A QUANTITATIVE STUDY OF THE INTERRELATIONSHIP OF OXYGEN AND HYDROGEN ION CONCENTRATION IN INFLUENCING TUBULARIA REGENERATION

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Increased hydrogen ion concentration of sea water has an inhibitory effect on *Tubularia* regeneration (Komori, 1933; Miller, 1939; Goldin, 1942). Goldin presented evidence supporting the view that the effectiveness of oxygen in regeneration is limited by the inhibitory action of accumulated acid metabolites. In addition, experiments in which the hydrogen ion concentration of the sea water was increased, while the oxygen tension was maintained at a constant level, showed that there is a pH range in which regeneration is retarded, and a critical pH below which regeneration will not occur. It was also indicated that the critical pH is related to the oxygen tension.

Thus the ability to form primordia appears to be dependent upon the hydrogen ion concentration as well as the oxygen tension (Barth, 1938a, 1940) of the sea water. These experiments were designed in order to study the interrelationship of these factors in their effects on *Tubularia* regeneration. The author wishes to thank Professors L. G. Barth and H. B. Steinbach for their guidance during the course of these investigations.

Methods

The general procedure for selecting the *Tubularia* stems, treating them and measuring rate of regeneration, was essentially the same as that described by Barth (1938a, 1938b). Young, unbranched stems of uniform diameter were selected from young colonies. Stem segments 6 mm. in length were used for the experiments, the required length being removed from the region 5 to 11 mm. proximal to the hydranth. In order to insure uniformity of material, random samples of these stem segments were taken for each experiment.

The oxygen tension and the hydrogen ion concentration of the sea water were adjusted by saturating the sea water with oxygen-carbon

dioxide-nitrogen gas mixtures. The gases were drawn from cylinders and the mixtures were prepared in a graduated 20 gallon carboy by the standard method of water displacement. The carboy was filled with water, and the water was then displaced by the required quantity of each gas. Adjustment to atmospheric pressure was made after the addition of each gas, so that the total pressure was one atmosphere. The nitrogen gas was used in order to bring all of the mixtures to the same total volume. For each experiment a series of gas mixtures was prepared containing carbon dioxide at increasing partial pressures and oxygen at the same partial pressure. Measured samples of filtered sea water (350 ml.) were introduced into one liter florence flasks and then saturated with the required gas mixtures. In this way, for each experiment, the hydrogen ion concentration (carbon dioxide tension) of the sea water was varied, while the oxygen tension was maintained at a constant level. The various experiments, however, were conducted at different levels of oxygen tension, so that a wide range of oxygen and hydrogen ion concentration was covered. Twenty stem segments were introduced into each flask, and samples of sea water were removed in order to determine the oxygen tension and the pH. Dissolved oxygen was determined by the Winkler method, and the hydrogen ion concentration was measured with a Beckman pH meter. The experimental flasks were kept at constant temperature ($19 \pm 1°$ C.) during the above operations and throughout the course of the experiments.

The stems were then examined for regeneration. They were removed from the flasks as the primordia were formed, by means of a long pipette. The time was noted, and the primordia were measured with the aid of an ocular micrometer mounted in a binocular microscope. The formula $R = L/t =$ R. U. (regenerative units) was used for measuring rate of regeneration, where L is the length of the primordium in micra, and t is the time in hours at which the primordium becomes constricted from the stem segment proper. At low oxygen and high hydrogen ion concentration many of the stems did not regenerate. Stems which failed to regenerate were removed from the flasks at the close of the experiment (106–120 hours), and placed in running sea water. The stems which regenerated after removal were considered to be inhibited, and the length of their primordia as well as their rate of regeneration were considered as zero. The stems which failed to recover were not included in the calculations. This treatment of the data is in general the same as that employed by Barth (1938a), and appears to give the most accurate index of regeneration rate.

