# RELATIVE DNA AND TOTAL PROTEIN CONTENT IN HUMAN CARDIOMYOCYTES

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The principal method of early postnatal growth of the myocardium is by polyploidization of the cardiomyocytes [2]. The subsequent increase in mass of this organ takes place through hypertrophy of its cells [5, 6]. Heart cell growth in mice has been shown [9] to lag behind the increase in size of their genome; the higher the ploidy of the cardiomyocytes, moreover, the greater the difference between the protein content expected on the basis of this ploidy and the amount actually measured.

The aim of this investigation was to study the characteristics of growth of human cardiomycytes. Contradictory information is given in the literature on the degree of polyploidy observed in the heart cells of man and primates. These differences of opinion may be due to the use of different technical approaches to the problem. The first studies in this field were undertaken on myocardial slices [4, 8, 11]. In investigations on films of isolated myocytes or their nuclei, ploidy of separate nuclei and not of cells as a whole was estimated [1, 7, 10, 13, 15] or only the percentage of binuclear and mononuclear cardiomyocytes was determined in the population [12]. It was impossible, on the basis of all these investigations, to judge the true cell ploidy in the human myocardium. Postmitotic growth of human cardiomyocytes has not hitherto been studied.

## EXPERIMENTAL METHOD

Isolated ventricular cardiomyocytes from four men dying accidentally at the age of 30-40 years were investigated. The cells were isolated by alkaline dissociation with 50% KOH after prolonged fixation of the tissue with formalin, which has been shown not to give rise to appreciable losses of DNA and proteins [3]. The DNA and protein content was determined in the same cell by staining the cells by a combination of Feulgen's method and with Naphthol Yellow S [14]. The use of this method to analyze DNA and protein concentrations in the same cell is based on the difference in absorption spectra of the dyes Fuchsine-$SO_2$ and Naphthol Yellow S. The absorption spectra of the proteins and DNA were measured on a Vickers M-86 scanning integrating microdensitometer. To determine the protein content a whole myocyte was introduced into the photometric field: magnification 20, probe 2 μ, wavelength 445 nm. Next, the nucleus of the same myocyte was introduced into the closest possible field (probe 0.2 μ, magnification 100) and photometry of DNA carried out at a wavelength of 580 nm.

## EXPERIMENTAL RESULTS

Analysis of ploidy of the cardiomyocytes revealed that on average 2.5% of them were diploid cells, 31.8% tetraploid; binuclear 4c × 2 cardiomyocytes predominated, i.e., octaploid cells in their total genome, which accounted on average for 41.7%, whereas allowing for the mononuclear cells, there were 52.7% of octaploid cells. Hexadecaploid cells (8c × 12, 4c × 4, 16 c) accounted for 12.5% of the cardiomyocyte population, and not more than 0.5% of cells had a DNA content of 32c (Fig. 1). The distribution of the cells among ploidy classes was found to be variable in different people. For instance, the number of diploid cardiomyocytes varied from 0.7 to 4%, the number of 2c × 2 cells from 6 to 15%, of 4c cells from 15 to 26%, of 8c cells from 5 to 24%, and of 8c × 2 cells from 8 to 16%. However, despite individual differences, the commonest class of cell in all cases was 4c × 2. Cells with higher ploidy (16c, 16c × 2, 8c × 4) were quite rare and had to be specially looked for, and many

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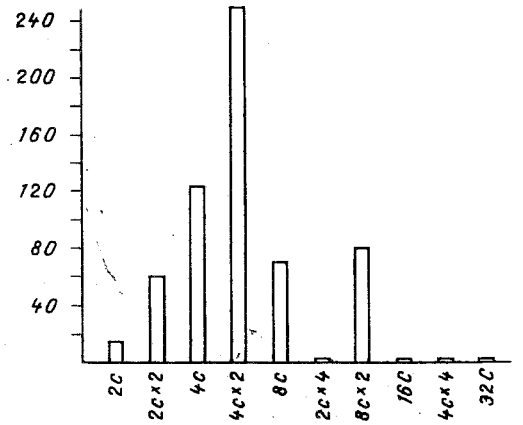


