CHANGES IN PROTEIN CONTENT AND PLOIDY CLASS OF MOUSE CARDIOMYOCYTES

G. V. Delone, N. N. Tsirekidze, and V. Ya. Brodskii

UDC 611.127-018.15+612.172.014.2:612.6/:012.173.1.015.348/:612.66

KEY WORDS: mitoses; DNA synthesis; myocardium.

The aim of the investigation was to determine whether the number of mitoses in the period of growth of the myocardium is limited or whether it can vary under different circumstances, so affecting the ultimate number and level of polyploidization of the cells. Heart muscle is a convenient object with which to study this problem. Before animals begin to feed themselves, three weeks after birth, DNA synthesis and mitosis in the ventricles of the rat and mouse heart are almost completely blocked [3]. Under normal conditions multiplication of cardiomyocytes in the ventricles of the mouse heart is basically complete by the 3rd-4th day after birth, and the period of polyploidization occupies the next 10-15 days [2, 4]. The weight of the growing heart can be varied experimentally, by distributing newborn mice among nests with different numbers of individuals [6].

The writers studied the ratio between ploidy classes of cardiomyocytes of the ventricles in mice growing at different rates. The protein content in single cells, corresponding to their total mass, also was studied.

EXPERIMENTAL METHOD

CBA/C57Bl/6 mice were used. The rates of growth of the animals in the early postnatal period of development were quickened and slowed by keeping the newborn mice in nests with different numbers of individuals per lactating female. In the first case (group 1) the number of young mice was limited to 4, in the second case (group 2) there were 16 of them. The animals were kept under these conditions until the 21st day, when they began to feed themselves ad lib. The animals were weighed after different times and killed on the 21st and 90th days after birth. The heart was removed, weighed, and fixed in 10% formalin in phosphate buffer (pH 7.0) for not less than 10 days. A cell suspension was obtained by alkaline dissociation of the formalin-fixed tissues. A transverse central slice was cut through both ventricles and the fragment was dissociated into separate cells with 50% KOH solution. Preparations of isolated cells were obtained from the suspensions. It was shown previously that the technique used does not lead to appreciable loss of DNA and protein [1]. To determine DNA and protein in the same cell, a combination of Feulgen's method with staining with Naphthol yellow S was used [7]. DNA and proteins were determined quantitatively by scanning cytophometry (Vickers M-86) at 2 wavelengths: about 580 and 445 μm [2]. To determine the DNA content and the ratio between the ploidy classes 16 animals were used and measurements were made on 350-500 cells. The protein content in the cardiomyocytes was determined in 8 mice, and 100-150 cells were measured in each animal.

EXPERIMENTAL RESULTS

Mice from nests with 4 young animals grew much more rapidly than mice from nests with 16 young. By the beginning of independent feeding of the animals (21st day after birth) the mean weight of mice of group 1 was 3.3 times greater than that of group 2, and the weight of their heart was 3.1 times greater. After 3 months the body weight of the animals and the weight of their hearts was roughly the same in the two groups, although mice of group 1 still remained heavier than those of group 2 on average by 26%, and their hearts were heavier by 18% (Fig. 1a, b).

Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. Department of Cytology, Histology, and Embryology, Tbilisi University. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 364-366, September, 1984. Original article submitted January 13, 1984.

 0007-4888/84/9809-1280$08.50

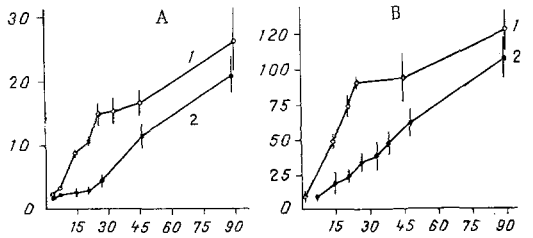

Fig. 1. Changes in body weight (A) and weight of heart (B) of mice growing at different rates ($\bar{X} \pm 2\sigma$, number of animals n = 4-10). Abscissa, animal's age (in days); ordinate: A) body weight (in g), B) weight of heart (in mg). 1) Mice of group 1; 2) mice of group 2.

