CHANGES IN PHOSPHATASE ACTIVITY OF THE MYOCARDIUM IN CATS DURING POSTEMBRYONIC DEVELOPMENT

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The literature contains information from comparative studies concerning the alkaline and acid phosphatase contents of the myocardium in adult cats and cat embryos,but we have found no investigations which followed the phosphatase activity of the myocardium during postnatal development [4, 5]. It is stated that alkaline phosphatase is absent from the heart wall [19]. Conflicting evidence in the matter may be due to problems of species peculiarities. Species differences in alkaline phosphatase activity of the vascular walls have been demonstrated [12, 13].

The present work is a study of the alkaline and acid phosphatase distribution in cat myocardium during postembryonic development.

METHODS

Material from various sections of the right and left ventricles was fixed in a 12% solution of neutralized formalin at 4° for 4 h. Sections 10-15 μ in thickness were obtained with the freezing mictotome. The sections were taken from water and placed in microscopic object slides and dried at room temperature. Phosphatase activity was detected according to Gomori. The length of exposure times in the medium with β-glycerophosphate for the alkaline phosphatase determination were 4 and 15 h; for the acid phosphatase determination the exposure times were 15 and 24 h. Investigations were made on newborn kittens and at the ages of 2 and 3 weeks, 1, 2, 3, $4^1/_2$ months and also on adult cats.

RESULTS

In the newborn kitten alkaline phosphatase was observed in the nuclei of the myocardial fibers and the walls of the arterioles when exposure was 15 h. The structures in the nuclei were clearly seen and especially in the nucleolus in which the most intense reaction was obtained. In certain portions of the muscle fibers the cross striations were stained.

In the kitten at 2 weeks of age, 15 h exposure of the tissue revealed a more intense alkaline phosphatase reaction in the arterioles and in isolated sections of capillaries. The nuclei of the myocardial muscular fibers were stained with decreased intensity.

Animals at 3-4 weeks of age showed significantly greater alkaline phosphatase activity in the arterioles and capillaries, the enzyme being observable after only a 4-h exposure (Fig. 1). The left ventricle in contrast to the right ventricle evidenced a greater number of capillaries having a positive reaction. The nuclei in the muscle fibers, as before, show evidence of alkaline phosphatase on 15-h exposure, but during the process of postnatal development the staining of the nuclei becomes weaker.

The adult cat showed a clear positive alkaline phosphatase reaction only in sections of the capillaries.

In the control series, in which incubations were made in a medium without the glycerophosphate, nonspecific color was not observed in any structure either in the adult or newborn subjects.

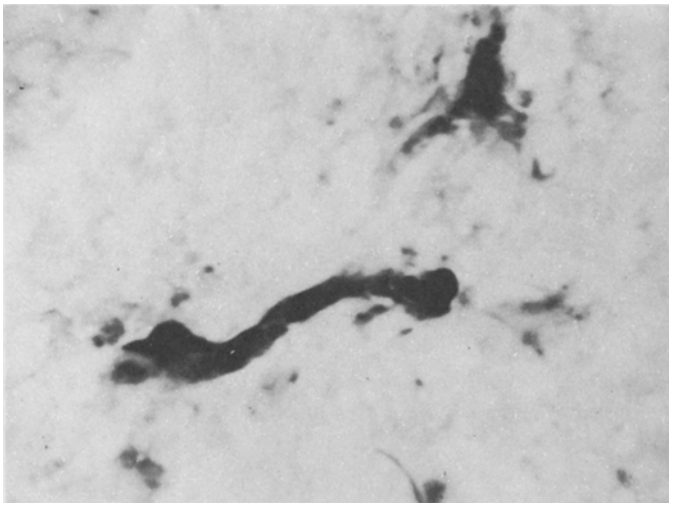

Fig. 1. Myocardium of kitten at 2 months of age. Positive reaction for alkaline phosphatase evidenced by capillary endothelium. Neutral formalin was the fixative. Detection according to Gomori. Four-hour exposure to the medium containing β-glycerophosphate. Photomicrograph. 60x objective, 10x ocular.