Fig. 1

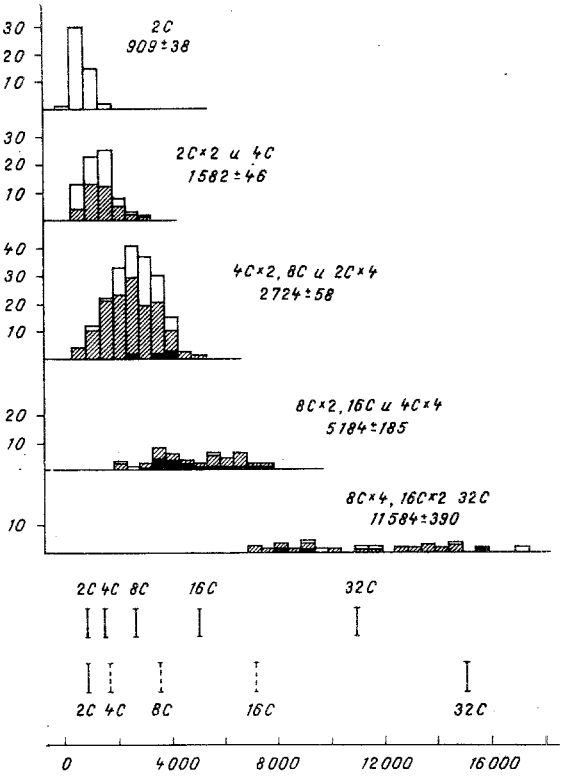


Fig. 2

Fig. 1. Distribution of cardiomyocytes of the human left ventricle by ploidy classes. Abscissa, type of cells; ordinate, number of cells (here and in Fig. 2).

Fig. 2. Combined histograms of protein content in mono-, bi-, and tetranuclear myocytes with different ploidy values. Abscissa, protein content (in conventional units). Unshaded columns — mononuclear myocytes; obliquely shaded columns — binuclear, black columns — tetranuclear. Numbers adjacent to histograms indicate ploidy and average protein content (M ± m). Continuous vertical lines below histograms indicate mean protein content in myocytes of different ploidy; broken lines indicate protein content expected in accordance with gene dose.

cardiomyocytes had to be examined before reliable data could be obtained on their total protein content.

Cytophotometric measurement of the protein content in cardiomyocytes of different ploidy showed that protein content is not proportional to gene dose. Tetraploidy cells containing on average 1582 conventional units (c.u.) of protein whereas octaploid cells contained 2724 c.u., or 13% less than there ought to be by doubling the amount (Fig. 2). The deficit of the protein content of octaploid and hexadecaploid myocytes was 25 and 29% respectively. To assess the significance of the changes the error of measurement was determined with respect to two parameters. First, the protein content in the same myocyte was measured 20 times. The mean protein content differed from the extreme values by not more than 2% and the coefficient of variation was about 1%. Second, comparison of errors of the mean for homogeneous populations of myocytes showed that the limit of deviation from the general mean was 3-4%. These two parameters, instrumental error and variability of the samples, were significantly less than the observed deviation from the expected doubling of the mean protein content, even for tetraploid myocytes. Summation of all the data shown on the histograms gives a ratio of DNA content (in mono- and binuclear myocytes) of 2:4:8:16:32, and a ratio of mean protein content 2:3.5:6:11.5:25.5, respectively. Thus in human cardiomyocytes, just as in mouse myocytes, the higher the ploidy of the cell, the greater the disparity between protein content and genome size. However, in the human myocardium this disproportion is manifested much less clearly than in the mouse myocardium, where ratios of genomes and protein were 2:4:8:16 and 2:3.3:5.0:6.3, respectively [9]. As has already been pointed out, binuclear cells (2c × 2,

4c × 2, 8c × 2) in different ploidy classes predominante in the human myocardium, just as they do in the myocardium of the mouse, rat, guinea pig, and dog [2]. Cells of equal ploidy but with 1, 2, and 4 nuclei did not differ in their protein content. This confirms yet again data showing that binuclear and tetranuclear myocytes are fully adequate analogs of cells of the same ploidy with only one nucleus [2].

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# IMMUNOCHEMICAL ANALYSIS OF LECTIN RECEPTORS IN THE STRUCTURE OF FERTILITY $\alpha_2$-MICROGLOBULIN


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Fertility $\alpha_2$-microglobulin (FAMG) is a placental protein [2, 7] which is found in a relatively high concentration in extracts of placental tissues and amniotic fluid during the first trimester of pregnancy, and is not detected by immunodiffusion analysis in blood serum in tissue extracts from organs of human adults and fetuses at different times of development [2]. FAMG also is found in the endometrium in the secretory phase [4, 6], and in the seminal vesicles and sperm [4, 9]. This protein has been isolated in a pure form [5] and its physicochemical properties have been studied [3]. FAMG is a glycoprotein containing hexoses (8.7%), hexosamines (6.3%), fucose (0.3%), and sialic acids (2.2%) [7].

Lectins, proteins of animal and plant origin capable of interacting selectively with one or more carbohydrate ligands in the glycoprotein molecule [1], have been used in recent years to study the carbohydrate receptors of glycoproteins [1].

The aim of this investigation was to study interaction between carbohydrate components of FAMG and various lectins, which can shed light on the structure of acceptors of the FAMG molecule and reveal the terminal radicals of the carbohydrates which can bind glycoprotein molecules with lectins.


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