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ORIGINAL ARTICLE

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Polyploidy in cardiac myocytes of normal and hypertrophic human hearts; range of values

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Abstract Two-wavelength scanning DNA cytophotometry was used for DNA and protein estimation in human ventricular myocytes. In many hypertrophic hearts weighing more than 500 g the DNA content assessed by ploidy of myocytes, was within the range of normal adult variation (4–10c, where c is the haploid DNA content). A correlation was found between the protein content of myocytes and the weight of the hypertrophic ventricle. In congenital heart disease, the excessive polyploidy (up to 15–20c) developed through the normal route of myocyte polyploidization in childhood. Excessive polyploidization was revealed only in overloaded hypertrophied ventricles. A correlation was identified between the ploidy level, the ventricular weight and age of the child. Excessive polyploidy was also detected in adults with congenital or acquired in childhood diseases. There was no correlation between the myocyte ploidy and age. We propose that childhood polyploidy excess persists in these adults. The ranges of polyploidy are compared with the recent data on genome: protein ratio in cardiac myocytes and the interrelationships allow us to discuss the significance of childhood heart polyploidy as a reserve utilised under pathological conditions in adults.

Key words Cardiac myocytes · Cell polyploidy · Cell growth · Human heart · Heart hypertrophy

Introduction

Polyploidization of cardiac myocytes is an essential stage of heart development in mammals (for recent review see Brodsky 1991). The level of polyploidy is particularly high under conditions of cardiac muscle hypertrophy in heart disease (for review see Adler 1991; Kupper and Pfitzer 1991). Significant hypertrophy of the human myocardium may lead to or be accompanied by further polyploidization of mature myocytes.

Hypertrophy and normal heart growth both depend on myocyte volume and mass (Bishop 1984; Rakusan 1988). Polyploid cells are always large and heavy, when compared with diploid cells, and therefore polyploidization which is associated with cell size and mass duplication may be considered to be one of the modes of tissue growth. Any mode of genome reproduction involves mitosis, either complete or incomplete. Evaluation of double (^{3}H and ^{14}C) thymidine labelling has demonstrated the mitotic origin of polyploid myocytes (Brodsky et al. 1980). Studies on the course of polyploidization in the rat heart in situ and in embryonic heart grafts implanted under the renal capsule of the adult rat revealed a similar process (Brodsky et al. 1988). The data show evidence of controls for the beginning and the end of myocardium polyploidization.

Both diploid and polyploid cells may proceed with growth after withdrawal from mitotic cycle. Unlike other polyploid cells, a difference has been detected for the genome: protein ratio in cardiac myocytes of normal hearts (Brodsky et al. 1985b, 1992, 1993). While the ratio of genomes is 2: 4: 8: 16..., the ratio of protein contents is 2: 3: 5: 8... The doubled protein ratio appears in hypertrophic hearts only and underlies increase in myocardial weight depending on the extent of ploidy in a given myocardium. If the myocardium consists mostly of tetraploid cells, their increase from 3 to 4 units results in 30% increase in the total myocyte weight. If octaploids predominate, the growth from 5 to 8 may results in 60% increase. Ranges of polyploidy in normal and hypertrophied hearts thus become interesting.

We have limited the topic of our article to polyploidy. It is known that polyploidization is preferred to cell division in mature cells (see Brodsky and Uryvaeva 1985). If mitoses are induced in adult myocytes, incomplete variants would predominate and polyploidization will occur rather than hyperplasia.

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Two confounding factors exist: – a histogram of nuclear ploidy may not adequately represent cell ploidy. Many ventricular myocytes are binucleate, and individual tetranucleate myocytes also can be found. It has been shown that the intensity of metabolism and functioning of the cell is determined by the sum of its nuclei rather than by a particular nucleus (see Brodsky and Uryvaeva 1985). The mononucleate, binucleate and multinucleate forms of cells with the same total ploidy (for example, octaploids, 8c, 4c times two and 2c times four) do not differ from one another by any of the variables studied while the nuclear histograms would be respectively octaploid, tetraploid or diploid. Secondly, great individual variability of cardiomyocyte ploidy in the normal human heart has been shown recently (Brodsky et al. 1991). In some normal myocardium the mean myocyte ploidy is about 4c and many diploid cells and tetraploid cells are found, while octaploids are rare. In other healthy men the mean myocyte ploidy is approximately 8–10c and octaploid cells predominate. In half of the hearts studied, the mean ploidy was about 6c with approximately equal numbers of tetraploid and octaploid myocytes.

