Development of the Eggs of *Phallusia mammillata* in Sea-Water Devoid of Sulphate Ions

The presence of sulphate ions ($SO_4^{=}$) is indispensible for the normal development of the sea-urchin[1,2]. Eggs of *Paracentrotus lividus*, which develop in sea-water free from $SO_4^{=}$, give rise to animalized larvae[3,4].

The present paper reports the results of an experimental study of the development of an ascidean, *Phallusia mammillata*, in the absence of $SO_4^{=}$ ions.

The eggs taken from the oviducts of *P. mammillata* were divided between 3 vessels: one of these contained artificial sea-water prepared by HÖRSTADIUS' method[5] (corresponding to the composition of the water in the Gulf of Naples); the second contained artificial sea-water in which the $MgSO_4$ had been replaced by an equivalent quantity of $MgCl_2$; and the third contained filtered sea-water.

The eggs were fertilized by introduction of a drop of sperm into each vessel; the vessels were then placed in a thermostat at 18 °C. A proportion of the larvae that developed were fixed in 10% formalin in sea-water, and photographed. The remainder were fixed in Bouin-Holland and Zenker-Helly solution, and embedded in paraffin. Sections 4 μ thick were stained by the trichromic staining method, with Carazzi haemalum, eosin-orange and brilliant green. In certain studies on unfixed eggs and larvae, the Nadi reaction[6] was used.

We studied the development of the eggs of *P. mammillata* up to the stage of absorption of the tail. Of the eggs that had developed in the absence of $SO_4^{=}$ ions, a considerable percentage showed no segmentation, or else segmentation had been arrested in its early stages. The larvae that did develop showed alterations to the tail only, which was much shorter, broader, and more curved than in the control larvae (Figures 1 and 2). The motility of the larva was arrested; the cephalic part showed apparently normal development with the exception of the palps, which were slightly larger than in the control larvae.

Microscopic examination revealed alterations to the tail only. Compared with the controls, the muscle cells were rounder and fewer; in the nucleus the chromatin stained very slightly and was arranged in small masses in contact with the nuclear membrane (Figure 3). The chordal cells did not appear to have undergone marked morphological changes; they were compressed and were rectangular in shape. The Nadi reaction was always positive.

The findings obtained clearly show that the absence of $SO_4^{=}$ ions leads to anomalies in the muscle cells of the tail of larvae of *P. mammillata*. The anomalies we see

Fig. 1. Control larva, developed in artificial sea-water. × 40.

Fig. 2. Larva developed in artificial sea-water free from SO_4 ions. × 40.

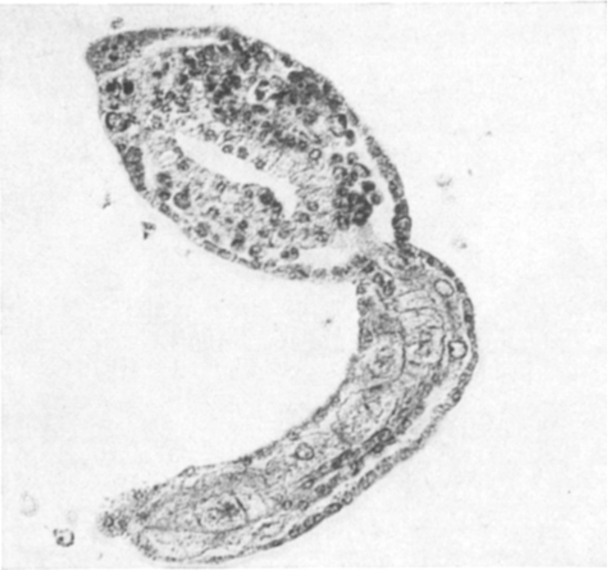

Fig. 3. Histological section of larva developed in artificial sea-water without $SO_4^{=}$ ions. Bouin-Holland. Trichromic staining. × 325.

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[1] C. HERBST, Arch. EntwMech. Org. *5*, 48 (1897).

[2] C. HERBST, Arch. EntwMech. Org. *17*, 306 (1904).

[3] J. IMMERS, Exp. Cell Res. *10*, 546 (1956).

[4] J. RUNNSTRÖM, S. HÖRSTADIUS, J. IMMERS, and M. FUDGE-MASTRANGELO, Revue suisse Zool. *71*, 21 (1964).

[5] S. HÖRSTADIUS, Pubbl. Staz. zool. Napoli *14*, 251 (1935).

[6] G. REVERBERI, Experientia *12*, 55 (1956).

here were similar to those obtained by REVERBERI with the use of sodium azide[7,8] and of sodium malonate and selenite[9], and to those observed by DE VINCENTIIS with the use of partial anaerobiosis[10] and in the presence of CO in the dark[11]. According to REVERBERI, the cytochrome oxidase, which is segregated at an early stage in the posterior vegetative blastomeres, plays a part in the morphogenesis of muscle cells by supplying the energy required for the construction of the molecules that form the first substrate of the specific substance of the muscle cells, or for the linkage of these molecules into fibrous chains, or for the elongation of the entire cell.

