THE RELATION BETWEEN OXYGEN CONSUMPTION AND RATE OF REGENERATION[1]

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Previous work (Barth, 1937, 1938*a*) showed that regeneration in *Tubularia* could be inhibited by placing the cut end of the stem in a glass capillary. A lowered O_2 tension was thought to be the cause of the inhibition and experiments in which the oxygen tension was varied showed that the rate of regeneration was closely dependent on the availability of oxygen. This relationship was also noted by Miller (1937), who found that the hydranth always appeared at the end where more oxygen was made available either by a higher oxygen tension or by circulating the sea water.

Further experiments by Zwilling (1939), in which the perisarc was removed from the middle of ligatured stems, showed that regeneration occurred on both sides of the perisarcal opening. This phenomenon could be interpreted to mean that the perisarc does not permit enough oxygen to reach the tissues to enable them to form a hydranth. The removal of the perisarc allows direct access to the oxygen of the sea water and starts the process of regeneration. If the foregoing experiments are to be explained on the basis of the concentration of oxygen at the tissues, then a study of the amount of oxygen consumed by the tissues is necessary. Some observations have been made by Child and Hyman (1926) on *Corymorpha* and Hyman (1926) on *Tubularia*. They found that the distal parts of stems consumed more oxygen than the proximal parts and further that young stems respired at a higher rate than old ones.

The experiments in this paper were designed to test whether: (1) the rate of regeneration of different levels of the stem is proportional to the rate of oxygen consumption at these same levels; (2) the regenerating portion of the stem uses more oxygen than the resting stem; and (3) whether the rate of regeneration of the stem is changed when the rate of oxygen consumption of a stem is increased or decreased.

[1] These researches were aided by a grant from the Rockefeller Foundation.

Methods

The stems of *Tubularia* were prepared from freshly collected material by cutting them off from the base of the colonies and selecting straight, unbranched stems of uniform thickness and healthy appearance. For the most part the more distal regions of the stem were used as these are free of parasitic growths which introduce an error in measurements of O_2 consumption. They were cut the desired length, care being taken to discard the region just adjacent to the hydranth as this region may exhibit a low rate of regeneration. The stems were then cut into halves, thirds, etc., according to the requirements of the experiment. The rate of regeneration was measured by taking the length of the primordium of the hydranth, the diameter of the primordium and the time at which the primordium becomes separated from the stem by a constriction. The rate of regeneration is then calculated as the volume of the primordium in μ^3 divided by the time in hours (Barth, 1938*b*). The units for rate of regeneration are $\mu^3/\text{hours} \cdot 10^5$.

The O_2 consumption was measured with Warburg manometers and the O_2 uptake calculated as the number of cubic millimeters of O_2 per hour per 10 mg. of dry weight. Weighings were made on a microbalance to 0.001 mg. In some of the earlier experiments the stems were merely selected of the same size and the oxygen uptake calculated for the mass of stems without weighing.

The O_2 Consumption of Parts of Stems

In these experiments the stems were cut into 2, 3, 4, or 5 parts and the rate of O_2 consumption determined. In some cases the rate of regeneration of the hydranth was also measured although previous experiments (Barth, 1938*b*) show that the rate falls off from distal to proximal region of the stem. The results have been calculated on the basis of $mm.^3$ O_2 used per hour per 10 mg. dry weight of the stem. Table I gives the results of using distal and proximal halves of stems. Since each half forms a hydranth at both cut ends, the rate of regeneration of the distal (oral, apical) hydranth and proximal (aboral, basal) hydranth of each half is measured. The table shows that the distal half consumes more oxygen than the proximal half (18.7 compared with 11.9) and the rate of regeneration of the distal half is greater (53.2 compared with 34.2). Likewise, in thirds of stems the rates of O_2 consumption and rates of regeneration are highest in the distal third, lower in the middle third and lowest in the proximal.

Table II records the rates of O_2 consumption of fourths of a stem.

