HISTOCHEMICAL EVIDENCE OF THE PRESENCE OF MYOGLOBIN IN HEART MUSCLE

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The continuous rhythmic activity of the heart requires a constant supply of oxygen. During contraction of the heart, when the blood vessels are constricted and the erythrocytes expelled from them, the hemoglobin of the blood temporarily ceases to supply oxygen to the heart muscle. In this period the muscle pigment myoglobin plays an important role. It stores oxygen during relaxation of the muscle and liberates it during contraction. Myoglobin thus acts as an intermediate link between the oxygen brought by the blood and the oxygen utilized by the muscle. Detailed information on myoglobin, its composition, its distribution, and its biological significance has been given in an extensive summary by P. A. Verbolovich [1].

Myoglobin is widely distributed in the muscle tissue of various animals. However, nearly all investigations of myoglobin have been carried out by the use of biochemical methods, which cannot determine the localization of the pigment within the muscle fiber. Not until the appearance of two papers in 1961, describing histochemical methods of determination of myoglobin [2, 3], was it possible to study the topography of myoglobin in the muscle.

In the present investigation the presence and distribution of myoglobin in the heart muscle of various members of the animal world were studied.

EXPERIMENTAL METHOD

The histochemical technique suggested by Verbolovich and co-workers was used because it is convenient and time-saving. This method is based on the peroxidase activity of myoglobin. In the presence of hydrogen peroxide myoglobin oxidizes benzidine at first into a substance which is initially blue, and later brown in color. The material was fixed in 10% formalin. The frozen sections were washed, and then treated with an alcoholic solution of basic benzidine with hydrogen peroxide (5-10 mg basic benzidine, 6 ml 96° alcohol and 1 ml H_2O_2). The sections were then dehydrated and mounted in balsam.

The specificity of the reaction was proved by Verbolovich by excluding from the reaction the hemoglobin of the blood and the true peroxidases which could oxidize the benzidine equally with the myoglobin. Some control sections were treated with a solution of sodium hydroxide (pH 10.0), which destroys hemoglobin but leaves the myoglobin, which is more resistant to the action of alkali, intact. Other control sections were treated with a 4% solution of H_2O_2 for 30 min. These concentrations and periods were adequate for suppressing the activity of the true peroxidases. In both control variants the localization of the brown granules in the preparations was unchanged. In addition, it was known that peroxidases in animals are found only in the leukocytes and the tissue of the mammary gland.

The reaction was carried out on the heart muscle of mollusks (Anodonta, pond snails), arthropods (crabs, crickets, locusts, and cockroaches), fishes (silver carp, vostrobriushka—Hemiculter leucisculus—, crucian carp, loach), amphibians (axolotls, tritons, frogs), reptiles (agamas, turtles), birds (pigeons, chickens), and mammals (mice, rats, hamsters, guinea pigs, rabbits, cats, fur seals, sheep, pigs and oxen). Chick, mouse, and fur seal embryos were studied. Altogether 27 species of animals were investigated.

EXPERIMENTAL RESULTS

No myoglobin was found in the heart muscle of the investigated invertebrates. It was found, on the other hand, in the vertebrates in every case.

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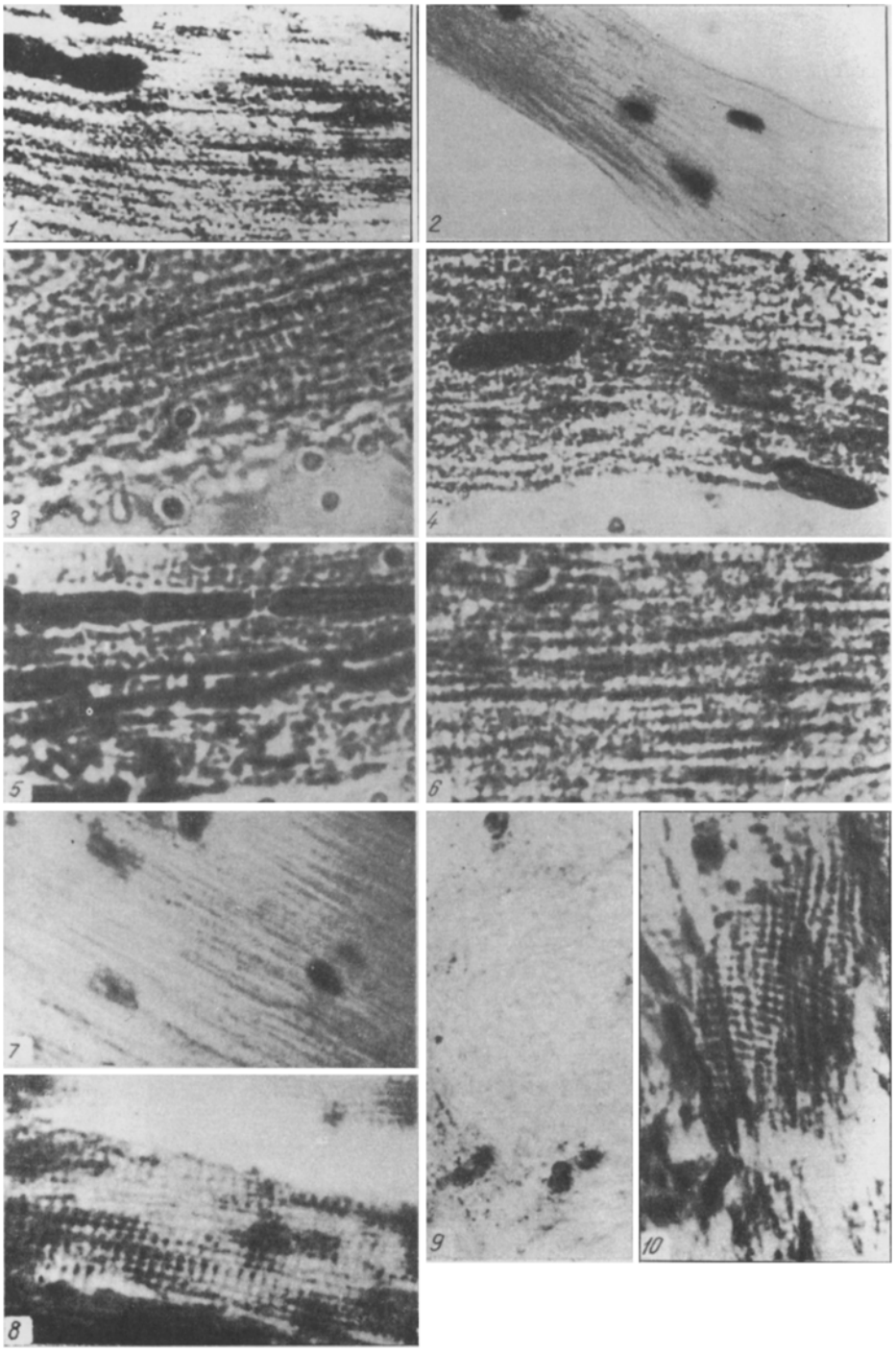