Results

The results are summarized in Table I, and the rate of regeneration is plotted against pH at the different levels of oxygen concentration in

Table I

The interrelationship of oxygen and hydrogen ion concentration in influencing Tubularia regeneration

Initial pH	Change in pH	Initial O_2 cc./l.	Change in O_2 cc./l.	L micra	t hours	L/t
			Experiment 1			
8.00	−0.17	8.4	−1.0	1246	31.3	39.8
7.00	+0.03	8.8	−1.3	969	43.8	22.1
6.60	+0.11	8.5	−1.1	629	65.5	9.6
6.64	+0.08	7.9	−1.1	630	66.4	9.5
6.30	+0.13	8.2	−0.9	0	106.0	0
			Experiment 2			
8.03	−0.12	11.1	−2.2	1693	28.8	58.8
7.52	−0.10	10.3	−2.0	1491	34.4	43.3
7.33	−0.01	9.8	−1.4	1623	33.6	48.3
6.97	+0.15	10.5	−1.9	1571	37.5	41.9
6.74	+0.25	10.2	−1.7	1406	40.4	34.8
6.26	+0.04	9.7	−1.2	1085	68.0	16.0
			Experiment 3			
8.00	−0.08	10.3	−2.2	1346	30.6	44.0
6.96	+0.15	10.5	−2.4	1230	37.8	32.5
6.69	+0.17	9.9	−1.7	995	56.3	17.7
8.00	−0.08	5.6	−0.1	921	41.6	22.1
6.96	+0.13	5.3	+0.2	643	69.1	9.3
6.69	+0.16	5.1	+0.3	255	70.5	3.6
			Experiment 4			
8.07	−0.03	6.5	−0.7	1054	33.2	31.7
7.07	+0.28	6.3	−0.3	848	46.4	18.3
6.82	+0.28	6.0	−0.5	699	71.2	9.8
6.63	+0.25	6.0	−0.7	29	91.0	0.3
8.10	−0.04	3.4	+1.3	786	52.3	15.0
7.44	+0.14	3.5	+1.1	577	58.3	9.1
7.16	+0.26	3.5	+1.3	426	78.8	5.4
7.00	+0.21	3.6	+0.6	53	81.0	0.7
6.81	+0.17	3.4	+0.6	0	120.0	0
6.64	+0.15	4.0	+0.8	0	120.0	0

Figure 1. In Figure 1 and in the text the data are presented in terms of initial pH and initial oxygen concentration. The hydrogen ion concentrations remained fairly constant during the course of the experiments, the greatest pH change being 0.28 units. The greatest change

in oxygen occurred at the high oxygen concentrations. However, as shown by Barth (1938a), at high oxygen the rate of regeneration changes slowly as the oxygen is varied, so that this probably does not enter as an important factor. The pH range studied was from 8.10 (normal sea water) to pH 6.26. The levels of oxygen concentration used extended from approximately 3.5 to 10.5 cc. O_2 per liter (normal sea water contains 4.5–4.8 cc. O_2/l.).

As the pH of the sea water is lowered, the rate of regeneration falls off at all levels of oxygen concentration. At the highest level of oxygen concentration studied (Exp. 2; $O_2 = 10.5$ cc./l. approx.) there was a

