REVIEWS

Polyploidy in the Myocardium and Compensatory Reserve of the Heart

V. Ya. Brodskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 454-459, May, 1995
Original article submitted March 27, 1995

The mammalian heart is a polyploid organ. Cardiac myocytes undergo polyploidization in the early postnatal ontogeny, and the degree of their polyploidy depends on the conditions of heart growth. The myocardium of healthy persons is characterized by considerable individual variability of polyploidy. The principal mechanism by which normal and compensatory growth of the heart occurs in adult mammals is through increases of cytoplasmic mass in postmitotic myocytes. In the normal myocardium, the protein mass of myocytes does not correspond to the gene dosage, while their protein mass in a hypertrophic myocardium becomes a multiple of their ploidy. The capacity of polyploid myocytes to grow so as to double their mass constitutes the reserve of cardiac growth. This reserve, which is laid down in the early ontogeny, materializes in response to functional overloading of the heart in adult life.

Key Words: *heart; cardiac myocytes; postnatal growth; hypertrophy; hyperplasia; polyploidy*

Long ago, DNA cytophotometry led investigators to conclude that cardiac myocytes are polyploid, evidence of polyploidy being first obtained for the human myocardium [34,42]. Performed on sections, i.e., on cell fragments, these early investigations guessed at rather than identified polyploidy, but later studies using isolated nuclei and cells [14-16,35,45-48] confirmed the conclusions reached in the pioneering work of Sandritter and associates. However, even these interesting publications and numerous experimental data from studies on mice and rats failed to provide a complete characterization of cardiac polyploidy. The polyploid series was constructed on the basis of DNA levels in individual nuclei disregarding the number of nuclei in the cell. Yet even formally, if the total genome is considered, a binucleate cell has four chromosome sets while a tetranucleate one has eight. Since these sets are all active [30] and since more than a half of cardiac myocytes are binucleate (i.e., at least tetraploid considering the total cell genome) in the mammals that have been studied [7,17,33,36,37,44], it was not correct to base the judgment of cell ploidy on nuclear histograms. For example, a histogram with 90% diploid nuclei led to the conclusion that the mouse myocardium is diploid [31], whereas the myocardium is actually polyploid as nearly half these nuclei occur in binucleate cells [19]. Previous assessments of polyploidy also neglected individual variability.

Evaluating the ploidy of the total cellular genome has enabled investigators to supplement or revise the data on the kinetics and mechanisms of myocyte polyploidization in ontogeny and on how this polyploidization relates to heart growth. New evidence has also emerged from analyses of the relations between hypertrophy and hyperplasia of the human heart and, in particular, of myocardial polyploidization in hypertrophy. The main results

Institute of Developmental Biology, Russian Academy of Sciences, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0005-0435$12.50

obtained in recent studies may be summarized under the following headings.

Polyploidy is inherent in the myocardium of all mammalian species examined. Massive polyploidization of myocardial cells has been found to occur in all mammals examined so far, including the mouse, rat, rabbit, guinea pig, cat, dog, sheep, cow, pig, and man [20,41]. No species with a diploid myocardium has been found as yet. In contrast, massive polyploidization of cells in the mammalian liver (the classical object for the study of polyploidy) has been detected in only 4 species out of the 30 or so examined [8,30]. Polyploid cells are found in both the ventricles and atria of the heart. The level of polyploidy in the atria is normally lower than in the ventricles, and the polyploidization kinetics and regenerative reaction of atrial myocytes are distinct from those of ventricular cells [9,18,38]. The following review is confined to ventricular myocytes, whose levels of ploidy in health are similar in the left and right ventricles and consistently the same in different layers of a ventricle [23].

The time of myocardial polyploidization in ontogeny is limited. The studies by Rumyantsev, summarized in his book [9], demonstrated that thymidine incorporation into rat or mouse myocytes, i.e., DNA synthesis in these cells, goes on intensively only during the first 2-3 weeks after birth, when many mitoses are also detectable there. It was found subsequently that most of the mitoses occurring during that period result in polyploidization rather than division of the myocytes [21,22,29]. As shown by DNA cytophotometry and direct counts of myocytes, their division is completed on the 2nd or 3rd postnatal day, after which the myocardium undergoes almost complete polyploidization for 10-15 days. Thus, over 80% of the cells are diploid, i.e., tetraploid in terms of the entire genome. In man, myocardial polyploidization is completed by the age of 10-12 years [14,15,28,35,40,46].