Table III shows that the proximal fifth may consume as much O_2 as the third fifth of the stem, although since the stems were not weighed

TABLE I

Rate of oxygen consumption of parts of stems of *Tubularia*.
Rate = mm.^{3} O_2/hr./10 mg. dry weight.

Description of Stems			Oxygen Consumption					Rate of Regeneration μ^3/hrs. 10^5	
No.	Length	Region	Temp.	O_2	Time	Dry weight	Rate	Distal hydranth	Proximal hydranth
	mm.		°*C.*	*mm.*^{3}	*hours*	*mg.*			
20	7.5	distal half	19 ±.02	89.5	9.54	5.04	18.7	53.2	12.7
20	7.5	proximal half	19 ±.02	60.5	9.54	5.35	11.9	34.2	19.3
15	8	distal half	18.5±.02	112	13.42	3.512	23.8		
15	8	proximal half	18.5±.02	91	13.42	3.645	18.6		
20	5–6	distal third	19 ±.02	46.5	6.0	2.81	27.6	49.0	24.4
20	5–6	middle third	19 ±.02	41.0	6.0	3.06	22.4	34.4	17.4
20	5–6	proximal third	19 ±.02	31.4	6.0	3.36	15.6	23.6	9.6
10	5	distal third	18.5±.02	124	24				
10	5	middle third	18.5±.02	97	24				
10	5	proximal third	18.5±.02	89	24				

the results are not conclusive. This latter result agrees, however, with the experiments of Child and Hyman (1926), who found that the proximal third might sometimes use as much O_2 as the middle third. This observation correlates with the earlier observation of Child (1907) that the extreme proximal end sometimes regenerates as fast as higher levels of the stem.

It is clear then that there are regional differences in rate of O_2 consumption of the stem after cutting and that the rate of regeneration of the hydranth is roughly proportional to the rate of O_2 consumption.

TABLE II

Rate of oxygen consumption of parts of stems of *Tubularia*. In these experiments the proximal pieces were cut a little longer than the distal ones in an attempt to compensate for differences in diameter. As seen from the weight measurements they were cut a little too long and consequently are a little heavy. Rate = mm.^{3} O_2/hr./10 mg. dry weight.

No.	Length	Region	O_2	Time	Dry weight	Rate
	mm.		*mm.*^{3}	*hours*	*mg.*	
12	3	distal fourth	39.2	16.83	.977	23.8
12	3	second fourth	32.3		1.086	17.7
12	3	third fourth	30.0		1.190	15.0
12	3	proximal fourth	31.8		1.357	13.9
20	3–4	distal fourth	27.0	8.92	1.281	23.6
20	3–4	second fourth	22.8		1.517	17.0
20	3–4	third fourth	14.6		1.614	10.2
20	3–4	proximal fourth	21.2		2.023	11.7

Changes in the Rate of O_2 Consumption during Regeneration

When the values for O_2 consumption are plotted against time as in Fig. 1 the curve is S-shaped, indicating that as the regeneration process proceeds it requires O_2 at an increasing rate reaching a maximum and then falling off in the later stages of regeneration. The rate is highest from about seven to sixteen hours and it is during this period that the size of the primordium is determined (Peebles, 1931). These changes in rate are observed only in the case of short (2–4 mm.) stems.

The S-shape of the curve is lost or almost lost when the data from long (8–15 mm.) stems are plotted (Fig. 2). Here we find that the rate of O_2 consumption is almost constant throughout the period of regeneration. The interpretation given is that the O_2 consumption of

TABLE III

Oxygen consumption of fifths of stems of *Tubularia*. Figures are for total amount of oxygen in mm.^{3} O_2 consumed at time indicated.

