MORPHOLOGY AND PATHOMPHOLOGY

PROTEIN CONTENT DOES NOT CORRESPOND TO DNA CONTENT IN HUMAN CARDIOMYOCYTES

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A familiar after-effect of cell polyploidization is doubling of the quantity of various substances, including proteins, and of enzyme activity, the number of structures, and the volume and mass of the whole cell [3]. Certain polyploid Drosophila cells and a few polyploid cells in plants are the exception [7]. It has recently been found that heart muscle cells may be another exception. In the ventricles of the mouse heart, in which the ratio of the number of chromosomes and the DNA content in the myocytes is 2:4:8:16, the protein mass of these cells corresponds to the series 2:3.3:5:6.3 [4], and in the rat heart to the series 2:3.6:6.1 [1]. The DNA content did not correspond not only to the total protein content, but also to the content of special contractile proteins [2]. The aim of this investigation was to determine absence of correlation between the protein content of the myocyte and its ploidy, and also to demonstrate inequality of mass of myocytes of the same degree of ploidy in different layers of the left ventricle of the human heart.

EXPERIMENTAL METHOD

Isolated myocytes from three layers of the anterior wall of the left ventricle were studied [6]. DNA and proteins were measured by photometry in the same cell after the Feulgen reaction and the reaction with naphthol yellow [8]. A Vickers M-86 scanning cytophotometer, with 100× objective and a wavelength of 580 nm was used, for measuring DNA in the nucleus, and a 20× objective and a wavelength of 445 nm for measurement of intracellular proteins.

EXPERIMENTAL RESULTS

In the normal left ventricle of the adult human heart both diploid (2c) and polyploid (2c × 2, 4c, 4c × 2, 8c, 8c × 2, 16c) myocytes are found, where c denotes the DNA content in the nucleus corresponding to the number of chromosomes, and ×2 indicates that there are two nuclei in the cell. Occasionally myocytes in the human myocardium have four nuclei, and in some cases, mononuclear and binuclear cells with 32 sets of chromosomes are found. Most frequently, 4c × 2 muscle cells predominate in the human myocardium.

Curves showing variations in protein content revealed considerable variability of the mass of the myocytes with the same level of ploidy, for example, tetraploid (4c, or 2c × 2) differed neither in average size nor in range of variability, and 8c and 4c × 2 cells likewise were indistinguishable. On the graphs in Fig. 1, cells of the same ploidy are considered together. The masses of the cells of different ploidy intersected: some tetraploid myocytes did not differ in protein mass from diploid myocytes, but certain other tetraploid myocytes did not differ in protein content from octaploid cells. Cells of the same ploidy differed in mass in the outer, middle, and inner layers of the left ventricle (Fig. 2; Table 1). Myocytes in the

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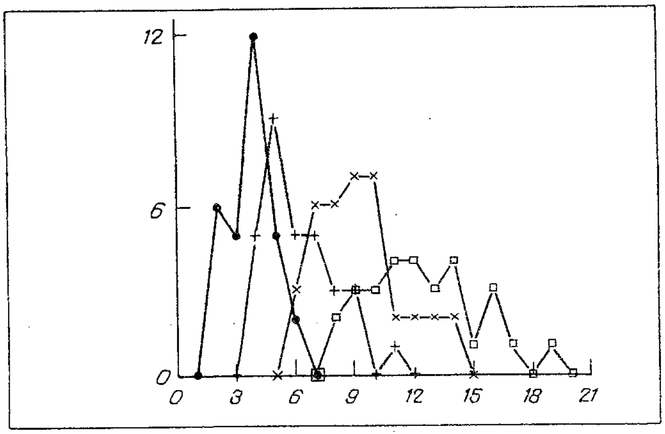

Fig. 1. Curves showing variation of protein content in cardiomyocytes Abscissa, protein content (in comparable units ×1000); ordinate, here and in Fig. 2, number of cells Line with filled circles indicates diploid cells, with plus signs — tetraloid (4c, 2c × 2), with multiplication signs, octaploid (8c, 4c × 2), and with squares — hexadecaploid (16c, 8c × 2).

