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Effects of Some Respiratory Inhibitors on Respiration and Reconstitution in Tubularia.*

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Morgan[1] showed that the polarity of stem sections of Tubularia can be reversed if the distal end, which has the higher tendency to reconstitute, is thrust into sand. Since that time several other researches have similarly indicated that access to oxygen may be the determining factor in polarity and rate of reconstitution; and recently Barth[2] showed that the rate of reconstitution can be elevated by increasing the oxygen tension of the water, and depressed by decreasing the tension.

These results indicated the desirability of studying the respiratory mechanisms underlying reconstitution. Accordingly a series of correlated experiments were performed to determine the effects of potassium cyanide, which is believed to attack cytochrome oxidase, and phenyl- and ethyl-urethane, which probably interfere with dehydrogenases, on respiration and reconstitution in Tubularia. Sodium azide was also used, although the nature of its effect at the pH of sea water is not known.

Methods. The solutions were made up fresh each week in filtered sea water, and when necessary adjusted to pH 8.2 with hydrochloric acid. Six mm sections cut from the most distal portion of freshly gathered stems, uniform in translucence, length, and diameter, were used. Most of the reported experiments were performed in July and August on Tubularia collected from the cold waters of Cape Cod Bay.

In the reconstitution experiments the stems were kept in partly filled, tightly stoppered flasks, which were shaken at intervals to redistribute oxygen. The stems were kept in flasks until they reconstituted, or otherwise for four or five days. They were counted as totally inhibited, with rate of reconstitution zero, if after being removed to fresh sea water they developed hydranths. The rate was

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1 Morgan, T. H., *Arch. Entw.*, 1903, **16**, 125.

2 Barth, L. G., *Physiol. Zool.*, 1938, **11**, 179; *Biol. Bull.*, 1940, **78**, 366.

determined according to the method formulated by Barth,[3] as length of the unemerged hydranth divided by the time to its constriction.

The respiratory measurements were made with Warburg manometers, 20 stems being used in 2 cc of fluid. Measurements were carried out over a period of approximately 24 hours, the time required for the primordium to be fully formed in sea water at the temperature used, 19°. For every sample in the Warburgs a parallel control was kept in standing solution for the accurate measurement of reconstitution rate. Although shaking caused the stems in the manometers to reconstitute at a slightly higher rate than the standing controls, it did not change the relative effects of the various inhibitors.

Results. Table I summarizes the results of a number of representative experiments involving all the agents used. The table shows the tendency of all values to vary with the batch of stems and with the season.

TABLE I.

Agent	Reconstitution rate				Q_{O_2} (mm³ O_2/stem/hour)			
	Exp. No.	Control	Experimental	% inhibition	Exp. No.	Control	Experimental	% inhibition
Potassium cyanide								
5×10^{-5} M	9	22.4	11.4	49.1	9	1.28	0.40	68.8
1×10^{-4} M	9	22.4	7.0	68.8	9	1.28	0.19	85.3
1×10^{-4} M	10	33.6	8.5	74.6	10	1.54	0.48	68.8
1×10^{-3} M	4	25.6	0	100	4	2.64	0.44	83.5
KCN + 0.002% methylene blue								
5×10^{-5} M + MB	9	22.4	10.5	53.1	9	1.28	0.08	93.8
1×10^{-4} M + MB	9	22.4	6.7	70.1	9	1.28	0.16	87.5
1×10^{-4} M + MB	10	33.6	10.5	68.8	10	1.54	0.40	74.0
Sodium azide								
5×10^{-5} M	12	15.7	13.8	12.1	12	2.24	1.98	11.7
5×10^{-4} M	12	15.7	15.4	1.8	12	2.24	1.92	14.3
1×10^{-3} M	12	15.7	6.3	60.2	12	2.24	2.04	8.8
2×10^{-3} M*	7	38.0	5.0	86.9	3	2.67	2.04	23.5
Phenyl urethane								
2×10^{-5} M	9	22.4	25.0	(11.3)†	9	1.28	1.24	3.0
1×10^{-4} M	9	22.4	2.7	87.8	9	1.28	1.24	3.0
1×10^{-4} M	10	33.6	4.9	85.4	10	1.54	1.39	9.5
Ethyl urethane								
5×10^{-2} M	11	21.8	0	100	11	1.20	0.92	23.4
5×10^{-2} M	9	22.4	0	100	9	1.28	1.36	(6.3)†
5×10^{-2} M	10	33.6	24.6	26.8	10	1.54	1.40	9.0

*Although these values are not from the same experiment, they were obtained from stems collected in the same locality within the same week.

†The figures in parentheses indicate a per cent increase above the rate of the control.

[3] Barth, L. G., *Biol. Bull.*, 1938, **74**, 155.

