PREPARATION OF SPECIMENS OF THE MYOCARDIUM FOR QUANTITATIVE CYTOCHEMICAL INVESTIGATIONS

L. N. Belov and M. E. Kogan

UDC 611.127-086.1

A simple method of preparing specimens of whole nuclei of the myocardium for cytophotometric and other quantitative investigations is suggested. The piece of tissue is crushed on a glass slide. Nuclei of muscle and nonmuscle cells and intact and injured nuclei can be distinguished in the crushed specimens.

Cytophotometric investigations of nucleic acids in the nuclei of heart muscle cells are usually carried out on histological sections. Sometimes nuclei isolated from a homogenate are used [3]. An essential disadvantage of sections is that they contain a varied number of sectioned nuclei. Since there are no sufficiently reliable means of verifying the integrity of nuclei in the sections, not even the use of thick sections can ensure that all nuclei measured are intact. This reduces the value of cytophotometric studies carried out on sections. The use of films of nuclei isolated from the myocardium is complication by the fact that nuclei of muscle and nonmuscle myocardial cells are very difficult to distinguish in the isolated state. Methods of obtaining isolated heart muscle cells which have been suggested [1, 2] are extremely laborious and they give no guarantee that the cells can be obtained in sufficient numbers or in an adequate state of preservation.

It was decided to test the use of crushed specimens in quantitative cytochemical research. The advantages of crushed specimens for cytophotometry have been reported previously [4], although it was impossible to find any reference to the use of this method on the myocardium.

The method suggested for the preparation of crushed specimens is simple. A piece of fresh tissue measuring 0.5-1.5 mm^3 in volume is placed on a clean, defatted glass slide, which is covered, first, with a piece of thin Teflon, and above it a celluloid film; the material is crushed by means of a roller. If the crushing force is correctly chosen, the nuclei in the specimen lie in one layer and are not expelled from the cell cytoplasm. After crushing is over the films are removed, and the specimen is left on the slide where it dries instantaneously. The specimens can be fixed and stained as desired. Crushed preparations of the myocardium and kidney can be fixed with 10% neutral formalin, ethyl alcohol, methanol, Carnoy's fixative, or mixtures of alcohol and acetic acid and alcohol and acetone, without any appreciable difference in the cytological picture of the nuclei. Fixation is rapid (20-30 min is sufficient) and uniform, for all the nuclei, being in the same layer on the side, are equally accessible to the fixative. Because the cytoplasm in crushed specimens of the myocardium remains intact, muscle and nonmuscle nuclei can be distinguished. Any nuclei injured by too vigorous crushing are easily found.

The writers have used crushed specimens of myocardium and kidney for cytofluorometric studies of DNA and quantitative autoradiography with uridine-H^3. The simplicity and reliability of the method of preparing specimens of whole nuclei for quantitative cytochemical investigations were satisfactorily demonstrated. This method is particularly convenient for the investigation of organs from which it is difficult to obtain impressions or isolated cells.

Laboratory of Pathomorphology, Institute of Physiology, Siberian Division, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 123-124, June, 1971. Original article submitted October 6, 1971.

LITERATURE CITED

1. A. L. Shnaper, Byull. Éksperim. Biol. i Med., No. 5, 123 (1968).
2. M. N. Berry, D. S. Friend, and J. Scheuer, Ciruclat. Res., 26, 679 (1970).
3. B. Fischer, G. Schlüter, C. P. Adler, et al., Beitr. Path. Anat., 141, 238 (1970).
4. W. Sandritter, J. Pilny, V. Novakova, et al., Histochemie, 7, 1 (1966).