No.	Length	Region	7 hours	26 hours	37 hours
15	2–3 mm.	distal fifth	31.4	125	162
		second fifth	23.0	104	140
		third fifth	18.3	95	125
		fourth fifth	16.8	83	108
		proximal fifth	22.0	94	122
			6.16 hours	17.33 hours	24.75 hours
17	2–3 mm.	distal fifth	17.3	52.0	63.0
		second fifth	14.6	41.5	51.0
		third fifth	12.4	38.9	49.5
		fourth fifth	13.3	36.6	48.5
		proximal fifth	11.8	34.4	46.0

the resting stem is so high that, in long stems where the regenerant comprises only about 10 per cent of the stem, the changes in rate caused by the regenerant are not noticeable. On the other hand in short stems, where 50 per cent of the stem may be regenerating tissue, the changes in rate during regeneration are more easily detected.

The Rate of O_2 Consumption and Rate of Regeneration at Varying O_2 Tension

The experiments of Miller (1937) and Barth (1937, 1938) indicated clearly that the rate of regeneration depended on the oxygen tension. In the following experiments the O_2 uptake was determined by placing the stems in different gas mixtures in the manometer vessels for most of the period required for regeneration. Then the stems were removed

and the rate of regeneration measured. Table IV gives the results. The rate of oxygen consumption falls from 5.4 in O_2 to 1.6 in N_2, while the rate of regeneration decreases from 177 to 0. It is clear that as the O_2 supply is reduced the O_2 consumption of the stem falls off and the rate of regeneration is decreased.