A new approach to studying polyploidy of the cardiac myocytes along with recent data on variability of the norm provided for a more detailed analysis of polyploidy in hypertrophied hearts. The purpose of this study was to verify the relationship between hypertrophy and the extent of myocyte polyploidization.

Materials and methods

Thirteen myocardiums scarred after infarctions with a history of generalized atherosclerosis and cardiosclerosis, were studied in men aged 44–68 years. Twenty-three cases of congenital heart diseases were examined; 13 children 5–15-years-old, and 10 men 16–42-years-old. The anomalies were mainly tetralogy of Fallot and also congenital stenosis of the outflow tract of the right ventricle, or congenital aortic failure or defect of the interventricular septum. Sixteen cases of acquired rheumatic heart diseases (aortic, mitral, aortomitral, aortomitral and tricuspid) were studied in men aged 19–56 years. Three cases of marked hypertension were studied in elderly men. All the hearts were hypertrophied, and were compared with 12 normal samples taken from men 20–30-years-old killed in accidents (see also Brodsky et al. 1991).

The weight of hearts and their left and right ventricles were measured. Myocytes from the middle layer of the left and/or right ventricle were studied. Tissue samples 2–3 mm thick were taken from the medial part of the middle layer no later than 24 h after death. Samples were quickly rinsed with cold (4–6° C) saline and fixed with cold formaldehyde solution in Sorensen's phosphate buffer (pH 7.0, 1: 10) for 2–4 weeks. Myocardium samples were dissociated into cells by treating them with 50% potassium hydroxide (Grabner and Pfitzer 1974). Cell smears were stained according to Feulgen and afterstained by Naphthol yellow S (Tas et al. 1980). For DNA measurement, an integrating microdensitometer Vickers M-86 was used (objectives 100× and 40×, scanning probe 0.4 or 0.6 μm, wavelength 560 nm). For protein estimation the cell was measured by objective 20× and the scanning probe 1 μm (see also Brodsky et al. 1993).

In each case, 200–300 myocytes were studied one by one and mononucleate, binucleate, as well as other cell types were distinguished. Fibroblasts and lymphocytes in the same smear were used as the reference diploid cells.

The results were treated statistically and plotted using an IBM PC/AT computer with the SuperCalc and the Harvard Graphics software.

DNA cytophotometry per se cannot be a source of variability in ploidy estimation. The reproducibility of modern versions of this method are known to constitute few percent from measured values. For cardiac myocytes, the mean 2c, 4c and 8c values (measured by Feulgen-staining cytophotometry) were determind in our previous study at 241, 492 and 974 arbitrary units (au). The average reference diploid value was 253 au (determined for lymphocytes present on the same cell smears). Variability of diploid myocytes was in the range 212–270 au, of tetraploids, 418–565 au, and of octaploids, 837–1110 au. Thus, the mean values of myocyte ploidy classes differed by no more than 4%, whereas limits of each class detected significantly. To estimate reproducibility of DNA-Feulgen cytophotometry, we performed repeated measurements. Three samples of the same left ventricle were studied. The mean DNA content of myocytes (for calculation the mean ploidy see the first paragraph of Results) of each sample was 5.3c, 5.0c and 5.8c, respectively.

Postmortem changes in cell ploidy can be ruled out since it is known that the DNA remains unchanged as late as 72 h after death (Adler and Costabel 1975). The effects of hypoxia were also excluded.

Ploidy differences have been found between layers of the same normal myocardium (Brodsky et al. 1991). Correlations in the myocyte ploidy between the layers was not established. The central and inner layers are known to form the main bulk of the myocardium. The ratio of mean ploidy between the layers (central: inner) was 1.14 (SD=0.23 and SEM=0.05). Therefore maximal (95%) deviation of interlayer ploidy did not usually exceed 10% for average values and 40% for rare extreme samples. Thus, data on the central layer alone do, to a certain degree, represent a whole myocardium.

