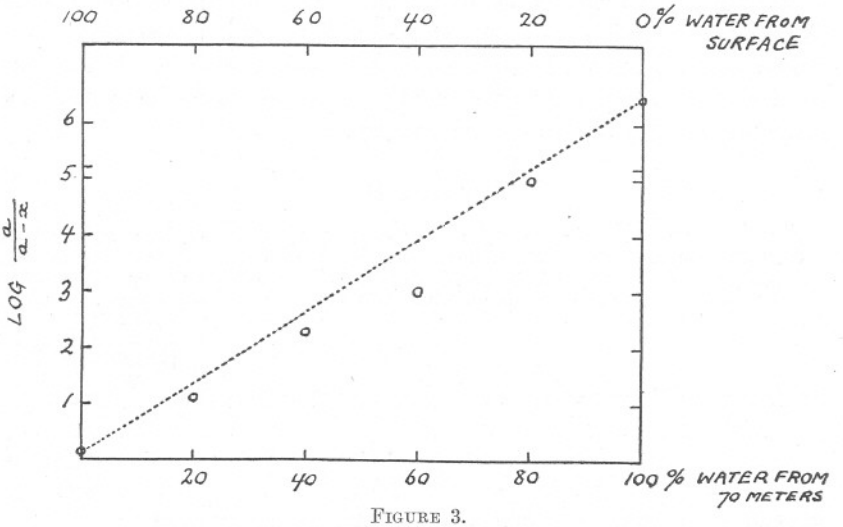


FIGURE 3.

Decomposition of hydrogen peroxide in mixtures of surface water and water from 70 meters at Station E1, after the same interval of time, starting with the same initial concentration of peroxide (a).

Fig. 3 shows the relation of $\log \frac{a}{a-x}$ to the relative amounts of the two waters in the mixture. The hydrogen ion concentration of the two waters was the same.

On the other hand, when aquarium tank water, containing an excess of inhibiting substances capable of practically stopping the reaction until oxidised by the peroxide, is mixed with an active deep water, the reaction velocity in the mixture is considerably less than the proportional value. The same quantity of peroxide was added to water from the surface at L3, to aquarium tank water brought to the same hydrogen ion concentration, to water from 65 meters at Station E1 collected on October 1st, 1924, and to mixtures. After 18 hours at room temperature the concentration a–x was determined.

| | $\log \frac{a}{a-x}$ (mean of duplicate determinations). |
|---|---|
| Surface water from L3 | ·054 |
| Water from 68 meters at E1 | ·734 |
| 50% surface water L3 with 50% water from 68 meters at E1 | ·334 |
| Aquarium tank water | ·006 |
| 50% Aquarium tank water, 50% water from 68 meters at E1 | ·186 |

The addition of 50% surface water from L3 has reduced the velocity in deep water rather more than the proportional amount (·334 against ·394), while the addition of 50% aquarium tank water has reduced the velocity considerably below the proportional amount (·186 against ·370).

With regard to the nature of the inhibiting substances occurring in the sea-waters it is noteworthy that aquarium tank water and inshore waters contain a much larger amount of organic matter in solution than open sea-water, as shown by the quantity of alkaline potassium permanganate solution they are capable of reducing.

TABLE II.

Quantities of 100 c.c. of sea-water, filtered through a porcelain candle, were boiled for 10 minutes with 10 c.c. of a solution consisting of 0·395 gms. per litre of potassium permanganate in 10% sodium hydroxide solution, cooled, acidified, and backtitrated with sodium thio-sulphate solution after the addition of potassium iodide.

| | Milligrams per litre of Oxygen consumed in oxidising contained organic matter. |
|---|---|
| Surface water from Station E1, 22 miles S.W. of Plymouth, collected 18.12.22 | 0·3 |
| | 0·22 |
| | 0·35 |
| Surface water from 22 miles S.W. of Plymouth, collected 16.1.23 | 0·52 |
| Surface water from close to Plymouth Breakwater, 1 hour before low water, collected 16.1.23 | 0·52. |
| Surface water from 150 yards offshore S. of Tinside in Plymouth Sound, collected 10.1.23, 1¼ hours before low water | 0·83 |
| Surface water collected at Saltash, 18.1.23, ½ hour before low water | 0·97 |
| | 0·98 |
| Surface water collected close to rocks near Tinside in Plymouth Sound at low water, 4.1.23 | 1·09 |
| Aquarium tank water, 4.1.23 | 1·77 |
| Aquarium tank water, 16.1.23 | 1·96 |
| Aquarium tank water, 16.1.23 | 2·09 |

Atkins (*Journal Marine Biol. Assoc.*, Vol. XII, p. 772, 1922) has shown that the upper layers of open sea-water turn more acid on standing, owing to bacterial action, thus proving that they contain more dissolved organic matter than the " deep " water.