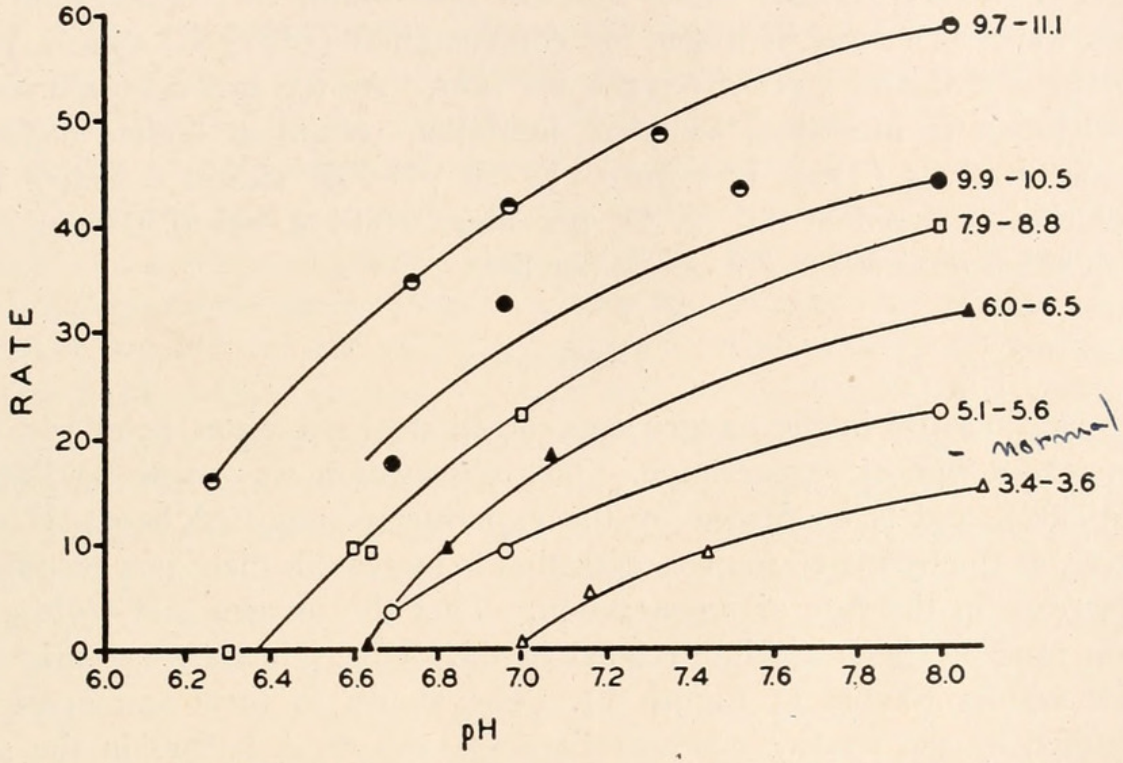

FIG. 1.[1] The interrelationship of oxygen and hydrogen ion concentration in influencing *Tubularia* regeneration. Data presented in Table I. Exp. 1 = □; Exp. 2 = ◓; Exp. 3 = ● and ○; Exp. 4 = ▲ and △. Rate = L/t where L = length of primordium in micra and t = time in hours. The pH values represent the readings at the beginning of the experiments. The figures to the right of each curve indicate the range of initial concentrations in cc./l.

fall of 42.8 R. U. as the pH was lowered from 8.03 to 6.26. At the lowest level of oxygen concentration (Exp. 4; $O_2 = 3.5$ cc./l. approx.), the fall was from 15 R. U. (pH 8.10) to 0 (pH 6.81). As the level of oxygen concentration is increased, however, the rate of regeneration is increased, not only at the pH of normal sea water but at all of the pH values studied. It should be noted that stems collected at different times, even though selected for uniformity, vary in their susceptibility

[1] The author wishes to thank Mr. Jack Godrich for his assistance in the preparation of the figure.

to oxygen changes (Barth, 1938a), so that the results of the individual experiments are only approximately comparable. The trend with increased oxygen is nevertheless quite clear. In the last two experiments the regeneration of stems from the same source was studied at two levels of oxygen tension so that the results are strictly comparable. Thus, in Experiment 3, increasing the oxygen concentration from 5 to 10 cc. O_2/l. increased the rate of regeneration 21.9 R. U. at pH 8.00, 23.2 R. U. at pH 6.96, and 14.1 R. U. at pH 6.69.

Complete inhibition of primordia formation may likewise be determined by both the oxygen and hydrogen ion concentrations of the sea water. Barth (1938a) found that the lower limit for regeneration in sea water at normal hydrogen ion concentration (pH 8–8.2 approx.) is between 0.35 and 1 cc. of oxygen per liter. As the hydrogen ion concentration is increased, complete inhibition occurs at higher oxygen concentrations (Table I; Figure 1). At pH 7.00 almost complete inhibition occurred at 3.6 cc. O_2 per liter, while at pH 6.30 complete inhibition occurred at 8.2 cc. O_2 per liter.

Discussion

An increase in the oxygen concentration of sea water results in an increased rate of regeneration. This observation was made by Barth (1938a), and is confirmed by the experiments reported here. However, as the hydrogen ion concentration is increased, there is a resultant decrease in the rate of regeneration. That the oxygen and hydrogen ion concentrations are interrelated in their effects is shown clearly by the results (Table I; Figure 1). The ability to form primordia is limited by the relative concentrations of both these factors in the sea water.