The next question concerns location of the studied samples within the myocardium. Some positional differences were detected but they were not large for the main bulk of a ventricle.

Results

The extent of ploidy was indicated in terms of the mean ploidy of ventricular myocytes. The value is a sum of products of frequency of the myocyte class by the genome weight of this class. Classes are defined according to the number of genomes: diploid (2c; genome number 2); tetraploid (mononucleates 4c and bidiploids 2c times two; genome number 4); up to binucleates 64c times two (genome number 128).

The main results are summarized in Figure 1. The dashed line shows the normal variability of the mean myocyte ploidy in adults (for detail see Brodsky et al. 1991).

In the left ventricle wall of all hearts with scars, mean myocyte ploidy was in the range of normal variability, irrespective of the heart weight. The highest values were found in hearts weighing 750 g (ploidy 8.9c) and 420 g (9.5c). In the first heart, the left ventricle was severely hypertrophied (weight 340 g) while in the second heart, the ventricular weight was within the normal range (125 g). To evaluate the cause of growth, protein content was estimated in octaploid (8c and 4c times two) myocytes by cytophotometry of Naphthol yellow S (Fig. 2). The stain is known to react quantitatively with the terminal amino groups of amino acids in cell proteins. The linear

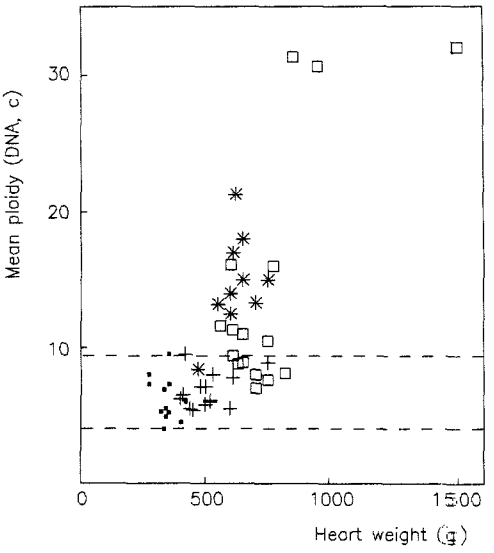

Fig. 1 Mean ploidy (DNA, c) of ventricular myocytes in hearts with different weight (abscissa) in adult men. *Dots* – normal hearts of healthy men, left ventricles; *hatched lines* – limits of the normal variability of cardiac myocyte ploidy; *crosses* – hearts with scars in the left ventricle wall; *asterisks* – congenital heart diseases; *squares* – rheumatic acquired heart diseases

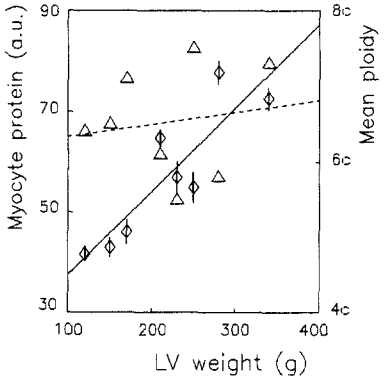

Fig. 2 Computer calculation (Harward Graphics software) of the regression line (trend) between the left ventricle weight in hearts with scars and the protein content in octaploid myocytes (left ordinate, *rhombs, solid line*) and between the ventricle weight and the mean myocyte ploidy in the ventricles (right ordinate, *triangles, dashed line*)

regression line demonstrates dependence of the ventricular growth on myocyte growth in conditions of relatively stable ploidy.

In all cases of congenital diseases in adults, the hearts were hypertrophied and overloaded ventricles were enlarged. The ploidy level exceeded the range of normal variability except one case (Fig. 1).