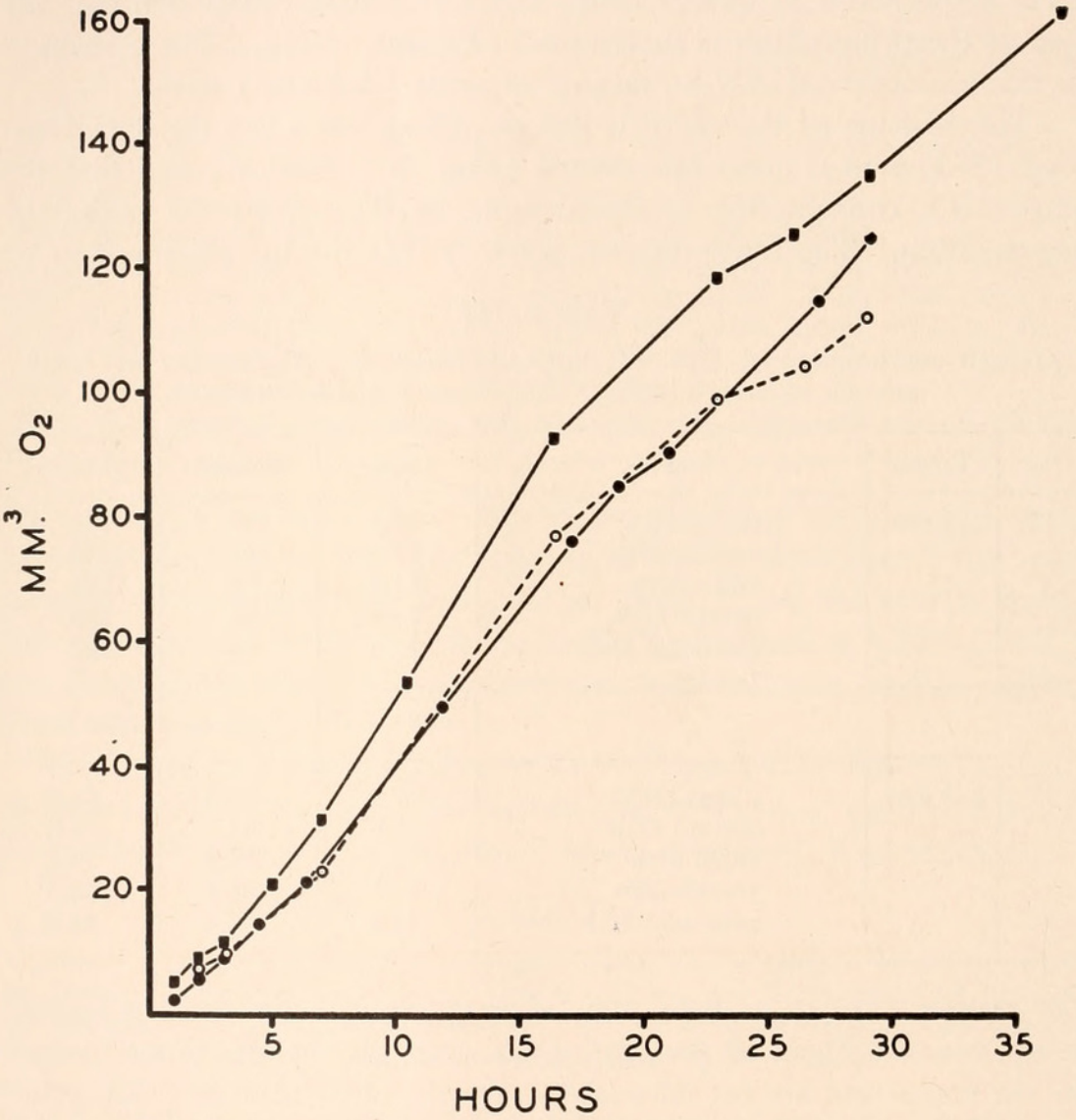

FIG. 1. Total amount of oxygen consumed by short stems, plotted against time. Squares give data from distal pieces of stems 2–3 mm. in length. Open circles are for more proximal pieces 2–3 mm. in length. Solid circles are for distal pieces 4 mm. in length. Hydranths are fully formed at 30 hours.

That the process of regeneration is closely dependent on the O_2 supply is shown by a comparison of the behavior of stems in the Warburg manometers and in open dishes. While stems never regenerate hydranths when ligatured at both ends and kept in open dishes, as many as 50 per cent of stems will regenerate hydranths if ligatured and shaken in the manometers with air. Thus, by keeping a high O_2 tension at the

surface of the perisarc, enough O_2 penetrates to start the process of regeneration. The O_2 consumption of these ligatured stems is about the same as for stems which have open ends (Table V). A similar result was obtained by Miller (1937) in comparing regeneration of ends in circulating sea water and standing sea water. More hydranths regenerate when the ends of the stem are bathed with circulating sea water.

