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VARIABILITY OF PLOIDY OF HUMAN CARDIOMYOCYTES

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Publications dealing with the study of ploidy of human cardiomyocytes give data on the DNA content in single nuclei [1, 5, 9, 10, 13]. It has been shown that the ventricles of the human heart contain many tetraploid nuclei, and indeed octaploid nuclei or nuclei of even higher ploidy have been found. However, such an interpretation is incomplete. Conclusions regarding polyploidy must always include an assessment of the number of nuclei in the cell: the binuclear cell with two diploid nuclei does not differ in the properties studied from a mononuclear tetraploid cell, one with two tetraploid nuclei from a single octaploid [8]. In mice and rats 80% or more of ventricular myocytes are binuclear 2c × 2 cells (where c denotes the haploid DNA content), which are tetraploid relative to the combined genome. The ventricles of the human heart also contain many binuclear cells [10, 12]; it has recently been shown that the modal class here is 4c × 2 [3]. Now for the first time we are comparing the composition of myocytes in different layers of the myocardium. Preliminary data on ploidy of mononuclear and binuclear myocytes in the hypertrophied heart are given below.

EXPERIMENTAL METHOD

Cardiomyocytes from four persons were studied: one died after burns (46 years), another died from knife wounds of the abdominal wall (age 15 years); in two cases the hearts of persons who died were hypertrophied due to coarctation of the aorta (31 years) and general atherosclerosis (61 years). Pieces of the inner, middle, and outer layers of the heart muscle were fixed with 10% formalin in phosphate buffer (pH 7.0) and dissociated into single cells with 50% KOH [2]. We previously improved this technique and demonstrated that DNA and proteins are preserved in the cells. After carrying out the Feulgen reaction in cardiomyocytes stained with naphthol yellow, we measured the DNA content on a Vickers M-86 integrating microdensitometer [6, 7].

EXPERIMENTAL RESULTS

In the two hearts with no marked pathological changes the classes of myocytes were similar in the inner, middle, and outer layers of the heart muscle (Fig. 1). In both cases 4c × 2 myocytes predominated. In one histogram there were many tetraploid mononuclear (4c) and binuclear (2c × 2) cells; 8c × 2 cells constituted a conspicuous group, but there were only single 16c and 16c × 2 cells. In another histogram (Fig. 1b) there were almost as

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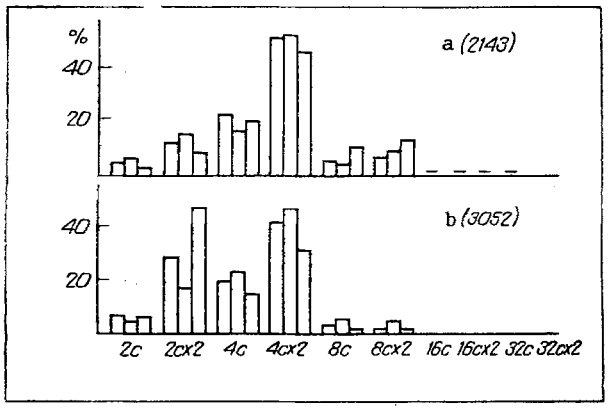

Fig. 1. Distribution of myocytes in left ventricle of normal heart by classes of ploidy. Total number of cells studied shown in parentheses. a) Man aged 46 years, b) youth aged 15 years. Columns on left to right denote inner, middle, and outer layers of myocardium. Abscissa, class of myocytes; c) quantity of DNA in haploid set (2c – diploid cells, 2c × 2 – binuclear cells with diploid nuclei, and so on, respectively); ordinate, percentage of cells (100% for each layer separately).

many 2c × 2 cells as 4c × 2, the percentage of 8c and 8c × 2 myocytes was lower than in the first case, and no 16c or 16c × 2 cells could be found.