Acquired heart diseases were also accompanied by heart hypertrophy. Heart weights always exceeded the limit of 500 g. In seven cases, polyploidy was within the normal range, close to its upper limit. In two cases associated with heart failure diagnosed during childhood,

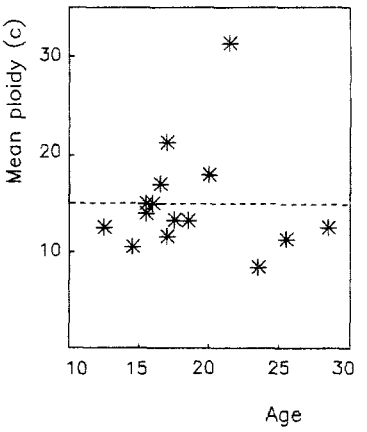

Fig. 3 Computer calculation of the regression line between the adult human age and the mean myocyte ploidy in cases of congenital heart diseases and rheumatic heart diseases acquired during childhood, during the usual ontogenetic course of myocyte polyploidization

ploidy exceeded the normal level only slightly. However, in one similar case, the mean ploidy was three times higher than the upper limit of the normal range. Such high values (about 30c) were also found in two cases of heart disease which were clinically manifest only in the adult. In four other cases, the mean ploidy was between 12c and 16c.

Thus, heart hypertrophy, even when extensive, does not necessarily coincide with excessive myocyte polyploidy. This is also true of ventricular hypertrophy per se. High polyploidy was observed in ventricles with normal weight, while the myocytes of hypertrophied ventricles were also often of low ploidy.

No correlation was found between myocyte ploidy and the age in adults (Fig. 3). Left ventricles were taken only from individuals with congenital malformations or with a disease acquired in childhood.

When histograms of myocyte ploidy are compared, the weight of the ventricle seldom correlates with myocyte ploidy. In two cases, for example, a normal histogram of myocyte classes was observed (Fig. 4) although the weights of the left ventricle different by a factor of 1.5. Tetraploid and octaploid cells, binucleate in particular, prevailed. The mean ploidy (5.8c and 6.2c) corresponded to usual normal values. In two severely hypertrophied left ventricles with an equal weight of 340 g, the mean myocyte ploidy was markedly different, 31.3c and 8.9c, respectively (Fig. 5). In the first case, 16c times two myocytes were common; cells with 64 and even 128 (64c times two) chromosome sets were also found. In the second case, a histogram of myocyte ploidy was normal, that is octaploid and tetraploid cells prevailed.

In hypertension (two cases), the mean ploidy of myocytes exceeded the normal level, being 12.5c and 29c. One further case had normal ploidy (6.2c mean).

Excessive polyploidy was inherent only in overloaded ventricles; the other ventricle of the same heart had nor-

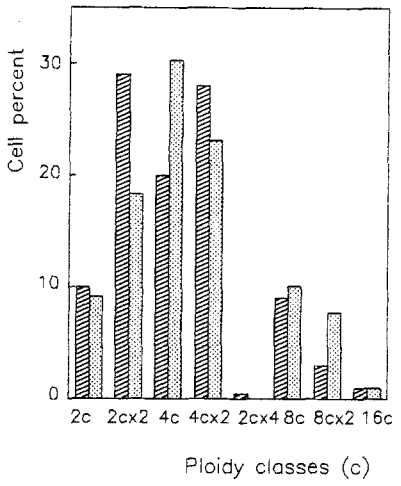

Fig. 4 An example of histograms of myocyte ploidy (2c – diploid cells, 2c times two – binucleate tetraploids, 4c – mononucleate tetraploids, etc.) in two left ventricles with weight differing by a factor of 1.5 (210 and 140 g). The myocyte population had similar mean ploidy – 5.8c in the first case and 6.2c in the second case (*dotted bars*)