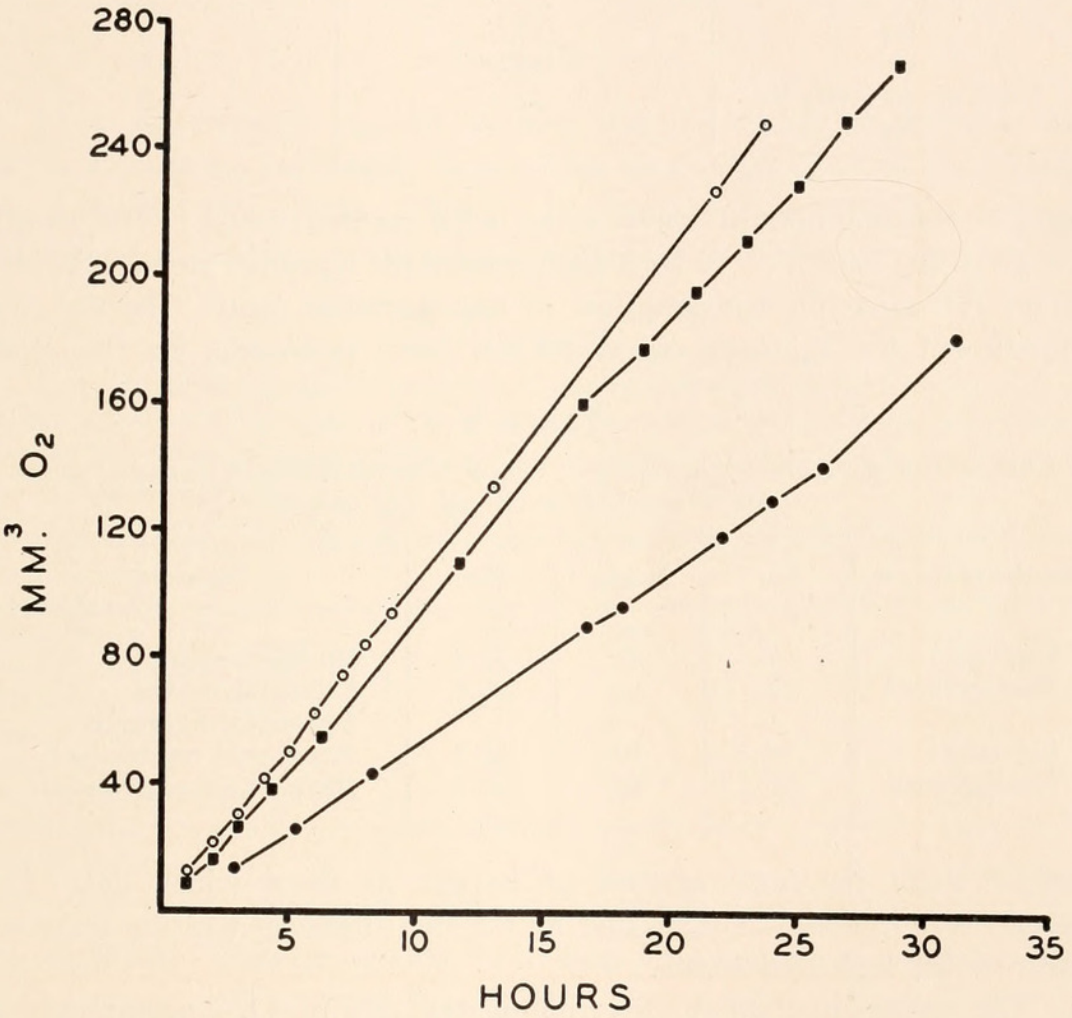

FIG. 2. Oxygen consumption of long stems, plotted against time. Squares = 12 mm. stems; open circles = 10 mm. stems; solid circles = 10 mm. stems. Hydranths fully formed at 30 hours.

Comparison of Rates of O_2 Consumption of Regenerating Stems with O_2 Consumption of Non-regenerating Stems

The rate of oxygen consumption of the regenerant itself must be only slightly greater than that of resting tissue. Attempts to measure the O_2 consumption of the regenerant itself were not very successful. The first attempt was made by ligaturing the stems and comparing the

TABLE IV

Rate of O_2 consumption and rate of regeneration at different O_2 tensions. Gas mixtures passed through vessels for 15 minutes with shaking. Twenty-seven stems (3 mm. in length) having a wet weight of 14 mg. were used for each gas mixture. Temperature 18.45 ± 0.02° C. Oxygen consumption measured for 28.75 hours. Stems then removed and the size of the hydranth measured.

Oxygen mixtures	Rate of regeneration μ^3 hrs. 10^5	Oxygen consumption mm.^{3} O_2/hr./14 mg. wet weight
O_2	177.0	5.4
air	155.0	4.9
1 vol. air to 5 vol. N_2	22.4	3.6
N_2	0	1.6

oxygen consumption of these stems with normal stems. The results are given in Table V. In both experiments the ligatured stems exhibited about the same O_2 consumption as non-ligatured stems. In the first experiment the ligatured stems did not form hydranths yet they con-

TABLE V

Comparison of oxygen consumption of ligatured stems with non ligatured stems. Rate = mm.^{3} O_2/hr./10 mg. dry weight.

Description of stems	No.	Length	Rate	Remarks
		mm.		
Ligatured	7	12	31.4	No regeneration
Nonligatured	7	12	30.4	5 distal hyranths 1 proximal hydranth
Ligatured	10	10	38.4	50 per cent regeneration
Nonligatured	10	10	35.0	100 per cent regeneration

sumed about the same amount of oxygen as those which did. The second experiment is complicated by the fact that regeneration occurred even in the ligatured stems.