1. *Cyanide.* Potassium cyanide effects a decrease in the reconstitution rate in concentrations as low as 6×10^{-6} M, and completely prevents reconstitution in solutions of 2×10^{-4} M, or a little higher. The action of cyanide is reversible, however, and stems kept in 1×10^{-3} M for 48 hours reconstitute when transferred to sea water. Cyanide cuts the reconstitution rate principally by lengthening the time of formation of the new hydranth, for the length of the hydranth is decreased only in the strongest solutions; this observation holds true also for the 3 other inhibitors used.

On the respiration of reconstituting stems cyanide also exerts a powerful inhibitory effect, but it is not capable of eliminating respiration altogether; even in 1×10^{-3} M respiration was maintained at 16% of the control value. Evidently there is a considerable residual respiration in stems in which reconstitution is completely blocked.

2. *Methylene Blue.* Because methylene blue is sometimes able to accept hydrogen and so to serve in place of the cytochrome system, 0.002% solutions were tried alone and with cyanide. But in no case did methylene blue alleviate the effect of cyanide on either reconstitution or respiration; nor did it have any perceptible effect when used alone.

3. *Sodium Azide.* Azide begins to affect reconstitution in 5×10^{-6} M solution, and completely suppresses it in solutions of a little more than 1×10^{-3} M. As in the case of cyanide the effect is reversible, for stems kept in 3×10^{-3} M for 48 hours reconstitute on transfer to sea water. That the intimate effect of azide differs from that of cyanide, however, is shown by the fact that azide solutions as concentrated as 2×10^{-3} M, which were found invariably to cut the reconstitution rate by at least 80%, alter the rate of oxygen uptake only moderately or not at all.

4. *Phenyl Urethane.* This narcotic inhibits reconstitution in concentration from about 5×10^{-5} M to 1.3×10^{-4} M. In this range inhibition is reversible, for weaker stems which failed to reconstitute in the urethane solutions do so when placed in fresh sea water, but in slightly higher concentrations the stems are rapidly killed. Below 5×10^{-5} M there is a decided acceleration of the reconstitution rate, which in one case rose to 135% of the control value. But in the respiration experiments, as the table shows, neither high nor low concentrations exerted any marked effect on the oxygen consumption.

5. *Ethyl Urethane.* Results obtained with ethyl urethane closely parallel those obtained with phenyl urethane. The inhibiting range is from 1×10^{-2} M to 6×10^{-2} M, and inhibition is reversible within this range. Concentrations a little above the upper limit consistently raised the reconstitution rate by not more than 10%, and solutions

slightly stronger than the lower limit were lethal. As can be seen from the table, however, a concentration which is capable of suppressing reconstitution completely does not exert a strongly inhibitive influence on respiration.

Discussion. The principal point established by these experiments is that only a very small part of the overall respiration is involved in the process of reconstitution. The urethanes and perhaps azide apparently act selectively on those dehydrogenases which belong to the system or systems directly associated with the metabolism for reconstitution.[4] Under these conditions only a small part of the respiration is affected, the remaining oxidative systems being unaffected or even accelerated. Cyanide, on the other hand, by attacking the ferro-catalyst in the main portion of the aerobic respiration, inhibits the corresponding oxidative systems equally and leaves only a low cyanide-insensitive respiration which is incapable of serving even the small respiratory needs of reconstitution. But this incapacity is not caused by quantitative insufficiency. Rather, the presence of cyanide blocks the flow of energy through many pathways, among which is that part of the respiratory cycle on which reconstitution depends. Barth's findings (1940, loc cit.) with varied oxygen tensions might be similarly interpreted on the basis of an unspecific stimulative or depressive effect on all oxygen-consuming systems.

This result is not surprising, for it has been found in numerous cases that an acitvity can be stopped without any marked effect on the respiration. If the oxidative system underlying the activity (in this case reconstitution) is only a small percentage of the total respiration, then neither stimulating nor suppressing that system would noticeably affect the overall respiration, although both events would be markedly reflected in the change in the rate at which the activity is carried on.

Summary. (1) Cyanide, azide, phenyl urethane, and ethyl urethane can slow or stop reconstitution in Tubularia; methylene blue does not alter the effect of cyanide. (2) Cyanide strongly inhibits oxygen consumption in the concentrations which affect reconstitution, but it does not suppress oxygen consumption entirely. (3) Azide and the urethanes do not have a marked effect on oxygen consumption in the concentrations in which they strongly decrease reconstitution. (4) These findings are compatible with the fact that oxygen tension influences the rate of both reconstitution and respiration.

[4] *Cf.* Fisher, K. C., *Biol. Bull.*, 1941, **80**, 282.