Surface water from well offshore was allowed to stand in a sterilised test tube with the top covered by an inverted tube in order to keep out

dust. After five months there was a well-marked scum consisting of short rod-shaped bacteria in a gelatinous mass round the meniscus.

Two glass stoppered bottles were sterilised and 100 c.c. of surface water from Station E1 was filled into each, together with 1 milligram of hydrogen peroxide in solution. Paper was tied over the stoppers to keep out dust particles. After three months there was a considerable growth of bacteria in each bottle, quite obvious to the naked eye. Similar treatment of water from the aquarium tanks gave rise to a gelatinous mass of bacteria several cubic centimetres in volume.

Sea-water, even from well out to sea, contains sufficient dissolved organic matter to permit the growth of a bacterial fauna, and such develops when the water is kept in a small vessel.

It appears from the following experiment that water from the surface contains more putrefactive bacteria than water from a depth. A series of test tubes were partly filled with 10 c.c. of a 0·02 per cent solution of Wittes peptone in sea-water. To one of these was added 1 c.c. of aquarium tank water, and all were steam sterilised. To each tube, the control excepted, was added 1 c.c. of one of the following sea-waters :—

Aquarium tank water.
Surface water from L1.
Surface ,, ,, L3.
Surface ,, ,, E1.
Water at 25 meters from E1.
Water at 70 ,, ,, ,,

After 48 hours the control tube, to which the aquarium tank water had been added previous to sterilisation, showed no opalescence due to the growth of bacteria. The other tubes showed a growth of bacteria in the order given above, most marked in the tube with aquarium tank water added, and least in the one with water from 70 meters at E1. The three surface waters showed about equal growth. The water from 25 meters at E1 fell intermediate between surface waters and water from 70 meters.

Experiments have shown that the addition of traces of the following soluble organic substances very materially reduce the activity of " deep " water in decomposing $H_2O_2$.

| | |
|---|---|
| Gelatin . . . | 5 mg. per litre. |
| Asparagin . . | 1 ,, ,, ,, |
| Tartaric acid . . | 5 ,, ,, ,, |
| Ethylamine . . | 2–3 ,, ,, ,, |
| Glycin . . . | 2 ,, ,, ,, |
| Tyrosin . . . | 3 ,, ,, ,, |

With regard to the oxidation of the inhibiting substances by hydrogen peroxide deduced from the increase in the velocity of decomposition of peroxide in surface or inshore water, Dakin found that amino and fatty acids were oxidised by hydrogen peroxide in the presence of iron salts. (*J. Biol. Chem.*, 1908, 4, pp. 63, 77, 227, also 1909, 5, p. 409). Later, Warburg (*Biochem. Zeitschr*, 136, p. 266, 1923) found that amino acids were oxidised by peroxide without the presence of iron.

That aquarium tank water contains the catalyst is indicated by the fact that after boiling with NaOH and redissolving the precipitate, it becomes active towards hydrogen peroxide. The inhibiting substances have probably been hydrolysed. Further if the precipitate of magnesium hydroxide, etc., obtained by boiling aquarium water is collected and redissolved in the least quantity of dilute hydrochloric acid, the addition of this to an offshore surface water increases its activity, the hydroxyl concentration having been adjusted to the original value of the surface water.

## Experimental.

It was found convenient to carry out the reaction in vessels coated with paraffin wax. These should be allowed to soak in distilled water for some time before use. To the sample of sea-water in the vessel 1 c.c. of a solution of hydrogen peroxide of suitable strength was added, and 5 or 10 c.c. portions of the mixture withdrawn for titration with 0·02% permanganate after addition of sulphuric acid. The hydrogen peroxide used should be a pure solution ; some proprietary brands are reputed to contain traces of " preservative," such as salicylic acid, to improve their keeping qualities.