Verbolovich's histochemical reaction for myoglobin in heart muscle. 1) Ventricle of a loach (objective 40, ocular 10); 2) ventricle of a frog (objective 10, ocular 10); 3) ventricle of a triton (objective 60, ocular 10); 4) ventricle of a turtle (objective 40, ocular 10); 5) ventricle of a pigeon, (objective 60, ocular 10); 6) ventricle of a rat (objective 60, ocular 10); 7) heart of a fur seal embryo (objective 40, ocular 10); 8) ventricle of an adult fur seal (objective 40, ocular 10); 9) conducting system of the ox heart (objective 60, ocular 10); 10) ventricle of an ox (objective 40, ocular 10). The large dark formations in 1, 2, 4, 5, 7, and 9 are erythrocytes.

This particular histochemical method does not allow accurate evaluation of quantitative differences between myoglobin in different objects. In some cases obviously small amounts of myoglobin were found, when the

sarcoplasm stained diffusely with a pale yellowish-brown color, and in other cases larger quantities were present, when brown granules were clearly visible, giving a dark coloration to the whole muscle fiber. Small amounts of myoglobin were found only in the axolotl and frog (Fig. 2). It was noted that more myoglobin was present in the myocardium of the same frog in autumn than at the end of winter or in spring. Evidently, the slowing of the metabolic processes associated with the period of hibernation was reflected in the myoglobin content. In all the other investigated vertebrates the myoglobin content was high (see figure, 1-6, 8, 10). In oblique and transverse sections through the muscle fiber the brown granules were irregularly scattered in the sarcoplasm. In a strictly longitudinal section it could clearly be seen that the granules were arranged in rows (see figure, 8, 10) and in some cases they evidently corresponded to definite disks (10). P. A. Berbolovich [2, 4], considers that myoglobin is localized at the level of the A-disks in cross-striated muscle and, possibly, may also be present in the mitochondria. Since it is now known that cytochrome oxidase, transferring the electron to oxygen, is located mainly in the mitochondria, it seems highly probable that the myoglobin is localized within or close to the mitochondria.

In most animals the myoglobin was studied in working heart muscle—in myocardium of the atria and ventricles. No differences were found in these parts of the heart. In the heart of the pig, sheep, and ox, specific muscle was investigated besides the myocardium. In all cases the conducting system—the bundle of His and the fibers of Purkinje—were very poor in myoglobin (see figure, 9). Only solitary granules could be found near the capillaries. The amount of oxygen supplied directly by the erythrocytes was evidently adequate for the functioning of the conducting system: in the first place, the conducting system is freely supplied with capillaries, and second, the function of conduction does not require the intensive contraction characteristic of the myocardium.

The moment of appearance of the myoglobin in the myocardium was determined in the myocardium of the developing chick embryo. Myoglobin was first found in the 6-day embryo. At this stage the granules were not regularly distributed, but squatted diffusely in the sarcoplasm. Initially accumulations of myoglobin were found only near the large blood vessels. Evidently, at this stage of embryonic development the newly appearing myoglobin at once begins to store the oxygen brought by the erythrocytes.

In the heart muscle of the 18-day mouse embryo the myoglobin is present only in individual muscle fibers, and in small concentrations. Most fibers contain no myoglobin. In the myocardium of the fur seal embryo in the late stages of embryonic development, very little myoglobin is present (see figure, 7). In this case also, only certain fibers contain myoglobin.*

LITERATURE CITED

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