HEART STUDIES VI.
HISTOCHEMICAL INVESTIGATIONS OF THE MUSCLE, ESPECIALLY OF THE CONDUCTION SYSTEM, IN SOME VERTEBRATES

by

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Recent reports show that the impulse initiating and conducting fibres in the heart of mammals (including man) differ from the normal heart muscles both histologically and histochemically (9, 10). On the basis of these findings certain differences in the metabolism of the conduction system and that of the *myocardium* can be assumed. On the other hand current histological procedures failed to reveal a specialized conduction apparatus in the heart of lower vertebrates (4, 5, 6, 7). Many workers are, therefor, inclined to suppose that the heart muscle of the lower vertebrates performes the initation and conduction of impulses in addition to its contractives, i. e. has more complex tasks. A comparative histochemical study was performed on the heart of mammals and lower vertebrates to test the correctness of this assumption.

Material and method

Myocardium and His' bundle of cattles, the heart of adult guinea pigs and axolotls *(Amblystoma tigrinum)* were used for the experiments. The conducting bundle is clearly visible and easily prepared from the heart of cattles. The conducting bundle can be demonstrated by means of adequate morphological analysis in sections preparad from the heart of guinea pigs, whereas no such bundle distinguishable from the normal *myocardium* has so far been found in the heart of the axolotl. This is an urodele and thus belongs to a class inferior to that of the mammals; being neotenic, axolotl represents a phylogenetically younger stage within the urodeles. The removed hearts were immediately frozen with carbondioxide snow, 10 μ sections were cut in the cryostat. The sections were treated to demonstrate the following enzymes: β-glucuronidase with a postcoupling azo-dye method (PEARSE, 1960); alkaline phosphatase (GOMORI 1952), acid phosphatase (BARKA, 1960), succinic dehydrogenase (PEARSE, 1960) and esterase (DAVIS, 1959).

Results

The histochemical reactions (Fig. 1—12.) revealed that three hydrolytic enzymes, namely non-specific esterase, β-glucuronidase and acid phosphatase, were strongly active in the axolotl heart. The conducting bundle of guinea pigs and cattles showed strong non specific esterase activity. The β-glucuronidase reaction was weaker in the *myocardium* of the guinea pigs and the cattles than in the axolotl heart. The acid phosphatase reaction was negative in the heart of both species of mammals, and the alkaline phosphatase reaction in all species examined. As expected, the succinic dehydrogenase activity proved to be very intensive in the mammalian *myocardium*, but was extremely weak in their conduction apparatus as also in the heart muscle of the axolotls. The difference is so big as to make it possible to distinguish between conducting bundle and normal cardiac muscles in hearts where the bundle cannot be isolated.

Discussion

The examined hydrolytic enzymes (alkaline and acid phosphomonoesterases, β-glucuronidase, non specific esterase) are group-specific enzymes. The histochemical methods demonstrate enzymes acting on a certain group of substrates. It will be thus understood that observations regarding the activity of the aforesaid few hydrolytic enzymes do not suffice for direct conclusions as to the corresponding metabolic processes. All we can say on the basis of our results in that metabolic processes are different in the *myocardium* of the higher and the lower vertebrates. We may add that, since the said enzymes belong to the biochemically isolated lysosome fraction, it is justified to suppose that the lysosome system is more active in the heart of the axolotl than in that of the examined mammals.

It is easier to assess the results of the succinic dehydrogenase reactions. Being a markedly substrate specific enzyme, its behaviour affords insight into the tricarboxylic acid cycle and thus the organ's oxidative metabolism. We have notes that the activity was especially strong in the heart of the examined mammals. This may have been due to the intensive physical work to be performed by the *myocardium* which receives the energy required for this work through oxidation. The reaction was, on the other hand, very weak in the conduction system of mammals and in the entire heart of the axolotls. The conducting fibres are known to be non-contractile so that they do not perform physical work like the rest of the *myocardium*. It is, therefore, understandable that the enzymes of these fibres which are at play in the oxidative metabolism, e. g. succinic dehydrogenase, show a less activity. The similarity observed between the conducting fibres of the examined mammals and the cardiac musculature of the axolotl in respect of the succinic dehydrogenase reaction is a noteworthy phenomenon. It admits of two interpretations. The first is that the heart of the axolotl receives the energy required for its physical work chiefly from glycolitic and not from oxidative processes. In order to obtain a clear picture in this respect it is intended to perform histochemical experiments with enzymes of the glycolysis. The second line of reasoning is this: if we accept that weak reaction is characteristic of heart muscles which serve

for the initiation and conduction of impulses, the fact that the reaction has been found to be weak in the entire heart of the axolotl would support our assumption that there is no distinct conduction apparatus in the heart of this animal, and that all fibres of the cardiac musculature perform physical work as also the initiation and conduction of impulses.