The experiments were carried out at room temperature, except those for the rate of reaction (Fig. 2). The values obtained in each experiment are comparable between themselves, but not with other experiments, owing to the varying room temperature.

## Oxidation of Organic Compounds in Sea-water.

It was found that the rate at which a number of compounds oxidise when dissolved in sea-water varied considerably according to the position and depth from which the water was taken, and according to the season.

A solution of pyrogallol or of hydroquinone in sea-water turns brown. A solution of equimolecular parts of paraphenylene diamine hydrochloride and $\alpha$ napthol in potassium carbonate solution (Röhman-Spitzer reagent) when added to sea-water oxidises to a blue dye, indophenol. Tincture of guiacum gradually takes on a green hue, more blue-green in the case of an " active " deep water. The most convenient reagent was

found to be either hydroquinone or Röhman-Spitzer reagent, the latter developing a strong colour within a few minutes.

The rate of oxidation increases with the alkalinity (OH concentration) of the sea-water. In order to show definitely that samples of water possessed different powers of oxidation when at the same hydrogen ion concentration, acid or alkali was added in some cases, so that the sample could be shown to possess both greater power of oxidation and lesser OH concentration, than the other sample with which it was being compared.

TABLE III.

| Date of experiment. | Position from which water sample was collected. | Depth from which sample was collected in meters. | Acid or alkali added. | pH. | Relative amount of oxidation of reagent. | Reagent used. | Date water sample was collected. |
|---|---|---|---|---|---|---|---|
| — | E1 | Surface | — | Same | + | Pyrogallol in | Nov. 23/23 |
| | ,, | 50 | — | ,, | ++ | distilled water | ,, |
| Dec. 3/23 | E1 | Surface | — | Greater | + | Hydroquinone in | Nov. 23/23 |
| | ,, | 50 | Acid | Less | ++ | distilled water | |
| Dec. 5/23 | Aquarium tank | | Alk. | 8·0* | + | Hydroquinone in distilled water | Nov. 23/23 |
| | E1 | Surface | Alk. | 7·85* | ++ | | ,, |
| | ,, | 50 | — | 7·0* | +++ | | |
| Dec. 12/23 | L5 | Surface | — | | + | Hydroquinone in | Dec. 10/23 |
| | E1 | ,, | — | Same | ++ | distilled water | |
| | ,, | 50 | — | | ++ | | |
| | ,, | 60 | — | | +++ | | |
| Jan.—/24 | E1 | Surface | — | Same | + | Hydroquinone in | Jan. 2/24 |
| | ,, | 70 | — | | +++ | distilled water | |
| Feb. 19/24 | E1 | Surface | — | Same | + | Hydroquinone in | Feb. 15/24 |
| | ,, | 70 | — | | ++ | distilled water | |
| April 9/24 | E1 | Surface | — | Same | +† | Hydroquinone in | April 8/24 |
| | ,, | 68 | — | | ++ | distilled water | |
| Aug. 26/24 | L6 | Surface | — | 8·25 | + | Hydroquinone in | Aug. 25/24 |
| | ,, | 60 | — | 8·20 | + | distilled water | |
| | L6 | Surface | — | 8·25 | + | Röhman-Spitzer | Aug. 25/24 |
| | ,, | 60 | — | 8·20 | + | reagent | |
| Sept. 4/24 | E1 | Surface | — | Same | + | Hydroquinone in | Sept. 3/24 |
| | ,, | 68 | — | | + | distilled water | |
| Oct. —/24 | E1 | Surface | — | Same | +‡ | Hydroquinone in | Oct. 1/24 |
| | ,, | 65 | — | | +++ | distilled water | |
| Oct. —/24 | E1 | Surface | | | + | Hydroquinone | |
| | ,, | 65 | | Same | +++ | in | Oct. 1/24 |
| | ,, § | 65 | $CO_2$§ | | +++ | distilled water | |
| Oct. —/24 | E1 | 0 | — | Same | + | Röhman-Spitzer | Oct. 1/24 |
| | ,, | 65 | — | | +++ | reagent | |
| Oct. —/24 | E1 | Surface | | | pale green | Tincture of guiacum | Oct. 1/24 |
| | L3 | ,, | | | ,, | ,, | ,, |
| | L4 | ,, | | | ,, | ,, | ,, |
| | E1 | 40 | | | pale blue-green | ,, | ,, |
| | ,, | 50 | | | ,, | ,, | ,, |
| | ,, | 65 | | | ,, | ,, | ,, |

* After addition of the hydroquinone solution.
† Difference not well marked.
‡ Dubosq colorimeter readings 100 and 125 respectively.
§ Heated on water bath for 30 minutes and pH adjusted by passing $CO_2$.

