MORPHOLOGY AND PATHOMORPHOLOGY

A HISTOCHEMICAL STUDY OF THE CHOLINESTERASE ACTIVITY OF THE NERVE CELLS AND CONDUCTING SYSTEM OF THE HEART

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During recent years there has been considerable development of histochemical methods of investigation, which enable the study of the histological and cytological structures to be approached from the point of view of the localization therein of various chemical components. For this reason the results of investigations using histochemical reactions are undoubtedly important for a proper understanding of the metabolic processes taking place in these structures.

The aim of the present investigation was to study the localization and degree of activity of the specific cholinesterase in the Purkinje fibers of the bundle of His in the conducting system of the heart, and also in the structures of the nervous system of the interventricular septum. We were particularly interested in the conducting system since this is known to differ only slightly in its histological structure in the majority of animal species from the rest of the heart muscle, although it has a special function.

EXPERIMENTAL METHOD

We studied the interventricular septum of the heart in the sheep, ox and pig, i.e. in animals in which the conducting system of the heart is particularly well expressed and suitable for study. Material was taken from animals only recently killed, placed in cold physiological saline, and dealt with not later than 30 minutes afterwards to avoid spontaneous diffusion of the enzyme. In order to reveal the activity of specific cholinesterase we used Koelle's method [2] which is based on the enzymic hydrolysis of the thioanalog of acetylcholine – acetylthiocholine – with the production of a precipitate of free thiocholine in the form of its copper mercaptide, and subsequent conversion of this into CuS. Copper sulfide, precipitated in cholinergic structures in quantities depending on the degree of activity of the enzyme, gives them a brownish color which varies in intensity from almost black to yellow. To minimise the possibility of diffusion of cholinesterase, the reaction was carried out with a 2.4% solution of sodium sulfate. Sections were cut to a thickness of 60 μ with the knife of a freezing microtome and placed for 30 minutes in a 10^{-7}M solution of di-isopropylfluorophosphate to inhibit the nonspecific cholinesterase; they were then transferred to glass slides and incubated for 1 hour 45 minutes in a medium at pH = 6.4.

In relation to the innervation of the bundle of His our observations confirm the findings of other workers [3].

EXPERIMENTAL RESULTS

Cholinergic nerve fibers or bundles of nerve fibers proceed in the layers of loose connective tissue between the Purkinje fibers, lying close to them and enveloping them on all sides. Anastomosing with each other, branching and altering the direction of their course, they form a single network of coarse loops; this can be

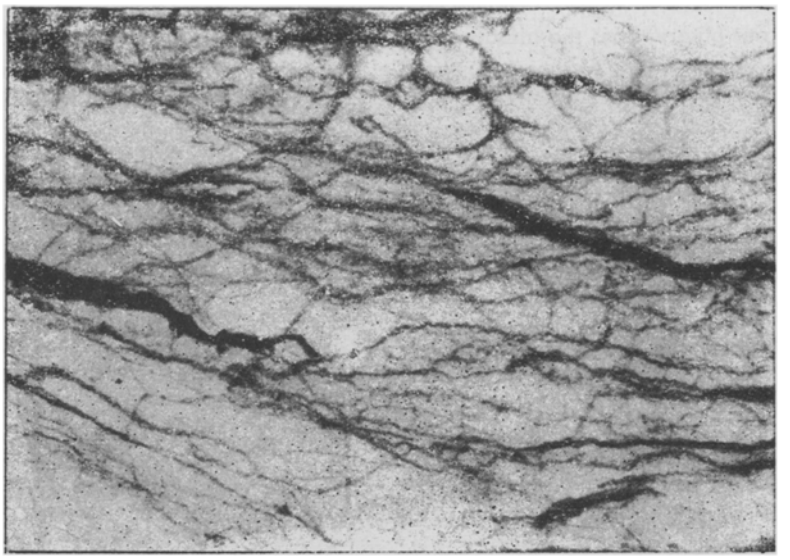

Fig. 1. The bundle of His from the heart of a sheep. Purkinje fibers, colorless and negative in relation to specific cholinesterase, are intertwined with highly active nerve fibers. Magnification 15 x 6 x 1 x 4.

observed with particular clarity in the bundle of His in the sheep and ox. Larger nerve trunks are also encountered, in whose path separate nerve cells may be seen.

All the nerve fibers and cells are highly active in respect of specific cholinesterase, as shown by the intensity of their staining. The separate trunks and fibers are almost black in color, which distinguishes them from the general mass of fibers forming the plexus; the majority of these fibers are stained a brown color, and the separate fine fibers brownish yellow. It is interesting that the Purkinje fibers themselves have negligible cholinesterase activity and appear in the films almost colorless (Fig. 1).

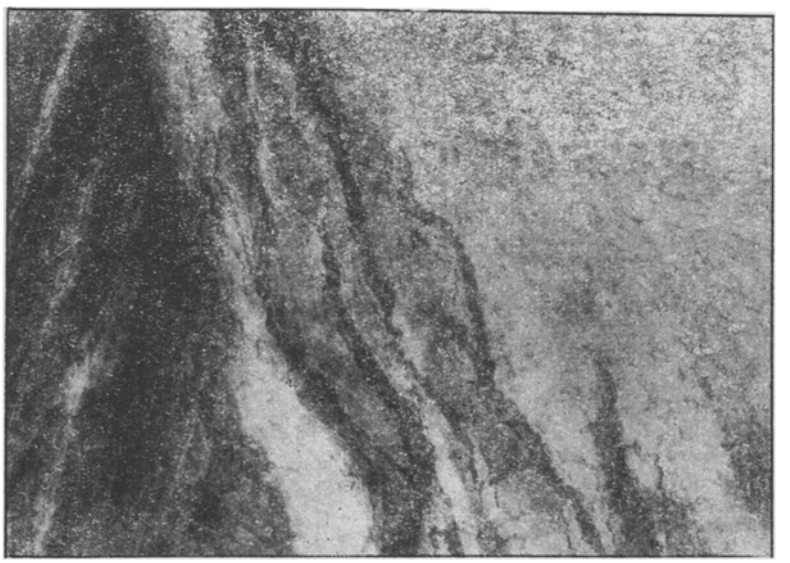

Fig. 2. Heart of a pig. Preparation counterstained with hematoxylin. The topographical relationships between the Purkinje fibers and bundles of nerve fibers passing between them are seen. Magnification 8 x 6 x 1 x 4.