The mechanism by which polyploid myocytes arise is ordinary but incomplete mitosis. Neither amitosis nor cell fusion plays a substantial role in the production of binucleate or mononucleate polyploid myocytes. The notion that endomitosis or endoreduplication plays such a role has not been validated. These conclusions were reached when double (^3H and ^{14}C) thymidine labeling and cytophotometry of DNA were performed in the same myocytes so that the composition of cells entering the cycle and completing mitosis could be evaluated [13,21]. In addition, mitotic figures and the kinetics of labeled mitoses and of labeled binucleate cells were studied, as was the time course of thymidine label dilution [22]. In contrast to hepatocytes, in whose population mononucleate and binucleate generations alternate in a consistent manner as the level of polyploidization rises, cardiac myocytes may convert into binucleate or mononucleate cells in each polyploidizing mitosis, apparently with equal probabilities. Myocyte nuclei containing doubled amounts of DNA also have a reduplicated chromosome set and are not blocked in the G_2 phase. Evidence for this has been furnished by observations of abnormal mitoses leading to polyploidy and of polyploid mitotic figures. Another indicator of polyploidy is the doubling of sex chromatin bodies in myocytes [1].

Polyploidization is a form of cell proliferation, and hyperplasia involves polyploidy. The common mitotic mechanism by which cells in the myocardium increase in number (complete mitosis) and undergo polyploidization (incomplete mitosis) and also the activity exhibited by all genomes give grounds for considering polyploidization as a form of cell proliferation that leads to the same main result as cell reproduction, namely a multiplied number of active genomes in the tissue [30]. It is also understandable why hyperplasia, which is excessive proliferation stimulated in certain disease states, may result in increased cell numbers in a given organ as well as in cell polyploidization, i.e., in a multiplied number of genomes at a constant cell number.

The timing of myocyte reproduction and proliferation is programmed in ontogeny, but the number of myocytes and their ploidy depend on the conditions of heart growth during the proliferation period. In an embryonal diploid myocardium transplanted under the renal capsule of an adult rat, the beginning and termination of polyploidization coincide with those in the heart *in situ*, but the percentage of polyploid cells in the transplant is considerably lower than in the myocardium *in situ* [4,5,22]. That the level of polyploidy and the conditions of heart growth during early development are interdependent has also been shown in studies of the polyploidization kinetics of myocytes in mice [24,25] and rats [26] raised from birth to the weaning period in nests containing 4, 8 (control), or 16 individuals each. Sucklings were fed to excess in the small nests and starved in the large nests, so that on the day of weaning (approximately 3 weeks after birth), the weights of the weanlings and of their hearts from the nests of these two types differed, on average, by a factor of 1.5 in the rats and of 2 to 3 in the mice. The times of polyploidization in these