CROWELL[12], using certain specific inhibitors of the –SH groups (mercapto-ethanol, thiomalic acid, phenyl-mercuric chloride) at various stages of development of the eggs and embryos of *Ciona intestinalis* L., was able to show recently that the larvae which developed had distorted tails, in which the muscle cells were only little differentiated and which in addition contained hypertrophic chordal cells, while the cerebral vesicle was much reduced in size and palps remained absent.

From these various findings it may be concluded that our results could be interpreted in two different ways: (1) on the one hand, the absence of $SO_4^=$ ions may act on the mitochondrial systems, the accumulation of which in the posterior vegetative blastomeres appears to be associated with differentiation of muscle cells. The positivity of the Nadi reaction does not in itself prove that the mitochondria function perfectly, as[13] the Nadi reagent is oxidized at the cytochromoxidase level, and yields no information concerning the potency of the succino-dehydrogenase and NADH-dehydrogenase systems. The function of these systems is known to depend on the presence of –SH groups. (2) Further, in view of the work done by IMMERS and RUNNSTRÖM[14], who found that absence of $SO_4^=$ ions causes a decrease of uptake of labelled uridine, thymidine and amino acids into the embryos of the sea-urchin, it is worth considering the hypothesis that in *Phallusia*, also, the effect observed may be due to a disturbance in synthesis of the specific proteins. Cytochemical and autoradiographic studies which are still in progress will probably throw further light on this problem[15].

Riassunto. Viene studiato lo sviluppo di *Phallusia mammillata* in acqua di mare priva di ioni $SO_4^=$. Le anomalie riscontrate a carico della larva riguardano principalmente il sistema muscolare della coda. Viene discusso il probabile ruolo esplicato dagli ioni $SO_4^=$ nel corso dello sviluppo.

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[7] G. REVERBERI, Pubbl. Staz. zool. Napoli *29*, 187 (1957).

[8] M. DE VINCENTIIS, Boll. Zool. *23*, 701 (1956).

[9] G. REVERBERI, Acta Embryol. Morph. exp. *1*, 12 (1957).

[10] M. DE VINCENTIIS, Experientia *12*, 381 (1956).

[11] M. DE VINCENTIIS, Ricerca scient. *26*, 3093 (1956).

[12] J. CROWELL, Acta Embryol. Morph. exper. *5*, 285 (1962).

[13] L. LISON, in *Histochemie et Cytochimie animales* (Gauthier-Villars, Paris 1960), p. 583.

[14] J. IMMERS and J. RUNNSTRÖM, J. Embryol. exp. Morph. *14*, 289 (1965).

[15] This investigation was supported by Consiglio Nazionale delle Ricerche of Italy.

[16] We are very much obliged to the Director, Dr. PETER DOHRN and to the staff of the Stazione for effective support and help in our work.

Biotin and Fatty Acid Biogenesis in *Aspergillus flavus*

Using avian liver preparations, it has been demonstrated that biotin has a role in the synthesis of fatty acid[1]. Biotin containing enzyme acetyl carboxylase catalyses the first step in the reaction; malonyl CoA, the product of this reaction, is the intermediate in the pathway. A great deal of data is available on the mode of biogenesis of fatty acid in animals and plants. In comparison, there is little information available on the mechanism of microbial fatty acid biogenesis. Recently, two reports[2,3] have been published which indicate that biotin deficiency results in the change in fatty acid composition of *Lactobacillus plantarum* and *E. coli*. WOODBINE et al.[4] reported high lipid production in *Aspergillus flavus*. In the present experiments we have used this mould to study the relationship between biotin and lipid synthesis.

Submerged cultures of *A. flavus* were grown at 28–30 °C on 50 ml medium pH 5.7 containing glucose 40 g/l, NH_4NO_3 1 g/l, $MgSO_4$, $7H_2O$ 0.3 g/l, and KH_2PO_4 0.3 g/l in 250 ml Erlenmeyer flasks. At the end of 6 days the mycelium was harvested and the lipid of the dry felt was extracted with ether and saponified by $1N$ alcoholic KOH. After acidification the fatty acids were extracted by ether and were determined by weighing the residue after evaporation of ether. Biotin-deficient mat of *A. flavus* was obtained by cultivating the mould on the medium containing 5 U of avidin. Reactivation of biotin-deficient mould was carried out by the addition of $0.05 \mu M$ of biotin per flask.

The results of the experiments on fat production in normal and biotin-deficient cultures are recorded in the Table. Biotin deficiency has been found to result in greatly diminished ability to synthesize fatty acid, which indicates a role of the vitamin in the biogenesis. Further, the data listed indicate that increase in the amount of avidin does not result in the total inhibition of the fatty acid synthesis, which suggests that there exist 2 pathways of fatty acid synthesis of which one is sensitive and the other is insensitive to avidin; biotin overcomes the avidin

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[1] S. J. WAKIL, J. Lipid Res. *2*, 1 (1961).

[2] J. A. CROOM, J. J. MCNEILL, and S. B. TOVE, J. Bact. *88*, 389 (1964).

[3] J. J. GAVIN and W. W. UMBREIT, J. Bact. *89*, 437 (1956).

[4] M. WOODBINE, M. E. GREGORY, and T. K. WALKER, J. exp. Bot. *1*, 204 (1951).