CYTOPHOTOMETRY OF DNA IN ISOLATED MOUSE HEART MYOCYTES

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The DNA content in the nuclei was determined in preparations of isolated mouse cardiomyocytes. Heterogeneity of the myocyte nuclei for ploidy was demonstrated.

KEY WORDS: cardiomyocyte; polyploidy.

The study of proliferation of cardiomyocytes has attracted attention both in connection with the character of differentiation of these cells and also from the point of view of the significance of the conclusions from these investigations for clinical cardiology [5]. One approach to the study of proliferation is to determine the ploidy of the cells: do mitoses in myocytes proceed to the end so that the cells remain diploid or, instead of completed mitoses, do polyploidizing mitoses occur in heart muscle?

Methods of obtaining histological preparations of isolated heart cells have been developed only recently. In a few investigations the DNA content has been determined in whole, undivided cardiomyocyte nuclei [6-8]. The object of the present investigation was to determine the DNA content in mouse cardiomyocytes.

EXPERIMENTAL METHOD

Cells from the heart, liver, and testis of CBA/C57Bl$_6$ hybrid mice aged three months were investigated. The ventricles of the heart were fixed for 10 days in 10% formalin in Sorensen's buffer at pH 7. The tissue was then kept in 50% KOH, and later transferred to distilled water, where the cells separated after the tube was shaken. The cell suspension was washed several times to remove alkali and to obtain a neutral medium [2]. Cells with a

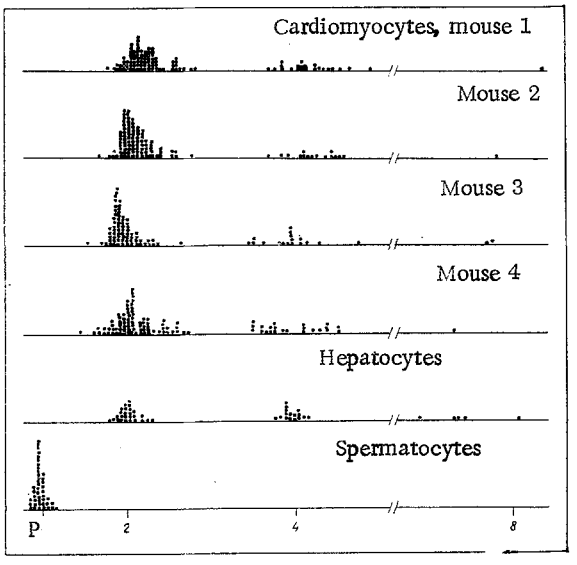

Fig. 1. Distribution of nuclei by DNA content. Abscissa, DNA content in ploidy units; ordinate, number of nuclei (each point represents one nucleus).

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standard DNA content (from the testis and liver) were treated in the same way. Films and drop preparations were obtained from the cell suspensions. The content of DNA-fuchsin was determined after the Feulgen reaction by the standard method [1] on a Vickers M-86 cytospectrophotometer.

EXPERIMENTAL RESULTS

Determination of the DNA content in the spermatids and hepatocytes showed that one was a multiple of the other (Fig. 1). Hence it follows that the DNA content either was unchanged during this method of treatment of the tissue, or it changed proportionally to the ploidy. The cardiomyocyte nuclei differed in their DNA content. Here also, the classes were multiples of each other. Besides diploid nuclei, nuclei with twice or four times their DNA content also were found. Among the binuclear cells there were myocytes with both diploid and tetraploid nuclei.

Heart muscle cells of the mouse, as in man, primates, pigs, and turkeys [8, 7], are thus polyploid cell populations. Besides a high proportion of binuclear [3] cardiomyocytes known to be polyploid, certain mononuclear and also binuclear cells have nuclei with twice the DNA content. In mice aged three months there were at least 20% of such nuceli. It will next be interesting to study the mechanism of polyploidization, at present most fully elucidated in liver cells [4], for heart cells.

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