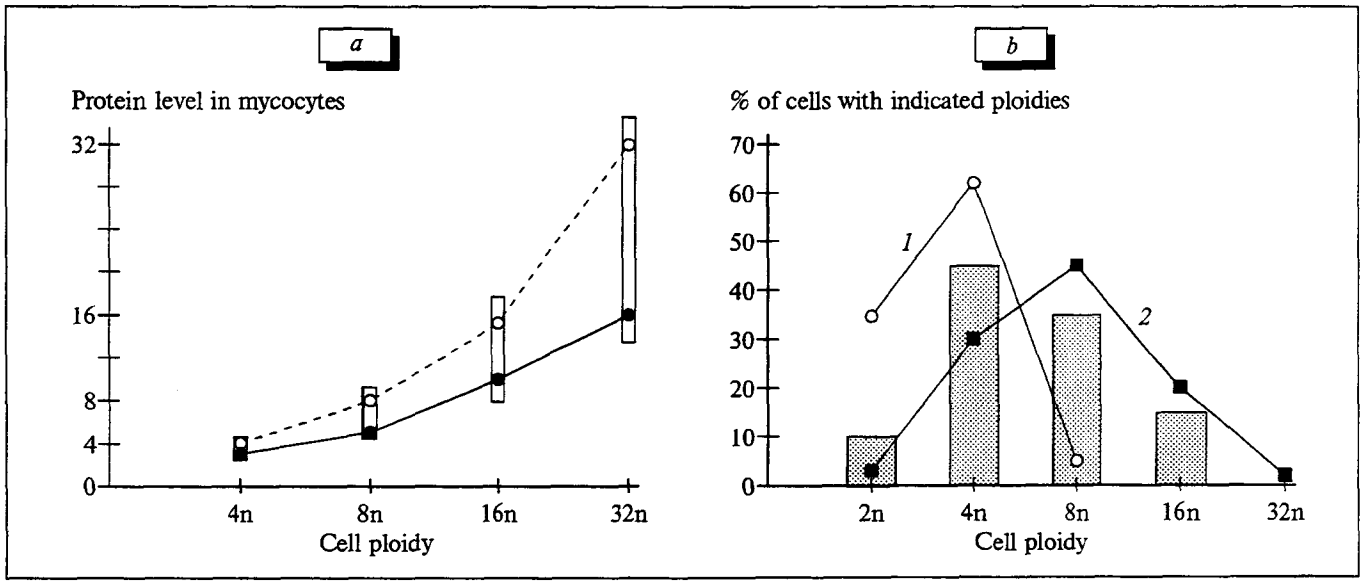

Fig. 1. *a*) reserve of cardiac myocyte growth calculated as the difference between the relative cellular protein masses in a myocardium of normal weight (solid curve) and a hypertrophied myocardium (dashed curve). *n* = number of chromosomes in mononucleate or binucleate cells. *b*) extreme examples of polyploidy variation in myocardia of normal human beings. *1* and *2*) mean myocytic ploidy of about 4n and about 9n. The bars are a histogram of myocyte ploidy occurring in approximately 50% of people.

groups did not differ, but the total number of myocytes and the level of their ploidy in the rapidly growing animals from the small nests were higher than in their slowly growing counterparts from the nests containing four times as many sucklings. The average intergroup differences in the total myocytic genome, as estimated from the number of cells and their ploidy, amounted to 40% on the day of weaning, the time when myocyte proliferation (polyploidization) is completed in the mouse and rat. Differences in the myocytic genomes persisted throughout life.

Variability of the myocytic genome can be described in terms of heritable variation and nonheritable modifications [2]. Examples of modifications are cited in the preceding paragraph. Heritable modifications were identified in comparing the number and ploidy of myocytes in normotensive rats and rats with hereditary hypertension (SHR strain). An enhanced myocytic genome arises in rats of this strain before the manifestations of hypertension and associated myocardial hypertrophy become pronounced.

Individual variation of cardiac polyploidy in healthy human beings is much higher than in the rat or mouse. It is widely accepted in the literature that the ploidy of myocytes in humans is always much higher than in mice or rats. Since Sandritter's first published paper [42], hypertrophic human hearts have been found in many studies to show even higher degrees of polyploidy than normal hearts [14,48], a fact which led investigators to conclude that hypertrophy of an adult human heart involves massive myocyte polyploidization, ignoring the variability of polyploidy in normal hearts and the possibility of excessive polyploidy occurring during childhood. Recent studies have revealed that the average myocyte polyploidy among healthy young males who died in accidents varies by a factor of 3 [23,28]. In approximately one half of the cases the average ploidy was 5-7 *c* (where *c* is the DNA content in a haploid chromosome set). These myocardia contained many octaploid cells, although tetraploid cells usually predominated. Cells with higher levels of ploidy were found in the myocardium of only a few individuals, and they had an average ploidy of 9-10 *c*. In such a myocardium octaploid cells and especially their binucleate variety 4 *c*×2 prevailed and quite a few cells with 16 chromosome sets and some with 32 sets were always found. However, about a quarter of the healthy adults had a myocardium of low ploidy, with nearly half of the myocytes being diploid.