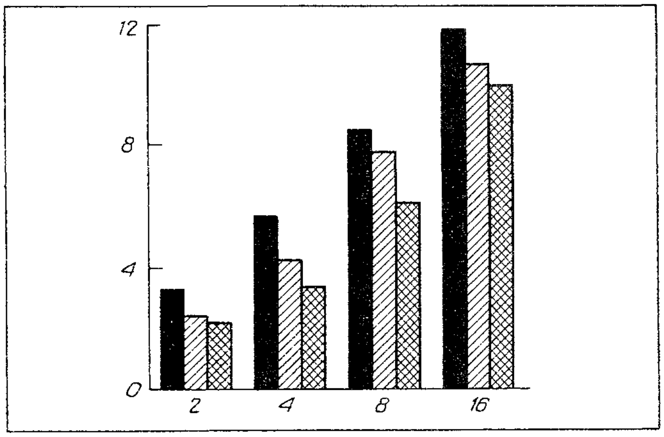

Fig. 2. Average protein content in diploid (2), tetraploid (4), octaploid (8), and hexadecaploid (16) cardiomyocytes in inner layer (black columns), middle layer (obliquely shaded), and outer layer (cross-hatched). Ordinate, protein content (in comparable units × 1000).

inner layer had the highest protein content. The mass of the tetraploid myocytes of the inner layer was greater than the mass of the central myocytes with the same level of ploidy by 35%, and of the outer layer by 70%. Correspondingly, the average protein content of octaploid myocytes differed by 10 and 40% and differences for these and hexadecaploid cells were less than for tetraploid, but also were significant.

In the example given in Fig. 2, myocytes in the three layers differed not only in the average mass of the cells but also in their ploidy. The average mass of a myocyte of a particular layer was inversely proportional to the average ploidy (Fig. 3). The number of cells in each layer is unknown, and we cannot therefore calculate the ratio between the total genome and the total mass. It is impossible as yet to exclude compensation of the mass of the layer with relatively low ploidy on account of hypertrophy of the myocyte cytoplasm. The difference in protein content of the cells thus characterizes, not the layer itself, but its ploidy, which varies in different myocardia and arbitrarily in different layers.

Given that the ratio of the DNA content in the myocytes with different ploidy values is 2:4:16, the protein contents of the myocytes formed a series 2:3.5:5.2:7.2 in the inner layer, 2:3.5:6 5:8.9 in the middle layer, and 2:3.1:56:9.1 in the outer layer. Previously, when myocytes of the outer layer only were investigated, a similar ratio was obtained [5].

TABLE 1. Average Protein Content in 4c × 2 Myocytes in Two Left Ventricles (comparable units)

Myocardium	Number of myocytes measured	Inner layer	Middle layer	Outer layer
1	37	8514±336	7805±251	6095±482
	109	8268±307	7443±284	6888±279
2	82	9536±256	8351±321	5593±165

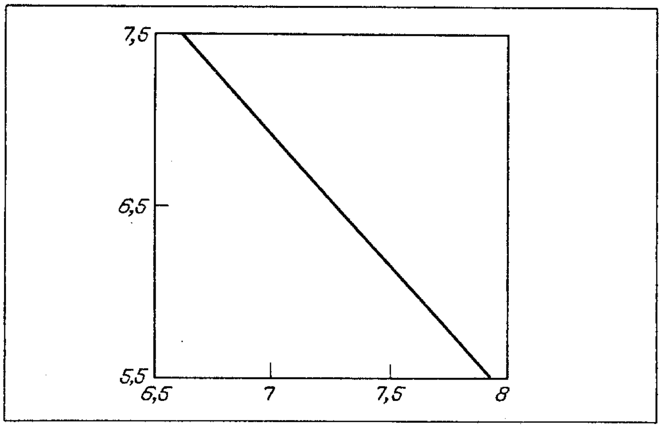

Fig. 3. Ratio between average protein content in myocyte (ordinate, in comparable units × 1000), and average ploidy of myocyte (abscissa). From top to bottom: in inner, middle, and outer layer Trend calculated by IBM AT PC (Harvard Graphics program).

Thus in man, just as in the rat and mouse, the protein content of the cardiomyocytes does not correspond to the DNA content. Relative to this feature, cardiomyocytes differ from hepatocytes and other mammalian polyploid cells which have been studied, in which doubling the DNA content means doubling of the mass and other tested characteristics of the cell.

Two important questions still await answers. First, what determines this absence of correlation between the mass of the cardiomyocyte and its genome? By analogy with *Drosophila,* we can suggest compensation of the DNA content, i.e., inactivation of some allelic genes. Another suggestion is insufficient activity of the protein-synthesizing apparatus in the polyploid myocyte, for example, a number of ribosomes that is not proportional to the genome. The second question is: what is the result of this lack of agreement between protein content and DNA content? Does it persist in hypertrophy of the myocardium or other pathological states of that structure? Continuation of the study of the mass of myocytes in different layers of the myocardium is evidently indicated.

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