The inhibitory effect of low pH is not dependent upon the source of the hydrogen ion. In the experiments reported here the change in hydrogen ion concentration was brought about by increasing the carbon dioxide tension of the sea water. Inhibition is also obtained when the pH of the sea water is regulated by means of acid, base, and buffer (Goldin, 1942).

The interaction of these two environmental factors may likewise determine whether regeneration will occur or be completely inhibited. For, as the hydrogen ion concentration of the sea water is increased the oxygen concentration required for regeneration is also increased. Thus the complete inhibition of regeneration which has been obtained after various treatments is clarified. Morgan (1903) found that the cut end of a *Tubularia* stem fails to regenerate when it is placed in sand. Ligat-

ing the cut ends of stems inhibits regeneration. Barth (1938a) and Rose and Rose (1941) demonstrated that the covering of the cut end of a *Tubularia* stem with a glass capillary is sufficient to cause inhibition. Inhibition is also obtained when coenosarc fragments of *Tubularia,* as well as stem segments, are inserted in glass tubing (Goldin, 1942). The inhibition in all of these cases may be attributed to both lack of oxygen and increase of hydrogen ion concentration. The inhibition of stem regeneration in glass tubing occurred at pH 6.9, while stems kept in open dishes (4.4 cc. O_2/l.) failed to regenerate in the neighborhood of pH 6 (Goldin, 1942). It was suggested that this difference could be accounted for if, in the glass tubing, a decrease in the oxygen concentration of the sea water occurred concomitantly with the accumulation of acid metabolites. This interpretation is supported by the experiments reported here, for almost complete inhibition occurred at pH 7.0 when the oxygen concentration was reduced to 3.6 cc. O_2/l.

The results lend support to the conclusions of Goldin (1942) on the origin of polarity in *Tubularia* regeneration. The conditions of oxygen and hydrogen ion concentration which are required for regeneration may likewise account for the origin of polarity. The polarity of dedifferentiated coenosarc fragments (Goldin and Barth, 1941; Goldin, 1942) as well as stem segments (Komori, 1933; Miller, 1937, 1939) may be determined by subjecting them to a differential environmental exposure. The differential exposure may consist of a gradient of oxygen availability, a gradient of hydrogen ion concentration (or CO_2), or a combination of both of these factors. The effectiveness of the gradients would be dependent upon the resultant concentrations of both oxygen and hydrogen ion at the exposed surfaces of the coenosarc.

In order to proceed with studies of the chemical processes involved in regeneration it is necessary to understand fully the physiological action of these environmental factors. Oxygen apparently plays an important role in the respiratory mechanism involved in regeneration. Barth (1940) studied the oxygen consumption of *Tubularia* stems, and showed that there exists a close correlation between oxygen consumption and the rate of regeneration. As the oxygen tension is increased, the tissues consume more oxygen, and the rate of regeneration is increased. Further, distal levels of the stem regenerate more rapidly and have a higher rate of oxygen consumption than proximal levels. The close relationship between the effects of oxygen and hydrogen ion concentration indicates that the same processes are affected. In this connection it would be important to test whether increasing the hydrogen ion concentration (or carbon dioxide tension) of the sea water decreases the rate of oxygen consumption.

Summary

The regeneration of *Tubularia* stem segments was studied in sea water at varying concentrations of hydrogen ion (carbon dioxide tension) and oxygen. As the hydrogen ion concentration was increased there was a fall in the rate of regeneration. On the other hand, as the dissolved oxygen was increased, the rate of regeneration was increased.

The effects of hydrogen ion and oxygen are interrelated, the resultant rate of regeneration being determined by the relative concentrations of both these factors in the sea water.

Complete inhibition may be effected by increased hydrogen ion, decreased oxygen, or by a combination of both of these factors. As the hydrogen ion concentration was increased, complete inhibition occurred at higher oxygen concentrations.

The origin of polarity in *Tubularia* regeneration was discussed in terms of these results.

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