some mitochondria were found to be arranged in a disorderly manner in the heart muscle. A marked alteration of mitochondria in the heart was observed 7 min after hypoxia. From a population of 780 mitochondria, 30% had greatly increased in size and were swollen, 4% of them were small bodies or degenerated mitochondria, and 25% had fused together. 1% of the sample was huge mitochondria with floating cristae or mitochondria forming an elongated giant structure of about 6 to 7 sarcomere in length (Figure 1). Mitochondria in the early stages of fusion formed a 'giant structure' of different shapes as illustrated in Figure 2 and 3. Narrow channels or bridges were seen along the border of the adjoining mitochondria; the interior parts of the organelles had already been fused in some instances. This phenomenon is probably due to the inherent property of mitochondrial structural protein to reform hydrophobic surfaces after rupture of the mitochondrial membrane. Some of the mitochondria contained clear matrices as well as disrupted cristae. The formation of vacuoles in the myoplasm was occasionally observed.

Fig. 1, 2 and 3. Electron micrographs were taken from right ventricles of isolated rat heart 7 min after hypoxia illustrating fusion of mitochondria. × 26,000.

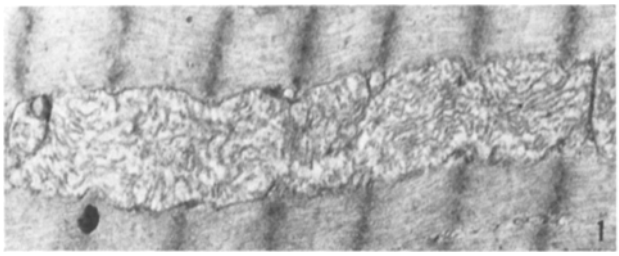

Fig. 1. A rod shaped mitochondrion ran parallel to the course of the myofibrils through 6 or 7 sarcomeres.

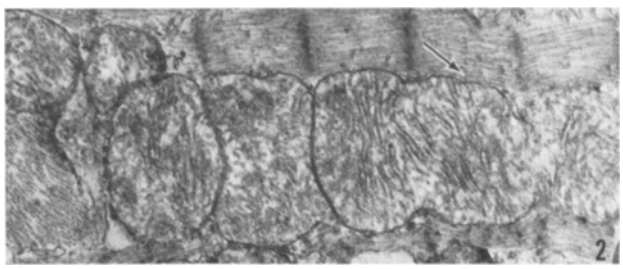

Fig. 2. A mitochondrion ran through 3 sarcomeres (arrow).

Fig. 3. Mitochondria were in different stages of fusion to form giant structures of different shapes.

Zusammenfassung. Herzen gesunder, erwachsener Ratten wurden mit Krebs-Henseleit-Bikarbonatlösung durchströmt (95% O_2:5% CO_2). Bei künstlich herbeigeführter Hypoxie (95% N_2:5% CO_2) erschienen die Herzmitochondrien innerhalb von 7 min bedeutend vergrössert und zu 25% miteinander verschmolzen.

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Observation on the Metamorphosis of Larvae of *Ascidia malaca* in Sea Water Devoid of $SO_4^=$ Ions

Previous investigations carried out on embryos of Ascidiaceae (*Ciona intestinalis* L., *Ascidia malaca* and *Phallusia mamillata*) allowed to develop in sea water devoid of $SO_4^=$ ions led to the development of larvae with marked abnormalities solely affecting the tails which were shorter than normal and exhibited anomalous muscle cells[1]. Subsequent cytochemical and autoradiographic studies led to the conclusion that the anomalies seen in the muscle cells of the tails were probably the consequence of a disorder of protein synthesis[2-4].

Preliminary studies carried out after metamorphosis had revealed changes of the morphogenesis of the adhesive papillae in *Ascidia malaca*[5]. In the present article, a report is given of the results of further experiments carried out during and after metamorphosis in embryos of *A. malaca*.

The embryos were allowed to develop in ordinary sea water, artificial sea water and $SO_4^=$-free sea water, using the methods previously described[1]; some of the embryos were photographed in vivo. In one experiment, groups of larvae developed in normal sea water or in artificial sea water to which 25 μC of $^{35}SO_4$ was added 10–12 h after hatching; after 30 min, these larvae were washed repeatedly in sea water and were then treated with Nile

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blue by the method of NUMAKUNAI, ISHIKAWA and HIRAI[6] to accelerate metamorphosis. 3 h after the addition of Nile blue the larvae were fixed, some in Bouin's fluid, others in Zenker formol. Sections of larvae fixed in Bouin's fluid and imbedded in paraffin were then subjected to autoradiographic tests, using Kodak NTB 2 emulsion[7].