Increased myocyte ploidy in a hypertrophic myocardium may be due to enhanced myocyte proliferation during the natural period of ontogeny. Until recently, investigators disregarded the fact that myocytes may undergo much greater polyploidization than during normal development in cases of congenital organic heart defects or those acquired in childhood, as well as in cases of prolonged strain on the heart of children aged 5-10 years. Indeed, polyploidy levels exceeding normal values were found in congenitally defective hearts of children at the age of 10-12 years [28], i.e., at the time when ontogenic polyploidization is nor-

mally completed. The average ploidy could exceed 20 *c* if hexadecaploid cardiac myocytes predominated and myocytes with 32 and even 64 chromosome sets were present in the myocardium.

Enhanced polyploidization is a feature of cardiac ventricles laboring under an increased functional load [28]. In children, excessive polyploidy values were invariably found in the functionally overloaded ventricle, for example the right ventricle in Fallot's tetrad and the left ventricle in aortal defects. The other ventricle of the same heart contained myocytes of normal ploidy. The same was also true of hypertrophic hearts from adults with heart defects.

Hyperplasia is not the major factor of normal or compensatory myocardial growth in adult mammals. In the rat and mouse, cardiac myocyte proliferation becomes strongly inhibited approximately 3 weeks or so after birth but is not completely blocked even in adult animals. Proliferating cells are then small in number, and the very fact of their detection together with the demonstration of some increase in the number of mitoses in hypertrophic myocardia is a fundamental result of studies by Rumyantsev [9], since it opens up prospects for enhancing the mitotic effect. That compensatory myocardial growth occurs by way of hyperplasia and excessive cell proliferation has been suggested by many studies (reviewed by Adler [14]), but the evidence provided to substantiate this hypothesis is inadequate. Two types of relevant data have been presented: estimates of cell numbers based on stereometric reconstructions and data obtained in evaluating myocyte ploidy in hypertrophic myocardia. However, these estimates are unreliable because of the many arbitrary assumptions made. Although myocyte ploidy in myocardial hypertrophy may be high, it remains uncertain how the hypertrophy relates to myocyte polyploidization. When hypertrophied myocardia from adult persons with myocardial infarction were examined, myocyte polyploidy was found not to go beyond the range of normal variation [28]. The weight of the hypertrophic ventricle correlated in those myocardia with the mean protein mass of the myocyte. Myocyte ploidy was also within the range of normal variation in about half of the examined hypertrophic hearts with acquired defects; some of the hearts weighed more than 500 g. Polyploidy values exceeding the limits of normal variability were found in hearts with congenital defects or defects acquired in childhood [28]. Another argument against massive polyploidization in the adult hypertrophic myocardium is the absence of a correlation between the level of myocyte polyploidy and age in individuals over 12 years of age who have congenital or acquired (in childhood) heart defects [28]. In such individuals, cells of high ploidy could arise during the natural ontogenic periods. The reported instances of polyploidy in hypertension or heart disease require more detailed study, taking into account the fact that hypertension may be an inherited condition, in which case polyploidization may precede the onset of hypertrophy, as it does in rats of the SHR strain. A heart defect may be acquired in childhood and become clinically manifest in later life.

The principal mode of normal postnatal or compensatory myocardial growth is through augmentation of cytoplasmic mass in postmitotic myocytes. This mode of normal cell growth was recognized long ago as the main one for neurons and as very important for cardiac myocytes. Recently, Kudryavtsev [8] assessed the implications of cytoplasmic growth for hepatocytes. Cytoplasmic growth may also be a feature of other cells. Cell mass augmentation accompanied by multiplication of intracellular structures increases the active mass of the tissue concerned, i.e., it is a form of regeneration - both physiological regeneration in normal ontogeny and that occurring as a compensation for defective functions [10-12,43].

Other mechanisms of tissue growth are cell multiplication and cell polyploidization. The relative contributions of cytological mechanisms to normal myocardial growth in ontogeny have been determined for the mouse [20,24,29]. In this species, myocardial weight increases approximately 2.5-fold by the age of 2-3 weeks through the proliferation of myocytes in the first few days after birth and their subsequent almost total polyploidization. As soon as myocytes leave the cycle, their cytoplasm begins to grow, and its growth continues throughout life. This mechanism is responsible for a 4- to 5-fold increase of myocardial weight by the age of one year. The increase in cytoplasmic mass, or postmitotic hypertrophy, compensates for the insufficient myocardial growth during the period of myocyte proliferation. For example, the hearts of mice grown in a nest containing 4 individuals weighed 2 to 3 times more at the time of weaning than did those of mice grown in a nest housing 16 individuals, but these differences became small by the age of 3 months due to increases of protein mass in the myocytes. Similar changes in the mass of all muscle proteins, including specialized contractile proteins, were found for rats [26]. It is compensatory growth of the active cytoplasm (intracellular regeneration as defined by Sarkisov [10-12]) which appears to ex-