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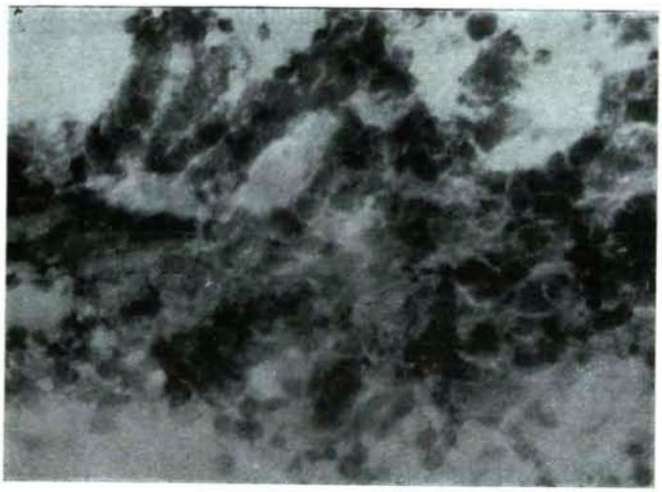


Fig. 1 Nonspecific esterase reaction in the *myocardium* of the axolotl. The cells give strong positive reaction. Magnification × 500.

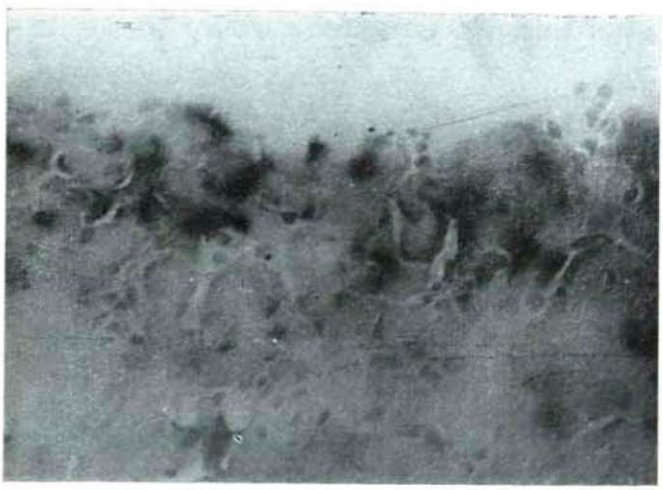


Fig. 2 Acid phosphatase activity in the *myocardium* of the axolotl. Note areas with positive reaction in the lower part of the picture. Magnification × 500.

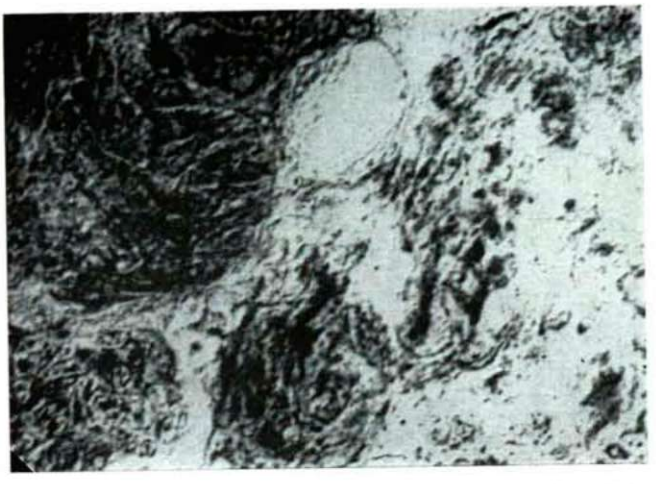
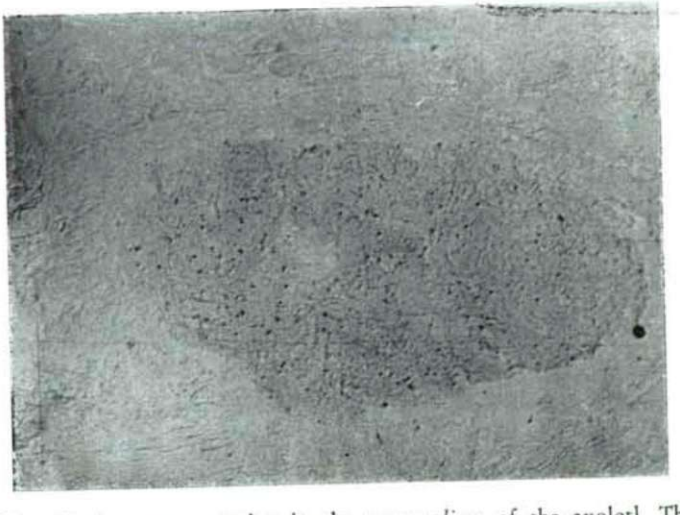


