METHOD OF OBTAINING LARGE FRAGMENTS OF MYOCARDIAL FIBERS FROM ADULT ANIMALS

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A method of obtaining fragments of native fibers from the myocardium of adult animals with the aid of collagenase and hyaluronidase is described. The method described is suitable for cytological and cytophotometric investigations of the myocardium of adult animals and man.

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With the need for cytological investigation of the pathologically changed heart in adult animals and man, a method is required for obtaining native myocardial fibers or sufficiently long fragments thereof. In particular, cytophotometric investigation of the absolute DNA content in the nuclei of the myocardium can be carried out successfully only on fragments of fibers. Investigations of the DNA content in the nuclei of the myocardium carried out on sections [1, 2, 8] have given conflicting results. This is evidently because of the necessity for reducing the results of cytophotometric measurement of DNA in thin sections of nuclei to an ellipsoid of rotation, which introduces a considerable error.

A series of investigations was recently carried out in which it was shown that myocardial fibers of newborn rats and chick embryos can be isolated [3-7]. Attempts to isolate myocardial fibers from adult animals by means of trypsin, widely used for de-aggregation of other tissues, proved unsuccessful. It was therefore necessary to study other possible methods for carrying out this task.

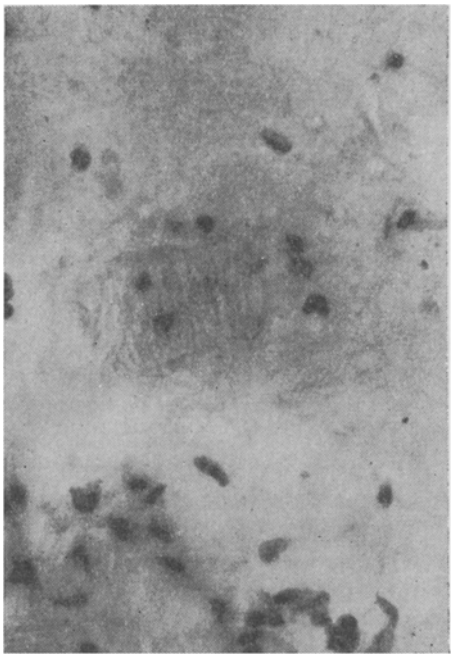

Fig. 1. Fragments of myocardial fibers. Hematoxylin-eosin, 500 ×.

In the present investigation, two enzymes (collagenase and hyaluronidase) which, according to data in the literature [9], cannot react with elements of the muscle fiber itself, were used to isolate fragments of myocardial fibers.

Collagenase (Serva) (working name in accordance with the classification of enzymes clostrifio-peptidase A) and hyaluronidase (Reanal) were used in concentrations of 2 mg/ml. Discs of myocardium, 1.5-2 mm in thickness, from adult male rats weighing 150-200 g, were used for isolation of the fiber fragments. The discs were cut out with a sharp razor.

Incubation with the enzyme at 37° was carried out in a test tube or bottle containing Earle's medium for 10, 15, and 30 min and 1 h. In some experiments a magnetic mixer was used. After incubation with the enzymes and shaking, the large pieces were allowed to settle and the suspension was transferred into a centrifuge tube and washed once with Earle's solution to remove enzyme. After centrifugation the supernatant was decanted, and 1-2 drops bovine serum were added to the residue and films were made. The films were fixed and stained with hematoxylin-eosin or by the methods of Brachet and Feulgen.

Prolonged exposure led to destruction of the myocardial fibers. Perfectly satisfactory results were obtained with

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an exposure of 15 min. With this duration of incubation with the enzyme, fragments of myocardial fibers were found in the films in the form of small "barrels" measuring 45-55μ, containing from 1 to 3 centrally situated nuclei, were found in the films. The transverse striation of these fragments was clearly visible (Fig. 1).

Treatment of pieces of myocardium with these enzymes for a short time thus yielded fragments of fibers for cytological and cytophotometric investigations. It can be concluded from the results obtained that the method described above can be recommended for cytological and, in particular, cytophotometric investigation of the myocardium of adult animals.

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