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Received October 10, 1958. P.S.E.B.M., 1959, v100.

Nutritive Substances and Reconstitution in Tubularia. (24566)

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Experiments of Barth(1) and of Rose and Rose(9) showed that substances circulate in the tubularian coelenteron which influence hydranth reconstitution. Barth(1,2,3) postulated that these substances are nutritive and that dominance of distal over proximal end is a result of the competition of the two for a postulated substance which he calls "S." His data can be interpreted either as the result of stimulatory or of inhibitory substances. The latter possibility was suggested by the experiments of Goldin(4,5) in which inhibition was produced by increasing hydrogen ions in the surrounding medium. In addition, Miller(6, 7,8) found that a decrease in pH of similar magnitude as found inhibitory by Goldin, occurred in the coelenteric fluid of stems which failed to reconstitute when placed in glass tubes. The experiments described below were designed to test the effects of reducing the amount of possible nutritive substances which might be available to the distal end of the stem during reconstitution.*

Material and methods. For the first experiments, Tubularia were collected from the Oceanographic Dock at Woods Hole. For later series they came from the U.S. Engineers Dock at Sagamore, Mass. The apparatus for maintaining a constant flow of seawater through the stems consisted of a battery jar 27 cm diameter, with wooden cover containing 21 holes in 2 concentric rings (Fig. 1). Filtered seawater was introduced through a glass tube inserted in the middle hole. In the other 20 holes were placed eye-droppers with tips drawn out to a diameter of less than ½ mm. These were inserted into proximal ends of 10 mm long segments of Tubularia stems. To insure uniformity the distal end of each stem was cut transversely 5 mm below the hydranth and to prevent possible mistakes in orientation the proximal cut was oblique (Fig. 2). After insertion each stem was checked to make certain that the eye-dropper was not occluded by tissue and that a free flow of seawater took place. Controls were given no further treatment and the eye-droppers gradually filled with seawater to the same level as that in the jar. A glass tube with 11 nipples and bent to fit the battery jar was placed on the cover. One nipple was connected through a water trap to an aspirator. The other 10 were connected to short lengths of glass tubing inserted into the eye-droppers as far as the constriction (Fig. 1). Flow of aspirated air was controlled by screw clamps (not shown in Fig.). By this means a difference of about 4 cm in the water level inside and outside of eye-droppers of experimental stems was maintained (Fig. 2). This produced a gentle flow of seawater which, usually, was sufficient to prevent closure of the ends of the stems but was not strong enough to flush the coenosarc out of the perisarc. Patency of the experimental stems was determined at intervals by stopping the aspirator for one-half hour and observing the rise of water in the eye-droppers. Stems which

* Biol. Bull., 1950, v99, 361.

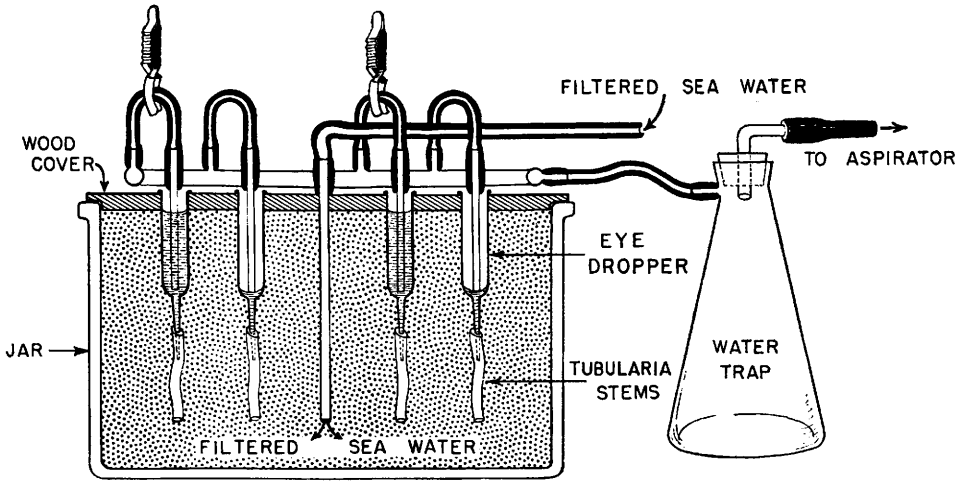

FIG. 1.