According to our observations the muscle fibers of the bundle of His have an even lower cholinesterase activity then the surrounding cardiac muscle tissue, i.e. they are practically without enzyme. The topographical relationships between the individual Purkinje fibers and their accompanying nerve fibers are clearly seen in the preparations counterstained with hematoxylin. In Fig. 2, in the heart of a pig, clearly outlined thick Purkinje fibers can be seen, anastomosing with each other, and with bundles of nerve fibers passing between them.

The brown nerve fibers are clearly distinguishable against the pale violet background of the counterstained Purkinje fibers, showing no signs of the presence of cholinesterase. In this respect we are quite unable to agree with the findings of Mohr [4], who studied the cholinesterase activity of the conducting system of the heart in guinea pigs, rabbits and the ox by means of the method of Koelle and Friedenwald as modified by Gerebtzoff [1], and who came to the conclusion that the conducting system is positive in respect of specific cholinesterase and that the enzyme envelops each Purkinje fiber in the form of a muff. In this case it is evident that diffusion of the enzyme took place, and on transverse section the divided nerve fibers, closely intertwined with the fibers of the conducting system, created the impression of a continuous ring.

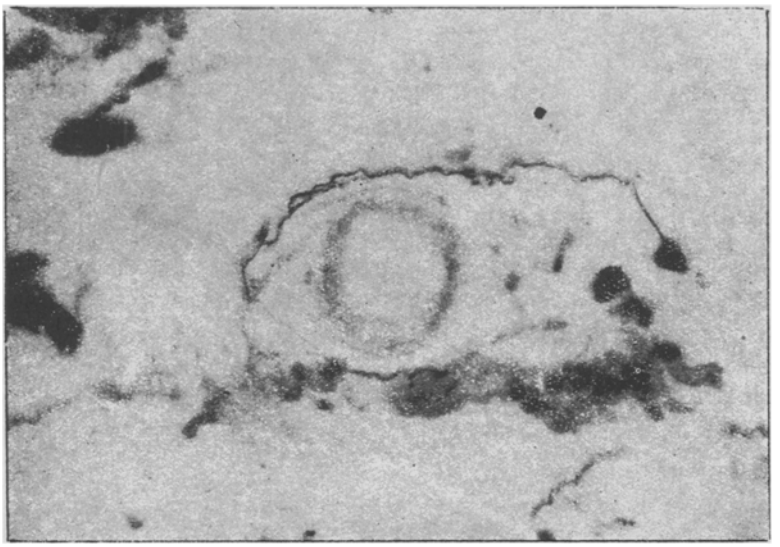

Fig. 3. Heart of a sheep. Side by side with a vessel lie single nerve cells with high cholinesterase activity. Processes from these cells, surrounding the vessel lose themsleves in its adventitia. Magnification 15 × 6 × 1 × 4.

The muscle tissue of the upper third of the interventricular septum is rich in its innervation. Fine nerve fibers run parallel with the muscle fibers, divide dichotomously, intertwine and form a continuous plexus. Individual nerve endings may also be met. Unfortunately the pale brown nerve fibers do not stand out well against the yellowish background of the muscle tissue, and this creates certain difficulties when taking microphotographs. In the lower third of the septum we found considerably fewer nerve fibers. In the upper part of the interventricular septum single microganglia and individual nerve cells are encountered in the layers of loose connective tissue. The cells of these ganglia stain with varying intensity – from almost black to light brown, which suggests different degrees of cholinesterase activity. Nerve fibers leave these ganglia, but it was not possible to trace them for any considerable distance. The nuclei of the nerve cells of these ganglia are not active in respect of specific cholinesterase; they are colorless in the sections.

In the sheep's heart, as a regular feature, microganglia were observed to be localized around small vessels and vessels of larger caliber. Single nerve cells could occasionally be seen lying near a vessel. When the course of the processes of these cells could be traced, it could be observed that they run towards the vessel, go around it, become thinner and lose themselves in its adventitia (Fig. 3).

The nerve cells of the ganglia and the nerve fibers show a different intensity of staining, i.e. unequal cholinesterase activity, which may possibly be associated with their differing functional state.

SUMMARY

The localization and the degree of activity of the specific cholinesterase was studied in the Purkinje fibers of the bundle of His of the cardiac conductive system, as well as in the nerve structures of the interventricular septum in sheep, hogs and oxen.

G. Koelle's method was used for the detection of the specific cholinesterase. It was established that Purkinje's fibers are richly innervated. The cholinergic nerve fibers form a continuous network and possess a high cholinesterasic activity. The Purkinje's fibers themselves remain negative with relation to the specific cholinesterase. Interventricular cardiac septum, especially in its upper third, contains numerous delicate nerve fibers with a low cholinesterase activity. The intramural nerve ganglia consist of the nerve cells with a different degree of cholinesterase activity, which is usually high. The groups of nerve cells in the myocardium are usually localized around the small and large blood vessels.

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