Other sections of larvae fixed in Zenker were subjected to the following tests for mucopolysaccharides: PAS; alcian blue-PAS[8]; toluidine blue buffered to graduated pH values with a constant dilution of 1:5000; high iron diamine-alcian blue[9]. (a) The control embryos exhibited normal metamorphosis, with absorption of the tail and appearance in the trunk region of the adhesive papillae (Figure 1). The embryos treated with Nile blue showed greatly accelerated metamorphosis, in agreement with the observations of NUMAKUNAI et al.[6]. (b) The embryos developed in sulphate-free sea water showed rather irregular and incomplete absorption of the tail; the adhesive papillae at the trunk failed to develop (Figure 2). (c) The cytochemical tests for mucopolysaccharides in normal larvae gave positive results with alcian blue, with toluidine blue and with the high iron diamine-alcian blue test in the testal membrane and the adhesive papillae, supporting the contention that these structures contain sulphated acid mucopolysaccharides as well as sialomucins.

The uptake of $^{35}SO_4$ was particularly marked only in the adhesive papillae (Figure 3).

The findings show that in the course of the metamorphosis of *Ascidia malaca*, absence of $SO_4^=$ ions leads to arrest of the metamorphosis of such structures as the adhesive papillae (which normally become differentiated in the trunk during this period), the absence of this ion also leading to disorders of the normal absorption of the structures of the tail.

The results of the above-mentioned cytochemical and autoradiographic tests (which revealed the presence of sulphated acid mucopolysaccharides in the adhesive papillae) and the results of earlier studies, which revealed a disorder of the uptake of H^3-phenylalanine in larvae allowed to develop in sea water devoid of $SO_4^=$ ions[4], suggest that the above abnormalities observed during the metamorphosis may be due to a disorder of metabolism of the sulphated acid mucopolysaccharides and of protein synthesis.

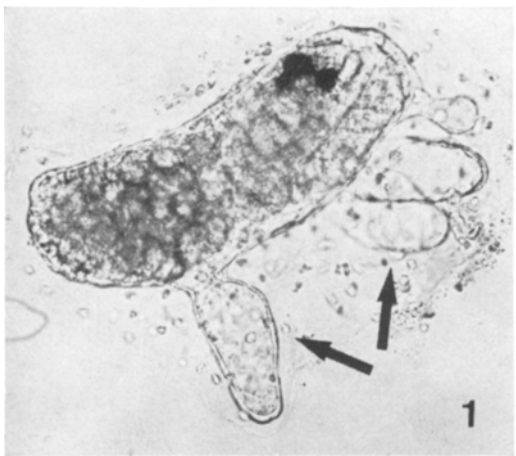

Fig. 1. *Ascidia malaca*. Larva developed in artificial sea water, after metamorphosis and photographed in vivo. The presence of adhesive papillae on the trunk, indicated by arrows, can be observed. × 120.

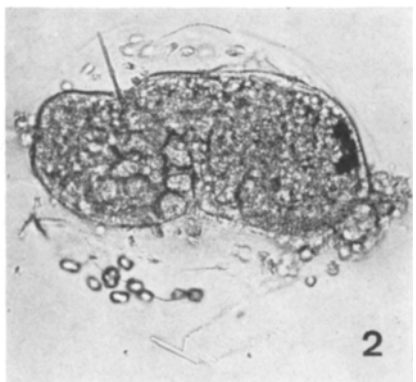

Fig. 2. *Ascidia malaca*. Larva developed in artificial sea water devoid of $SO_4^=$ ions, after metamorphosis and photographed in vivo. Same stage as larva in Figure 1. No differentiation of adhesive papillae on the trunk. × 120.

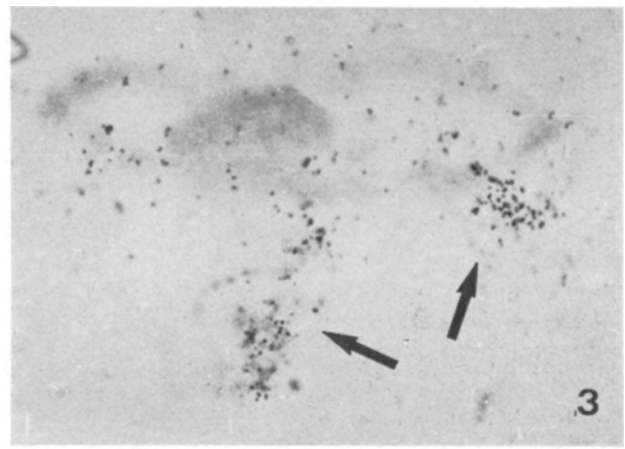

Fig. 3. *Ascidia malaca*. Larva developed in artificial sea water after metamorphosis. $^{35}SO_4$. Bouin. Autoradiography. An elective incorporation in the adhesive papillae on the trunk, indicated by arrows, can be seen. × 280.

Riassunto. Viene condotto uno studio su larve di *Ascidia malaca* durante la metamorfosi in acqua di mare priva di ioni $SO_4^=$. Si riscontra arresto del differenziamento delle papille adesive presenti nel tronco.

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