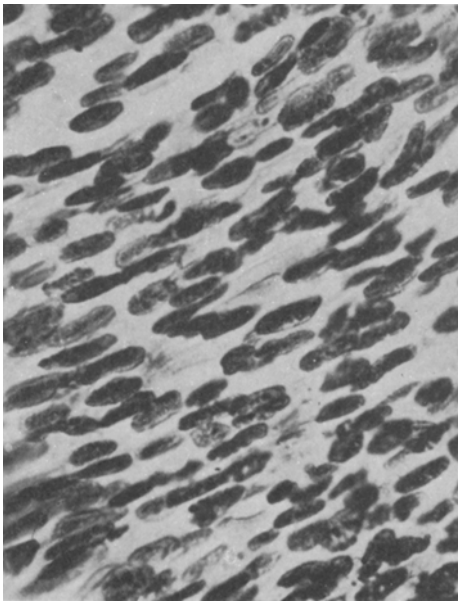

Fig. 2. Myocardium of the newborn kitten. A positive reaction for acid phosphatase is seen in the muscle fiber nuclei. Determination according to Gomori. 24-h exposure. Photomicrograph. 60x objective, 10x ocular.

Thus, in the process of postembryonic development, the activity of the alkaline phosphatase in the cardiac muscle fiber nuclei decreases, but it increases in the capillary endothelium.

It is known that there are difficulties in the interpretation of data on the localization of alkaline phosphatase [15]. Most workers in the field are inclined to view the coloration of the nuclei during determination of alkaline phosphatase as the result of diffusion of the enzyme or diffusion of its hydrolysis products from the cytoplasm where the enzyme is topographically associated with the mitochondria. However, numerous other workers have detected the enzyme in the nuclei [1, 3, 8, 10]. It is of interest that an intense positive alkaline phosphatase reaction is noted in the nuclei of fibroblasts and myoblasts actively growing in vitro [6] during the phenomenon of cellular proliferation and differentiation [9, 16]. It has been observed that in the process of cellular morphological differentiation the alkaline phosphatase becomes evident first in the nucleus and then later in the cytoplasm, and in this case the nucleus first develops alkaline phosphatase and only later on does it develop the enzyme specific for the nucleus, namely, 5-nucleotidase [9]. There is evidence that the alkaline phosphatase is contained in the chromosomes [7]. Thus, there is a basis for believing that the alkaline phosphatase, observed in the nuclei of the myocardial muscular fibers in newborn and young kittens, represents enzyme material localized therein.

An intense reaction for acid phosphatase occurs in the muscle fiber nuclei in the myocardium of the newborn kitten. In most of the nuclei the structure is clearly visible and this is especially true of the nucleoli, which give the most markedly positive reaction (Fig. 2).

Young kittens also show a positive acid phosphatase reaction in the nuclei of the cardiac muscle fibers.

In adult cats the acid phosphatase activity in these nuclei is significantly diminished.

Fig. 3. Myocardium of cat at 2 years of age. A positive reaction for acid phosphatase is evident in the myofibrils in which the cross striations may be clearly seen. Determination according to Gomori. 24-h exposure. Photomicrograph. 60× objective, 10× ocular.

In the control sections in which incubation was carried out in a medium without glycerophosphate, it was found that the newborn and young kittens gave a nonspecific color reaction only in the nuclei of connective tissue elements and the vascular walls in the various parts of the myocardium. The adult cats, however, in addition evidenced a weak nonspecific coloration in the cardiac muscle fiber nuclei. Consequently, the color of the nuclei in these fibers during the staining reaction for acid phosphatase can be attributed only partially to the presence of this enzyme.