failed to show a normal rise were discarded. Filtered seawater was provided at a rate 1 to 1½ liters/minute by a rapid filter constructed from a 2 gallon carboy with a nipple just above the bottom. In this was placed first, 4½ inches of thoroughly washed sand and on top, 2 inches of glass wool to prevent loss of sand when the carboy was inverted. The mouth was closed by a one-hole stopper which in turn was wired to the neck of the carboy. Pressure tubing was used throughout and connections wrapped with wire for security. The

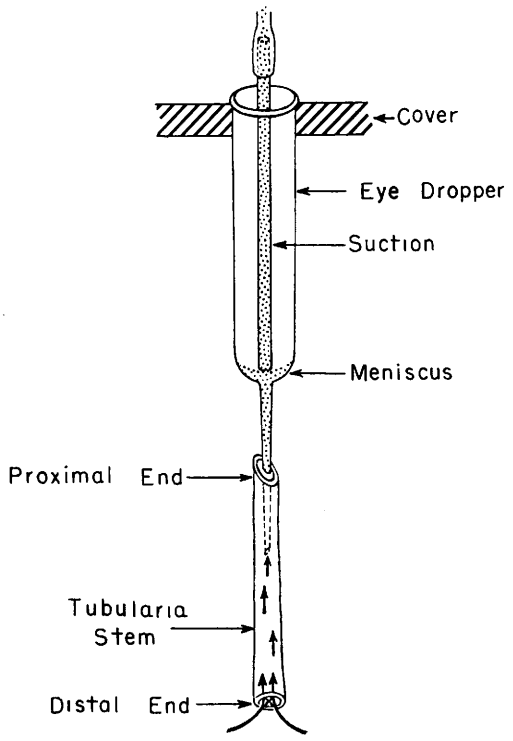

FIG. 2.

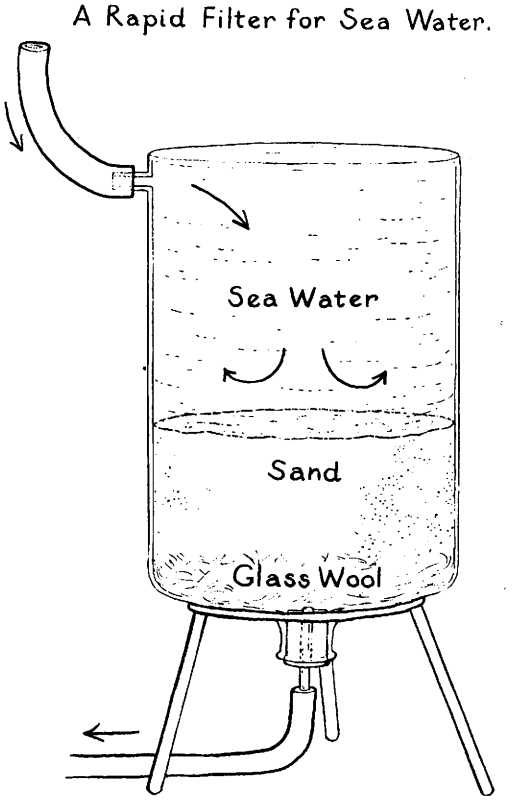

FIG. 3.

TABLE I. Reconstitution of Stems in Running Filtered Seawater.

	No.	No. discarded: Plugged	No. discarded: Coenosarc lost	No. used in calculations	Reconstitution: Primordia	Reconstitution: Emerged	%
Exp. I							
Control	10	0	0	10	7	7	70
Exp.	10	1	2	7	7	7	100
Exp. II							
Control	10	0	0	10	8	8	80
Exp.	10	2	2	6	6	6	100
Totals							
Control	20	0	0	20	15	15	75
Exp.	20	3	4	13	13	13	100

filter was inverted, connected with the experimental jar and completely filled with seawater through the upper opening. Then tubing connected with the seawater line and completely filled was attached to the carboy. Finally the spigot of the seawater line was opened slowly until the desired rate of flow was attained. When, after 3 or 4 days of continuous use, debris on surface of the sand reduced the rate of flow, it was restored by tilting the carboy sufficiently to break up the surface film.†

Results. In a preliminary experiment no provision was made for circulating the seawater in the battery jar. Neither the experimental nor the control stems reconstituted under these conditions.