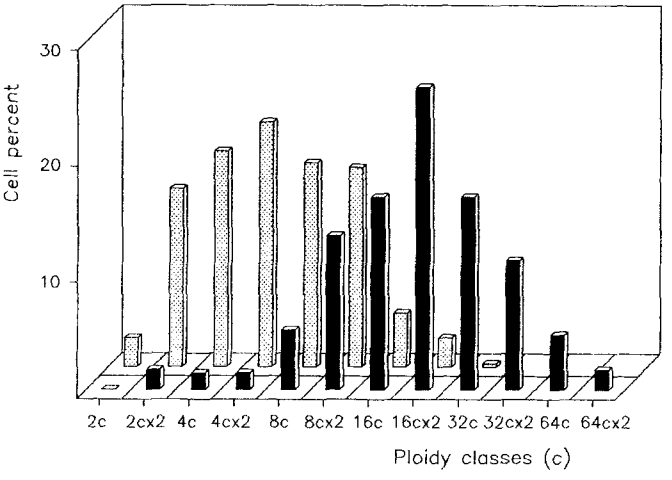

Fig. 5 An example of histograms of myocyte ploidy in two severely hypertrophied left ventricles (weight 340 g). The myocyte population had normal ploidy (8.9c) in one case (*dotted bars*) and excessive ploidy (31.3c) in the other case (*black bars*)

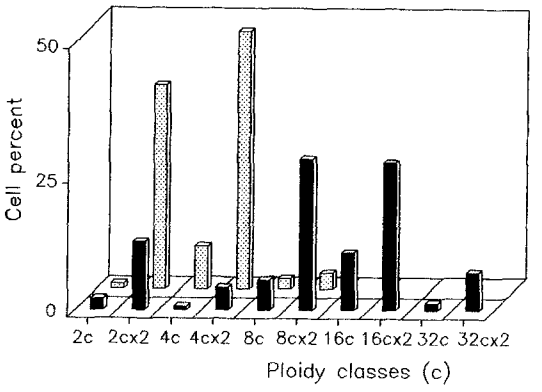

Fig. 6 Histograms of the myocyte ploidy in a case of tetralogy of Fallot. *Black bars* – the right ventricle. *Dotted bars* – the left ventricle of the same heart

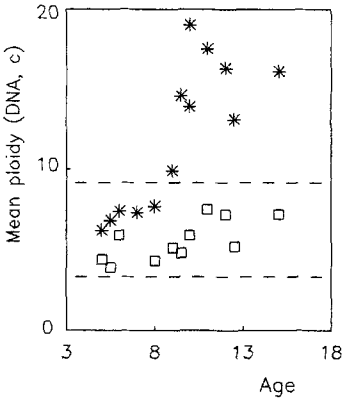

Fig. 7 The mean ploidy of cardiac myocytes in children hearts with tetralogy of Fallot at the age of 5–15 years. *Asterisks* designate hypertrophic right ventricles; *squares* designate the left ventricles of the same hearts (these ventricles were normal by their weight); *dashed lines* indicate the ranges of normal variability of the myocyte ploidy in the adults

mal ploidy. Thus, in congenital stenosis of the outflow tract of the right ventricle, the mean myocyte ploidy of the right ventricle was 21.3c, while in the left ventricle the value was only 6.3c (Fig. 6). In a Fallot's tetralogy, the mean ploidy was 13.3c in the right ventricle and 5.8c in the left; in a second case 15.1c and 6.2c; in the third 12.5c and 4.9c. In cases of rheumatic acquired heart diseases (aortic, mitral and mixed) excessive polyploidy was seen only in the left ventricle. The mean ploidy was, for example, 11.6c in the left ventricle and 6.4c in the right in a heart with aortomitral rheumatic disease.

To evaluate the origin of excessive polyploidy in myocytes, polyploidization was studied in hearts with Fallot's tetralogy in children aged from 5 to 15 years. The hypertrophic right ventricle was compared with the left ventricle of the same heart. A marked increase of the myocyte ploidy was found in the hypertrophic ventricles (Fig. 7). In the left ventricles, which were normal by weight, polyploidization was completed by 10–12 years, confirming earlier data (see Discussion). Even 5–6-year-old children have polyploid myocytes (mainly bidiploids). In these children, the myocyte ploidy corresponded to the lower limit of variability in adults.

In hypertrophic ventricles in children, the mean normal ploidy was achieved already by 5–6 years; 6–7 years before the completion of ontogenetic polyploidization of the myocytes. Later, at the age of 9–10 years, the myocyte ploidy began to exceed the upper limit of normal. In these cases, octaploid and hexadecaploid classes prevailed and some higher ploidy cells were detected.