The second method of determining the rate of O_2 consumption of the regenerant consisted in a comparison of the oxygen uptake of a whole stem with that of its parts. Thus in Table VI, first experiment, 24 stems 12 mm. long were selected and 12 were cut into 4 pieces and the oxygen consumption of the fourths were measured. The remaining 12 were placed in a manometer vessel at the same time for comparison. In the case of the stems cut into fourths there are eight regenerating ends, while in the whole stem only two. The expectation was a more rapid rate of O_2 consumption with four times as many regenerants. However, Table VI shows that the O_2 uptake is about the same in the whole stem as in the sum of its parts.

Neither of these methods shows a measurable difference between the

amount of O_2 consumed by the regenerant and resting stem. However, the curves for O_2 uptake of long stems (Fig. 2) can be interpreted on the basis that in long stems the regenerant is small in comparison with the resting stem and thus any variation in O_2 uptake caused by the regenerant could not be detected. The S-shaped curves for short stems where the amount of regenerant is relatively larger give evidence that the regenerant uses more oxygen than the resting stem (Fig. 1).

Discussion

These experiments support the idea that the tissues that exhibit the higher O_2 uptake regenerate faster than those that use less O_2 (Child and Hyman, 1926; Hyman, 1926). It might be argued that the regional differences in ability to regenerate caused the difference in rate of O_2

TABLE VI

Comparison of the rate of oxygen consumption of whole stems with the rate of oxygen consumption of parts of the stem. Rate = mm.^{3} O_2/hr./10 mg. dry weight.

Description of stems	No.	Length	Rate
		mm.	
Distal fourth	12	3	30.6
Second fourth	12	3	22.1
Third fourth	12	3	20.9
Proximal fourth	12	3	19.2
Whole stems	12	12	23.4
Sum of fourths			22.8
Distal half	20	7.5	18.7
Proximal half	20	7.5	11.9
Whole stem	20	15.0	14.8
Sum of halves			15.2

consumption. This is unlikely, as in long stems where the regenerating region forms only a small fraction (1/10 or less) of the resting stem the difference in O_2 consumption of distal and proximal halves is present. Since the greater part of these stems is resting tissue, the difference in rate of O_2 uptake must be due to this resting tissue and not to the regenerant. The regenerant would have to consume O_2 at ten or more times the rate of the resting stem in order to produce the observed difference in rate of O_2 consumption of proximal and distal halves. (Table I.)

However, since the regenerant consumes only slightly more O_2 than the resting stem, its oxygen consumption cannot account for the regional differences in O_2 uptake measured in distal and proximal parts of stems. Therefore, the gradient in oxygen consumption is an inherent character-

istic of the resting tissues at various levels of the stem. The evidence from varying the concentration of O_2 to which the tissues are exposed indicates that as the tissues increase their O_2 consumption they are able to regenerate faster.

It will be of interest in further experiments to see whether the O_2 consumption can be varied without changing the rate of regeneration and also whether the rate of regeneration can be changed without affecting the O_2 consumption. A word of caution is necessary here since in the sea urchin's egg it is quite possible to increase the rate of O_2 uptake of the unfertilized egg until it equals that of the fertilized egg without stimulating the egg to develop. Thus the rate of regeneration and the rate of O_2 consumption may be dependent upon two different processes which thus far are affected by the same treatment and conceivably some treatment might be found where either could be changed independently of the other.

Summary

The rates of oxygen consumption and the rates of regeneration of parts of the stem of *Tubularia* are greater in the distal levels of the stem than in the proximal levels.

The regenerating end of the stem consumes oxygen at a rate which is not much greater than the rate of the resting stem.

In different gas mixtures the rate of oxygen consumption of the stem and the rate of regeneration of the hydranth vary in the same direction.

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