Fig. 3 β-glucuronidase activity in the myocardial fibres of the axolotl. Note intensive positive reaction along the fibres. Magnification × 500.



Filg. 4 Succinic dehydrogenase reaction in the *myocardium* of the axolotl. The sole visible muscle fibre appears to be negative. Occasional crystals of formazan indicate weak positivity. Magnification × 500.

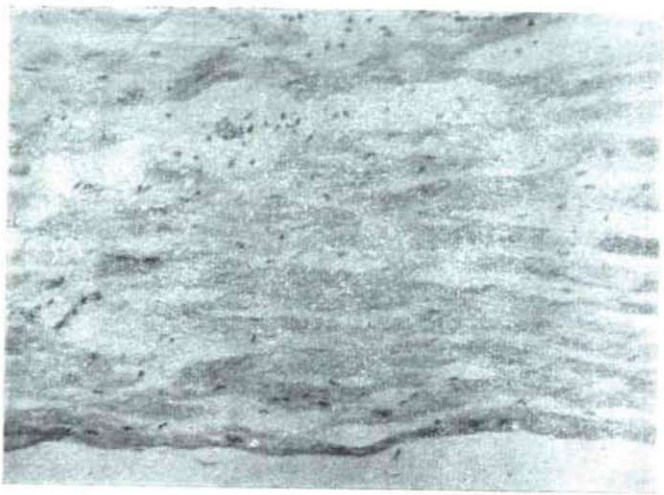


Fig. 5. Non-specific esterase activity is weak in the normal heart muscles of the cattle. Magnifications × 300

Fig. 6. Strong non-specific esterase reaction in the Purkinje fibres of the cattle-heart. Magnifications × 500

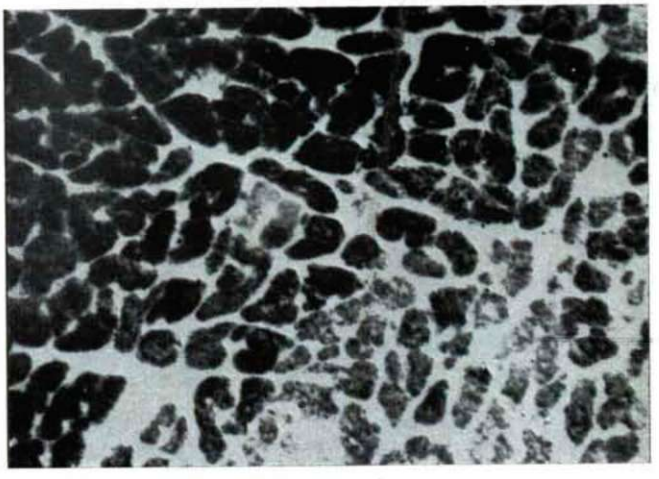


Fig. 7 Strong non specific esterase reaction in the *myocardium* of the adult guinea pig. Magnification × 350.

Fig. 8 β-glucuronidase reaction in heart-muscle fibres of the guinea pig. It is somewhat weaker than in the *myocardium* of the axolotl. Magnification × 500.