On reference to Table III it is seen that there is no marked difference between surface and deep water collected on August 25th and September 3rd, 1924, while the samples behaved very differently towards hydrogen peroxide. This points to the difference in behaviour towards peroxide and these reagents between surface and deep water being due to different causes.

A notable point of similarity is that a trace of cyanide slows the oxidation (of Röhman-Spitzer reagent) very considerably. The rate is reduced to the same order as the rate of oxidation in distilled water brought up to the hydrogen ion concentration of sea-water with sodium peroxide.

A trace of glycin very considerably slows the oxidation in sea-water.

By boiling surface water with sodium hydroxide, redissolving the precipitate in hydrochloric acid and adjusting the hydrogen ion concentration to the original value by adding sodium hydroxide, the oxidising power of the water is actually increased. It is suggested that this treatment hydrolyses part of the inhibiting substances present in the surface water.

These points of similarity suggest that sea-water contains in solution a compound or compounds of iron which act catalytically by increasing the rate of oxidation of a number of easily oxidisable organic substances. An iron compound in sea-water which is an active catalyst towards the decomposition of hydrogen peroxide is not necessarily active towards these other reagents (August and September, 1924) and vice versa (April, 1924). Surface water and inshore water contains inhibitory substances which are partly hydrolysed by boiling in sodium hydroxide solution.

Dr. Keilin suggested to the writer that the catalytically active iron compounds in sea-water may be enzymes—catalases and oxidases—excreted by, or dissolved out from dead organisms. He further pointed out that there are several known cases of oxidising enzymes withstanding a temperature of 100° C. or more for some hours.

## General Conclusions.

The preliminary experiments showed that the products of putrefaction of organic matter occurring in the sea, if they accumulated and were not oxidised, would in time exert an influence upon the life of marine organisms.

The oxidation of such products may be expected to vary in velocity in sea-water from different depths and localities; this was found to be the case with a variety of easily oxidisable organic substances.

The reaction of sea-water with hydrogen peroxide, being readily measured, permits conclusions to be drawn regarding the mechanism of this oxidation in sea-water.

The conclusion that dissolved organic substances, which inhibit the

action of the catalyst, are present in the upper layers of water, is in agreement with Atkins' conclusion regarding the vertical distribution of dissolved organic matter occurring in the water of the open sea (Atkins, W. R. G., *Journ. Marine Biol. Assn.*, Vol. XII, p. 772). The minute trace of dissolved organic matter plays a part in reducing the oxidation potential of the water.

The action of cyanide in stopping oxidation, the presence of iron in solution in sea-water, the catalytic action of hæmatin which remains in solution in sea-water upon hydrogen peroxide, and also the analogy to the action of iron on the oxidation of cystein, all point to organic compounds of iron being the active catalyst in sea-water.

It is suggested that the greater amount of dissolved organic matter in estuarine and polluted water, and the greater concentration of physiologically active products of putrefaction and their lesser rate of oxidation to non-active products, may *in part* account for the difference in fauna between such waters and those of the open sea. The major physiological difference undoubtedly is, that such water, being relatively shallow, is subject to greater and more rapid changes of temperature.

## SUMMARY.

1. Putrefaction in sea-water sets free compounds toxic to Calanus. A portion of these compounds withstands oxidation to non-toxic forms in air saturated water for considerable periods.

2. "Deep water" from the English Channel contains a catalyst, probably an iron compound, which increases the rate of oxidation of a number of easily oxidisable organic compounds. Its action is inhibited in surface and inshore waters by dissolved organic matter.

3. Hydrogen peroxide decomposes relatively quickly in "deep water" from the English Channel, at a velocity corresponding to that of a monomolecular reaction. A catalyst, probably an iron compound, active towards hydrogen peroxide, occurs in sea-water. The action of the catalyst is inhibited by dissolved organic substances in surface and inshore water, until such substances are oxidised by the hydrogen peroxide.