plain why the hearts of rats growing at different rates in the first few weeks of life, and thus differing by a factor of about 1.5 in the total myocyte genome during that period, did not differ appreciably in their physiological characteristics by the age of 3 months, showing similar contraction and relaxation rates, similar responses to isoproterenol and to short-term coarctation of the aorta, and similar blood flow rates in coronary vessels [39]. Persons who died in accidents and were found to differ by a factor of 3 in the average myocardial ploidy [23] did not have any detectable signs of heart disease.

The protein mass of individual cardiac myocytes does not correspond to the gene dosage in a normal myocardium. In a hypertrophied myocardium, proteins accumulate and the cell mass becomes a multiple of the ploidy. A lack of correspondence between cell mass and gene dosage is shown only by myocytes that have left the cycle. At the time of polyploidization, the protein mass of myocytes (and of other polyploid cells as well) doubles, and so does their genome. After the postmitotic growth of myocytes has begun, the proportionality between myocyte genome and mass disappears; in mice and rats, for example, this proportionality was canceled out in the very first weeks of life. At a 2:4:8:16 ratio of the genomes, the mass ratio of proteins in murine myocytes was approximately 2:3:5:9 [3,29], and similar disproportions have been found in rat [26] and human [27] myocytes. The disparity between cell mass and cell ploidy is a feature distinguishing myocytes from all other polyploid cells that have been examined [30]. With the disparity between cell mass and genome continuing, these cells grow until the body and heart growth stops, so that each cell is able to reach a huge size without altering its genome. In a myocardial hypertrophy due to infarction, cardiac defects, hypertension, or some other cause myocytes exhibit additional growth whereby the ratio of protein masses doubles to match the cell ploidy [27].

The normal heart has a reserve for myocyte growth, and this reserve is used in the compensatory response occurring in disease. Until recently, the significance of myocardial polyploidization remained unclear. But even before the phenomenon of cardiac polyploidy was discovered, Zhinkin [6] pointed out the advantages of polyploidization over cell division, the main advantage being the economy of energy achieved because of the incomplete mitosis. Another key advantage for cardiac function may be the absence of a need for reorganizing cellular structures and reinstituting intercellular connections if the cell cycle is incomplete [30,38]. An additional important factor may be cell enlargement as a result of polyploidization. Incomplete mitosis can indeed be preferable to complete mitosis, though only during a very short period of cardiac functioning. It should be realized that cell enlargement simplifies tissue regulations and intensifies cell functions. However, the low degree of ploidy found in the hearts of some healthy persons and the usually low polyploidy of normal atria argue against the importance of cell enlargement for normal cardiac functioning. The specific significance of polyploidy for the heart has been recognized recently in studies of myocytic proteins in hypertrophied hearts. In these studies a reserve for heart growth was identified, this consisting in a capacity for continued growth of polyploid cells to reach a mass that matches the gene dosage. The approximate magnitude of this reserve is shown in Fig. 1, where extreme examples of polyploidy in normal human myocardia are also given.

The myocardium is composed of muscular and connective tissues, and the connective tissue component of a hypertrophied myocardium may be changed disproportionately to the muscular tissue component. However, as the volume and weight of muscle cells in various states of the cardiac muscle are severalfold greater than those of connective tissue cells [41,49], estimates of the reserve based on the muscle cells alone (as in Fig. 1) closely reflect changes in ventricular weight. In most hearts, where the mean myocardial ploidy is somewhere between 5 and 7n (5-7 *c* in terms of DNA), the reserve may amount to one-third of the cardiac muscle weight, while in hearts with a cell population of high ploidy it may exceed 50% of this weight. It should be stressed that protein accumulation to a double level is a normal process in the biology of polyploid cells. In myocytes this process is delayed in its development. Occurring under conditions of functional overload, such myocyte growth completes, as it were, the polyploidization stage usual for other cells. The ventricular weight may increase by almost a half during this stage. In certain cases the hypertrophy oversteps this limit, and in such cases the growth of myocytes leading to a doubling of their mass is only the beginning of their extra growth, being the first phase of hypertrophy. While this phase may be considered as normal for the nucleocytoplasmic ratios, the further growth of the cells and myocardium may result, after some time, in adverse effects of which pathologists are well aware [11,12]. In many instances, however, hypertrophy of the myocardium (or, more precisely, that of the hyperfunctional