The two cases with pathological changes differed sharply from one another and from the normal hearts. In one case (Fig. 2a) the ploidy of the ventricular myocytes was much lower than normally, in the inner and middle layers. A difference was observed also in the ploidy of the cells by layers. For instance, the percentage of diploid (2c) myocytes was twice as high in the inner layers as in the middle layer, and 6 times as high as in the outer layer. Conversely, the percentage of 4c × 2 myocotes was twice as high in the outer layer as in the middle layer and 5 times as high as in the inner layer. The average ploidy of the myocytes in the inner, middle, and outer layers was 2.9, 4.1, and 5.2c respectively. Compared with the normal state, where this ratio was 6.9, 7.7, and 7.8c in one case and 5.8, 6.5, and 5.1c in the other case, the first hypertrophied heart appeared to be either undeveloped or reduced with respect to ploidy.

The second example of hypertrophy showed opposite ratios between ploidy classes of the myocytes. Ploidy layer by layer was 14.1:10.5:6.4c, i.e., just as in the first case asymmetry of the layers was observed, but the inner and middle layers were distinguished by excessive polyploidation. In these layers hexadecaploid cells (8c × 2 and 16c) were only a little fewer than octaploid; cells with 32 (as DNA) sets of chromosomes (16c × 2 and 32c) constituted an appreciable group; solitary enormous 32c × 2 cells were found.

Study of the DNA content in single nuclei revealed changes in ploidy classes in cardiac pathology; changes in the number of binuclear cells also have been discovered independently [1, 9, 11, 12]. Unfortunately, ratios between classes of mono- and binuclear cells have not previously been given. In most cases an increase in ploidy of the cardiomyocytes was observed in ventricular hypertrophy, but the actual values varied greatly. A second point to make is that ploidy of myocytes does not necessarily reflect myocardial hypertrophy. We know that the principal factor is postnatal growth of the heart is an increase in weight of the cytoplasm in the myocytes which have undergone division and polyploidization [4, 7]. Just as in the cases of hypertrophy which we investigated, the total weight of the heart was 750 and 600 g; in the first case of a heavier heart polyploidization, moreover, was appreciably lower than in the second case or in the normal heart.

Our own observations and data in the literature suggest two alternative hypotheses on the nature of variability of myocyte ploidy in myocardial hypertrophy: 1) under pathological conditions the monocyte genome is modified due to proliferation (additional polyploidization) of the cells or due to selective death of cells of the highest level of ploidy; no more concrete evidence in support of this hypothesis is yet available; 2) the difference lies in

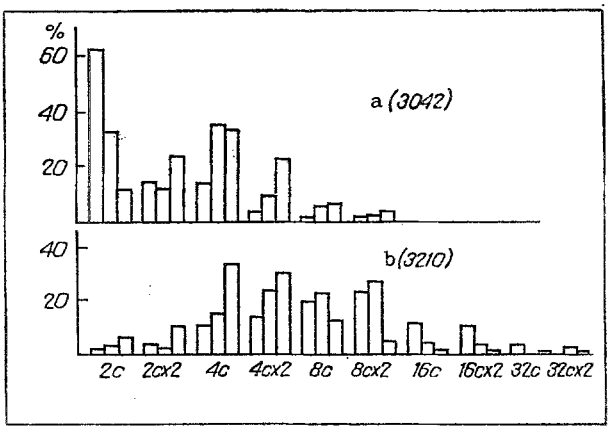

Fig. 2. Distribution of left ventricular myocytes in hypertrophied heart by classes of ploidy. a) Man aged 31 years, b) man aged 61 years. Legend as to Fig. 1.

initial variability of the normal value, and in that case its extreme variants, namely increased or (and) reduced ploidy of cells of the normal heart, may be risk factors of heart diseases. Variability of the composition of the myocytes in the normal adult human heart has not yet been studied. The second hypothesis is supported by observations of variability of the myocytic genome in a population of normal mice and experimental data on significant changes in the genome of animals with different rates of growth in the period of cardiomoycyte proliferation [6].

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