A correlation has been established between the ploidy

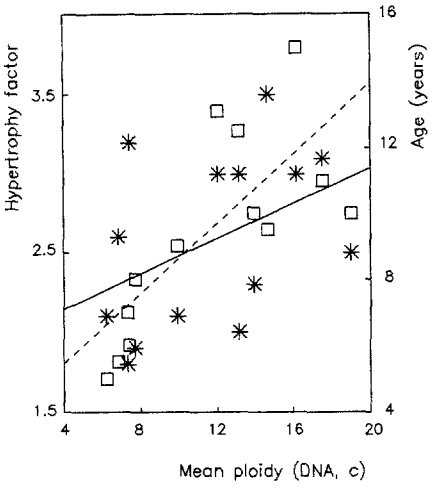

Fig. 8 Computer calculation of the regression line between the mean ploidy of myocytes in hypertrophied ventricles and the age of children (right ordinate, *squares, solid line*), and between the ploidy and the hypertrophy factor calculatd as ratio of ventricular weights measured: normal (left ordinate, *asterisks, dashed line*)

of myocytes, age of the children and the degree of hypertrophy in overloaded ventricles (Fig. 8). The latter was calculated as a ratio between the measured ventricle weight and its normal weight for a given age.

Discussion

The first result of this study is the finding of great variability of myocyte ploidy in hypertrophic hearts – as in normal hearts (Brodsky et al. 1991). As in other reports (Kompmann et al. 1966; Ebert and Pfitzer 1977; Adler and Sandritter 1980; Kondo 1981; Zak 1984; Adler 1991) high ploidy myocyte populations were found in hypertrophied hearts, with enlarged myocyte size and enhanced protein content (for data on the myocyte protein see Brodsky et al. 1993). Many cases of hypertrophy did not show high polyploidy.

In hypertrophy developing in adults (cardiosclerosis, infarction), myocyte ploidy did not exceed the normal range, irrespective of the heart or ventricle weight. Mean ploidy in the normal hearts varied for 4c to 10c; the mean was 6.2±0.5c (see also Brodsky et al. 1991). The mean myocyte ploidy for infarcted hearts was 7.1±0.6c. In some cases of heart disease the individual ploidy values were also in the normal range despite severe cardiac hypertrophy (700–800 g).

In many cases of excessive polyploidy the disease was congenital or acquired in childhood; during the natural schedule of polyploidization which proceeds until 10–12 years in Man (Pfitzer 1972; Adler and Costabel 1975; Kondo 1981; Takamasu et al. 1983; Adler 1991). The heart was already overloaded by that time. The growth of myocardial muscle fibres under such conditions is known to be accelerated (Sarkisov et al. 1966) and the most pronounced increase in size takes place 5–15 years from birth, during the period of myocyte polyploidization. Recently, the first evidence for an accelerated polyploidization of myocytes in hypertrophied myocardium of children has been published (Adler 1991). The same finding is evident in our observations. Polyploidization is actually accelerated in the overloaded ventricle with congenital malformation when compared with normal ventricle of the same children's heart. At the age of 9–10 years; 2–3 years before completion of polyploidization, the extent of polyploidy exceeded the normal level. After completion, the mean ploidy of myocytes may be close to 20c in overloaded ventricles. Thus, an excess of ploidy may appear in childhood and persist in the adult, without further increase.

The absence of a correlation between the myocyte ploidy and age is an argument against polyploidization in adults. Under the conditions of permanent functional overload with congenital or early acquired heart diseases, a positive correlation between myocyte ploidy and age (duration of disease) might be expected. However, such a correlation was observed only in children during ontogenetic period of polyploidization. The conclusion is evident, polyploidization may take place during childhood but the genome in adults remains unchanged, further evidence against hyperplasia in adults. The mechanisms of cell division and polyploidization are basically the same, moreover, incomplete (polyploidizing) mitoses prevail in cardiac myocytes, as well as in many other mature cells (Brodsky and Uryvaeva 1985). Our data on correlation between ventricular and cellular weights (cell protein content) also contradict the possibility of myocyte division in adults.