ventricle) does not exceed 30-50% of its original mass, i.e., it remains within the limits of the reserve growth inherent in most hearts. The situation is different with myocardia of low ploidy whose capacity of reserve growth is also low. The risks posed by infarction and other derangements of myocardial trophism and function in such hearts are higher than in hearts of increased ploidy. In man, myocardial polyploidization takes place in the early years of life. As described above, mice and rats raised under conditions of normal nutrition differed in cardiac polyploidy from their counterparts that had been nourished inadequately. Similarly, living conditions of children may affect the genome of their cardiac myocytes and, accordingly, the reserve for future compensatory processes. In this context, polyploidy in the heart may be viewed as a strategy for survival, as an additional factor operating to enhance restorative processes in an organ incapable of substantial proliferative growth. There is an urgent need for identifying the mechanisms by which the doubling of the polyploid cell mass is delayed during normal development in order to ensure accelerated growth of these cells in the event that pathological conditions arise.

REFERENCES

1. E. M. Antipanova, I. L. Erokhina, and P. P. Rumyantsev, *Tsitologiya,* **29**, № 7, 782-786 (1987).
2. V. Ya. Brodskii, *Ontogenez,* **25**, № 5, 29-44 (1994).
3. V. Ya. Brodskii, N. N. Tsirekidze, and A. M. Aref'eva, *Tsitologiya,* **25**, № 4, 434-440 (1983).
4. V. Ya. Brodskii, B. Carlson, and A. M. Aref'eva, *Ontogenez,* **17**, № 2, 138-145 (1986).
5. V. Ya. Brodskii, B. Carlson, and A. M. Aref'eva, *Ontogenez,* **21**, № 1, 89-95 (1990).
6. L. N. Zhinkin, *Arkh. Anat.*, № 1, 3-21 (1962).
7. M. E. Kogan, L. N. Belov, and T. A. Leont'eva, *Arkh. Pat.*, **38**, № 1, 77-80 (1976).
8. B. N. Kudryavtsev, *Cellular Mechanisms of Normal and Reparative Growth of the Mammalian Liver* (Author's synopsis of doctoral dissertation) [in Russian], St. Petersburg (1991).
9. P. P. Rumyantsev, *Cardiac Muscle Cells in Processes of Reproduction*, Differentiation, and Regeneration [in Russian], Leningrad (1982).
10. D. S. Sarkisov, *Arkh. Anat.*, **10**, № 10, 3-12 (1963).
11. D. S. Sarkisov, *Regeneration and Its Clinical Significance* [in Russian], Moscow (1970).
12. D. S. Sarkisov, V. O. Arutyunov, L. D. Krymskii, and L. S. Rubetskoi, *Myocardial Hypertrophy and Its Reversibility* [in Russian], Moscow (1966).
13. I. V. Uryvaeva, A. M. Aref'eva, and V. Ya. Brodskii, *Byull. Eksp. Biol. Med.*, **89**, № 2, 219-222 (1980).
14. C. P. Adler, in: *The Development and Regenerative Potential of Cardiac Muscle*, New York (1991), pp. 227-252.
15. C. P. Adler and U. Costabel, *Virchows Arch. [B],* **16**, 343-345 (1975).
16. C. P. Adler and H. Friedburg, *J. Molec. Cell. Cardiol.*, **18**, 39-53 (1986).
17. S. P. Bishop and P. Heine, in: *Recent Advances in Studies of Cardiac Structure and Metabolism*, Baltimore (1975), pp. 77-98.