In the second experiment circulation was provided but the water was unfiltered. Reconstitution was very slow in all stems and contaminating growth of ciliates and other microscopic organisms developed on the stems. One control and 3 experimental stems lost their coenosarcs either through the action of parasites or (in the case of the experimental stems) because of excessvie flow through the stems. A fourth experimental stem was discarded because it became plugged. Only 4 of the remaining 9 control stems reconstituted (44%). Their hydranths were very small and required 6 days to emerge as contrasted with the usual time of 48 hours. Three of the 6 experimental stems (50%) started reconstitution although in only one did the hydranth emerge. It appeared probable that contaminating growth on the other 2 was responsible for bringing reconstitution to a halt at the hydranth primordium stage. Since a slightly higher percentage of experimental stems than of controls began reconstitution, the results suggested that nutrient materials within the stem may not be essential for reconstitution. However, the only conclusion that could be drawn was that seawater flowing through the jar would have to be filtered.

Table I illustrates results of 2 experiments using seawater which had been passed through the rapid filter. Seven stems were discarded from the experimental group, 3 because they became plugged and 4 because of loss of coenosarc. The remaining 13 stems produced hydranths which emerged. Fifteen of 20 control stems (75%) reconstituted. Thus, stems from which all circulating substances were aspirated produced higher percentages of hydranths than did those which were permitted to close off cut ends and thereby retain any substances liberated into the coelenteron. Consequently, it was concluded that there is no substance essential for hydranth reconstitution which circulates within the tubularian coelenteron.

Discussion. It is very difficult to distinguish between results produced by stimulators and those produced by inhibitors of reconstitution. Barth(1,2,3) postulated that the 2 ends of a stem segment differ in their ability to utilize circulating nutritive substances. However, his results can be interpreted equally well by assuming a differential tolerance of circulating inhibitors. The results reported here cannot be reconciled with

† The author wishes to thank Dr. Arthur L. Colwin for suggesting this type of rapid filter.

the concept that cells in the middle of the stem liberate substances which are necessary for or contribtue to reconstitution. The constant flow of seawater into the distal end and out of the proximal would prevent any substances liberated into the coelenteron from reaching the distal end. By contrast however, the higher percentage of reconstitution which was recorded for experimental as contrasted with control stems is consistent with expectations if there were inhibitory substances within the coelenteron. The direction of flow would be as successful in preventing inhibitors from reaching the distal end as it was in siphoning off nutritive materials. Since increased acidity of the coelenteron occurs during reconstitution, and builds up to concentrations which are known to be inhibitory when externally applied, the suggestion is made that removal of such substances was responsible for the increased percentages of reconstituted hydranths in our experiments.

Summary. An apparatus was constructed which produced a constant flow of filtered seawater through the coelenteron of Tubularia stems. This arrangement was designed to prevent substances liberated into the coelenteron from reaching and influencing reconstitution at the distal end. Reconstitution occurred in spite of removal of such substances and was, in fact, superior to that of control series. Accordingly, it was concluded that although circulating inhibitory substances have been demonstrated, there is no evidence that any substances in the coelenteron influence reconstitution in a favorable direction.

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Received October 20, 1958. P.S.E.B.M., 1959, v100.

Total Blood, Erythrocyte and Plasma Volumes in Muscular Dystrophic Mice.* (24567)

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The marked decrease in skeletal muscle mass and lowered body weight observed in mice with hereditary muscular dystrophy evoked an inquiry whether or not the circulatory system was in any way altered to adjust to this condition. Since no reports of total blood volume in either mice or man afflicted with muscular dystrophy were found in the literature, experiments to determine total blood, plasma and erythrocyte volumes of muscular dystrophic mice were performed and the results are presented here.

Methods. Dystrophic mice were obtained from Roscoe B. Jackson Memorial Laboratory and after a week of acclimatization were studied at 43-52 days of age. Littermate normals of the same sex were used in all except 3 instances in which controls were obtained from other litters but of the same stock and age. Other normal but older mice of the same stock were also used, some of which had become very obese. Plasma volumes were measured by T-1824 dye dilution technic and hematocrit values were obtained by using Van Allen tubes. These procedures were used as previously described(1) except for the dye volume. The plunger of a 0.25 ml hypodermic syringe was calibrated to deliver 0.05 ml of dye through a 26 gauge, ½ inch needle inserted into exposed jugular vein in mice under light ether anesthesia. Dye injection was ac-

* Aided by grant from Muscular Dystrophy Assn. and the Sage and Sackett Research Funds of the Dept. of Zoology, Cornell University.

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