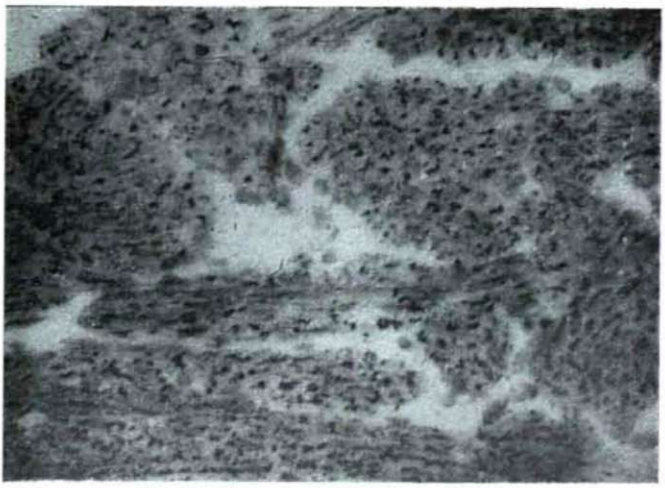


Fig. 9 Succinic dehydrogenase activity in the *myocardium* of the guinea pig. Note crystals of formazan indicating positive reaction. Magnification × 500.

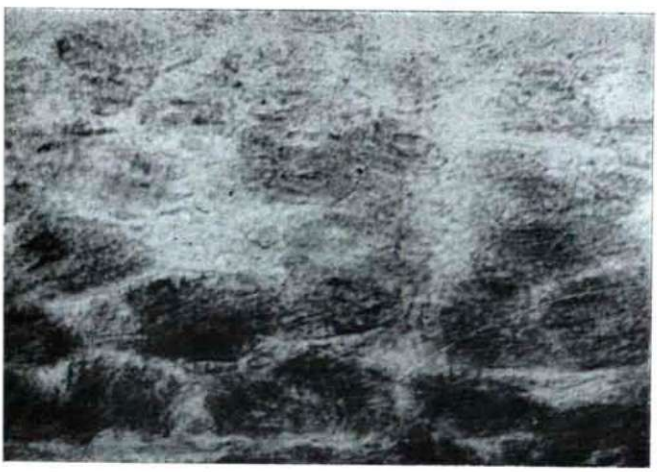


Fig. 10 β-glucuronidase activity in the normal heart muscles of the cattle. Note positive reaction along the microfibrils. Magnification × 450.

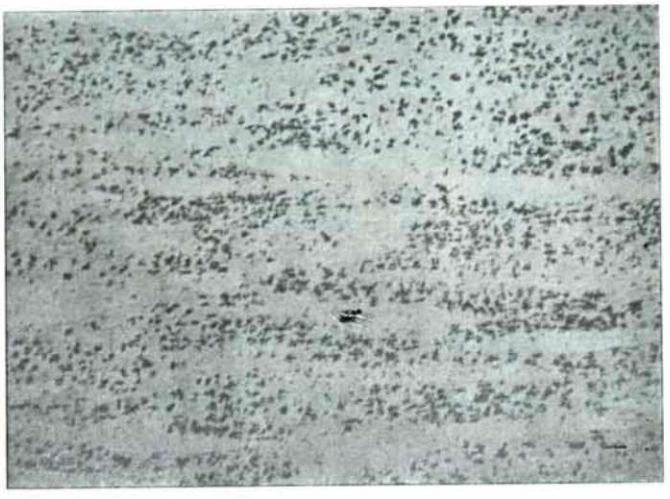


Fig. 11 Succinic dehydrogenase activity in the normal heart muscle of the cattle. Note strong reaction along the fibres. Magnification × 350.

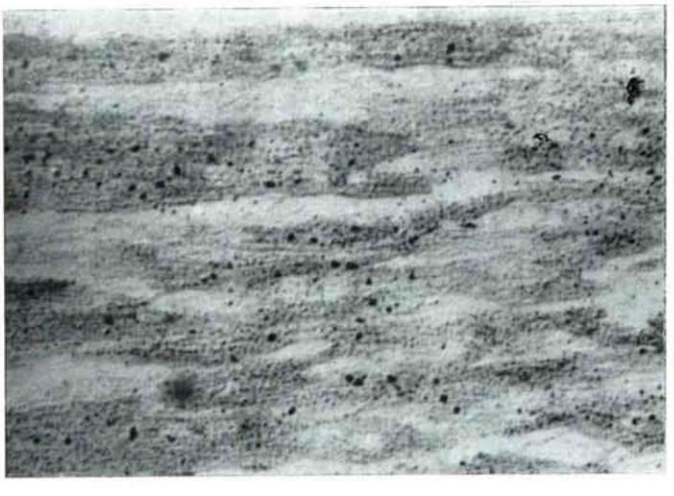


Fig. 12 Succinic dehydrogenase activity in the Purkinje fibres of the cattle-heart. Comparison with Fig. 11 shows reaction to be considerably weaker here. Magnification × 300.