18. A. B. Borisov and P. P. Rumyantsev, in: *The Development and Regenerative Potential of Cardiac Muscle,* New York (1991), pp. 115-139.
19. V. Y. Brodsky, *Cell Tissue Kinet.*, **20**, 367-368 (1987).
20. V. Y. Brodsky, in: *The Development and Regenerative Potential of Cardiac Muscle,* New York (1991), pp. 253-293.
21. V. Y. Brodsky, A. M. Arefyeva, and I. V. Uryvaeva, *Cell Tissue Res.*, **210**, 133-140 (1980).
22. V. Y. Brodsky, B. Carlson, A. M. Arefyeva, and I. A. Vasilieva, *Cell Differ. Dev.*, **25**, 177-184 (1988).
23. V. Y. Brodsky, A. L. Chernyaev, and I. A. Vasilieva, *Virchows Arch. [B],* **61**, 289-294 (1991).
24. V. Y. Brodsky and G. V. Delone, *Biomed. Sci.*, **1**, 467-470 (1990).
25. V. Y. Brodsky, G. V. Delone, and N. N. Tsirekidze, *Cell Differ.*, **17**, 175-181 (1985).
26. V. Y. Brodsky, V. Pelouch, A. M. Arefyeva, *et al., Int. J. Dev. Biol.*, **36**, 339-342 (1991).
27. V. Y. Brodsky, D. S. Sarkisov, A. M. Arefyeva, and N. W. Panova, *Europ. J. Histochem.*, **37**, 199-206 (1993).
28. V. Y. Brodsky, D. S. Sarkisov, A. M. Arefyeva, *et al., Virchows Arch. [B],* **64**, 429-436 (1994).
29. V. Y. Brodsky, N. N. Tsirekidze, and A. M. Arefyeva, *J. Mol. Cell. Cardiol.*, **17**, 445-455 (1985).
30. V. Y. Brodsky and I. V. Uryvaeva, *Genome Multiplication in Growth and Development*, Cambridge (1985).
31. F. Cluceaut and B. Maurer-Schulze, *Cell Tissue Kinet.*, **19**, 267-274 (1986).
32. W. Grabner and P. Pfitzer, *Virchows Arch. [B],* **15**, 279-294 (1974).
33. A. A. Katzberg, B. B. Farmer, and R. A. Harris, *Amer. J. Anat.*, **149**, 489-500 (1977).
34. M. Kompmann, I. Paddags, and W. Sandritter, *Arch. Pathol.*, **82**, 303-308 (1966).
35. H. Kondo, *Acta Med. Okayama,* 37, 281-293 (1981).
36. B. Korecky, S. Sweet, and K. Rakusan, *Can. J. Physiol. Pharmacol.*, **57**, 1122-1129 (1979).
37. B. Korecky and K. Rakusan, *Amer. J. Physiol.*, **234**, H123-H128 (1978).
38. J. O. Oberpriller and J. C. Oberpriller, in: *Cardiac Morphogenesis,* New York (1985), pp. 12-23.
39. B. Ostadal, A. M. Arefyeva, and F. Kolar, *Basic. Res. Cardiol.* (in press).
40. P. Pfitzer, *Verh. Dtsch. Ges. Kreislauff.*, **38**, 22-34 (1972).
41. K. Rakusan, in: *Growth of the Heart in Health and Disease,* New York (1984), pp. 131-164.
42. W. Sandritter and G. Scomazzoni, *Nature,* **202**, 100-102 (1964).
43. D. S. Sarkisov, *Zentralbl. Allg. Pathol.*, **137**, 14-19 (1991).
44. G. Schmidt and P. Pfitzer, *Virchows Arch. [B],* **48**, 59-67 (1985).
45. R. Schneider and P. Pfitzer, *Virchows Arch. [B],* **12**, 238-258 (1973).
46. T. Takamasu, K. Nakanishi, M. Fukuda, and S. Fujita, *Histochemistry,* **77**, 485-494 (1983).
47. H. W. Vliegen, A. M. Vossepoel, A. Van der Laarse, *et al., Histochemistry,* **84**, 348-359 (1986).
48. Y. Yabe and H. Abe, in: *Advances in Myocardiology,* Baltimore (1980), pp. 553-564.
49. R. Zak, *Circulat. Res.*, Suppl. 2, 17-25 (1984).