Acid phosphatase is also localized in the myofibrils. In the newborn kitten there is a weak staining of the myofibril discs in separate areas of the muscle fibers. At 2-3 weeks of age and older, as well as in adult cats, most of the muscle fibers show clearly stained cross striations (Fig. 3). Some intercalated discs evidence enzyme activity in the adult animals. There was no staining of cross striations in the control series and, consequently, the color in the discs in the myofibrils may be viewed as specifically due to the presence of acid phosphatase.

Thus, in the postembryonic development process the acid phosphatase subsides progressively in the muscle fibers and increases in the myofibrillar discs.

Despite the widespread view that acid phosphatase is not present in the nuclei [15], we consider that the data obtained herein indicate the presence of the enzyme in the nuclei of the myocardial muscular fibers at least during the early stages of postembryonic development in the period of accelerated growth and differentiation of the muscle fibers.

There is evidence in the literature of increased acid phosphatase activity during mitosis and formation of nuclei in newly developing young cells [2], in the cardiac muscle fiber nuclei of the goat embryo [4]. Acid phosphatase contained in the nuclei of numerous cells and especially in the nuclei of striated muscle fibers in the heart and skeletal musculature, has been observed by various workers [1, 2, 18]. That the acid phosphatase is localized in the muscle fiber nuclei in the newborn kitten is demonstrated by the absence of any histochemical evidence of such activity in the cytoplasm. The coloration of the nuclei could be explained by diffusion of the ferment into them only in the event that an even more significant enzyme activity were observable in the cytoplasmic structures [14].

The high phosphatase activity in the cardiac muscle fiber nuclei in the newborn kitten must be associated with the energy processes required for synthesizing nucleic acids [11, 20].

Localization of alkaline phosphatase in separate portions of the capillaries is consistent with the function of the capillary endothelium in transfer of phosphorus-containing substances from the blood to the muscle fibers [17].

The presence of acid phosphatase in the myofibrils, perhaps is due to the role of this ferment together with ATPase in the process of muscle contraction [2].

LITERATURE CITED

1. G. I. Roskin and M. E. Teodoresku, Tsitologiya, No. 1 (1963), p. 97.
2. A. A. Savinovskaya, Dokl. AN SSSR, 139, No. 6 (1961), p. 1467.
3. A. A. Savinovskaya, Dokl. AN SSSR, 151, No. 3 (1963), p. 697.
4. E. B. Beckett and G. H. Bourne, Acta Anat., 35, Basel (1958), p. 224.
5. G. H. Bourne (Ed.), The Structure and Function of Muscle, 1, New York (1960).
6. M. Chévremont and A. Firket, Int. Rev. Cytol., 2 (1953), p. 261.
7. M. Dixon and E. Webb, Enzymes [Translation from English into Russian], Moscow (1961).
8. H. Elftman and W. M. Copenhaver, Anat. Rec., 97 (1947), p. 385.
9. W. Fortak, M. Karasek, and J. Kolaszynski, Folia biol., 10, Krakow (1962), p. 221.

10. J. C. George and S. M. Nair, Curr. Sci., 27 (1958), p. 177.
11. T. Hoshino, J. Tohoku, et al., Agricult. Res., 12 (1961), p. 9.
12. Z. Lojda, Csl. Morfol., 10 (1962), p. 46.
13. M. R. Malinow, et al., Nature, 183 (1959), p. 1262.
14. G. E. Palade, Anat. Rec., 106 (1950), p. 310.
15. E. Pearse, Histochemistry [Translation from English into Russian], Moscow (1962).
16. J. J. Pritchard, J. Anat., 81, London (1947), p. 352.
17. F. C. A. Romanul and R. G. Bannister, Nature, 195 (1962), p. 611.
18. A. Stolk, Proc. Kon.Med. Akad. Wet., Bd. 63, S. 53 (1960).
19. R. C. Truex and W. M. Copenhaver, Anat. Rec., 106 (1950), p. 314.
20. A. Vorbrodt, Exp. Cell. Res., 15 (1958), p. 1.

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