But two (of three) cases of extraordinary polyploidy (Fig. 1) do not allow us to exclude cardiomyocyte proliferation in adults. The exact time of the acquisition of disease is not known for these cases, heart damage by rheumatism could have taken place during childhood as occurred in the third case of extraordinary polyploidy in the adults. In such a case, polyploidization might occur early, while clinical symptoms of the disease appear only in the adult state. But the two other cases of extensive polyploidy indicate that stimulation of polyploidization may occur. Mitoses of mature myocytes occur though they are extremely rare in rat hearts (Rumyantsev 1977). The number of cycling myocytes may be increased in overloaded myocardium (in hypertension, for example). Two examples of high ploidy myocyte population in hypertensive men seem to provide evidence for polyploidization of myocytes in the adults. The third case was normal in terms of the myocyte ploidy. However, the data on hypertensive patients cannot be interpreted unambiguously. Early differences in the myocyte genome of spontaneously (hereditary) hypertensive rats have already been shown (Brodsky et al. 1994). Hypertension in Man may also have a genetic origin; excessive polyploidy of heart myocytes might then appear during childhood, even without hypertension during this period. The data obtained by Pfitzer (Pfitzer 1972; Pfitzer et al. 1977) are of great interest in terms of cardiomyocyte polyploidiza-

tion in adults. An increased number of polyploid nuclei was found in cardiac myocytes of Rhesus monkeys subjected to coarctation of the aorta. If such an increase does exceed limits of the normal variability, it is an argument for the possibility of the myocyte proliferation in adults.

Polyploidization of mature ventricular myocytes looks less certain then at the past. Apart from speculations on the aetiology of heart overload, assumptions about expression of mitotic activity seem to be unreasonable. If polyploidization occurs it has to result in increasing the mean ploidy by 20–30c from normal ranges 4–10c. One had to assume re-entering the mature myocyte population into mitotic cycle once or even twice.

What is the significance of polyploidy in different tissues? Replacement of cell divisions by polyploidization itself may be favourable for permanently functioning cells, since polyploidization does not require cell junctions and extracellular structures to be reconstructed (Oberpriller and Oberpriller 1985). The multiplied (polyploid) genome itself also can improve the functional ability of a large cell. For instance, only large polyploid cells of the bladder epithelium are adequate for synthesis and translocation of membrane elements, which are necessary for contraction and distension of the bladder wall (Hicks 1975). Only polyploid megakaryocytes can produce blood platelets (Dupont et al. 1983). Multiplied genome may be a factor providing for the resistance of polyploid liver cells to injury (Uryvaeva 1981). Thus, polyploid genome may have some advantage in comparison with the diploid and this advantage is specific for different tissues.

Can we discover any specific role of polyploidy in myocardium and, especially, variability of the myocyte genome? Under normal conditions, the variability is not essential for heart function; a minimal genome can provide for normal performance. In adult rats grown from birth until weaning in litters of 4 or 16 and differed in the total weight of the myocyte genome (ploidy and number the cells) by 40%, no differences were detected in terms of speed of contraction and relaxation, coronary blood flow, and in the heart response to isoproterenol and aortic constriction (Rakusan et al. 1978; Brodsky et al. 1985a, 1992; Ostadal et al. 1993). In healthy men ploidy of cardiac myocytes varied by a factor of 2–3, to a far greater extent than in rats, but function of the heart was not impaired at any ploidy level. The size of myocyte genome may, however, be important under the conditions of excessive heart growth.

In contrast to other polyploid cells, growth of cardiac myocytes results in a lack of proportionality between a cell ploidy and protein content of the cells (Brodsky et al. 1985b, 1992, 1993). The more the ploidy of a myocyte the more its protein content falls behind the duplicate series. Unlike hearts with normal weight, in hypertrophic human hearts the protein ratio comprises the duplicated series. Thus normal heart growth forms a reserve for an additional growth under pathological conditions. The greater the ploidy of a normal myocardium the greater is the growth reserve for future hypertrophy. Considering the normal variability of myocyte ploidy, the range of the reserve is between 1/3 to 1/2 of the weight of cardiac muscle before hypertrophy. A number of cases of adult hypertrophy are exactly of this kind. Increasing ploidy of cardiac myocytes during childhood may be a key factor for the additional heart growth under pathological conditions in adults.

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