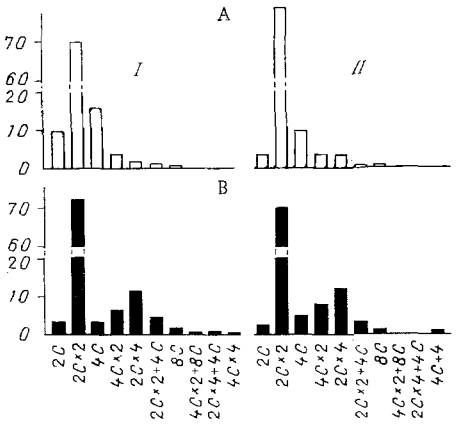

Fig. 2. Diagrams of distribution of ploidy and number of nuclei of ventricular cardiomyocytes of mice growing in nests containing different numbers of young. Each diagram represents mean for 4 animals. Abscissa, types of cells; ordinate, frequency of cells of given type (in %). A) Group 2, B) group 1. I) Mice aged 21 days, II) mice aged 90 days.

The main class of ventricular cardiomyocytes of mice aged 3-4 weeks and older consisted of binuclear cells with diploid (2c, where c is the haploid DNA content) nuclei. These cells (2c × 2) constituted the majority of the cardiomyocyte population in mice of both groups 1 and 2: 70-72 and 70-79% respectively. In the mice of group 2 (16 in the nest) there were more diploid and mononuclear tetraploid myocytes than in the mice of group 1. However, the main difference in ploidy of the myocytes in the animals of the two groups concerned the number of high-ploidy multinuclear cells. Cells of this kind (2c × 4, 2c + 2c + 4c) are found in very small numbers in adult mice growing at the usual rate. In these experiments cells with 3 or 4 nuclei were found in small numbers in mice of group 2 also. However, there were significantly more multinuclear cells in the animals of group 1, roughly 15-16% compared with 2-4% under normal conditions and in the hearts of the mice of group 2 (Fig. 2).

Cardiomyocytes with a tetraploid DNA content (2c × 2 and 4c) are the result of the first polyploidizing mitosis. The number of these cells in normal mice is 85-90% [2]. To form octaploid cells of different types, a second polyploidizing mitosis is necessary (complete divisions of tetraploid cells are possible between polyploidizing mitoses). Usually not more than 7-9% of the cells are octaploid. Cardiomyocytes formed as a result of passage through a 3rd polyploidizing mitosis, mainly hexadecaploid in their total DNA content, account for fractions of 1% during normal development. A very small number of these cells were observed also in mice of group 2. However, in the animals of group 1 the fraction of cardiomyocytes which had passed through 2 polyploidizing mitoses was considerably increased (by 2-4 times). In these mice with more rapid rates of growth of the organ in the early postnatal period, there were also more hexadecaploid cardiomyocytes, up to 1% (normally 0.1-0.3%).

TABLE 1. Mean Protein Content in Cardiomyocytes of Mice Reared under Different Conditions

Group of animals	Mouse No.	All cells		Binuclear cells with diploid nuclei (2c×2)	
		age of animals, days			
		21	90	21	90
1	1	475±15,8	1227±50,3	300±14	937±48
	2	546±21	1158±57	450±20	1064±52
2	1	189±5,9	939±34	183±7	808±42
	2	216±10,1	991±48	180±6,6	811±35

Correlation between ploidy classes in mice of the two groups was similar in animals aged 21 and 90 days, further evidence that the basic proliferative processes in the myocardium are complete by the 3rd week, and that thereafter the number and composition of the ventricular myocytes remain stable. An increase in the number of multinuclear cells in the group of mice with faster rates of growth will be noted. Multinuclear cardiomyocytes are a rare phenomenon in mice and rats. However, multinuclear cells constitute a high proportion of the population of ventricular cardiomyocytes in pigs [5] and cows.

Advantages of the multinuclear cardiomyocyte over the mononuclear cell have been discussed previously [2]. Their formation as a result of the 2nd and 3rd polyploidizing mitoses is possible in appreciable numbers if the periods of polyploidization of the myocardium are long enough. A considerable increase in the number of multinuclear cardiomyocytes found in the present experiments suggests that acceleration of rates of growth of the organs increases the intensity and duration of proliferative processes in the growing myocardium.

The increase in the protein content in the cardiomyocytes corresponded in general to the increase in weight of the ventricles (Table 1). The mean protein content in the cardiomyocytes of mice of group 1 aged 21 days was 2.5 times greater, and the mean weight of the binuclear cells (2c × 2) which were the most numerous group, was 2.1 times greater than in the mice of group 2. By the age of 3 months the weight of the cardiomyocytes correlated with the body weight of the animals: Differences with respect to these parameters in the two groups of animals averaged 24%.

Acceleration of the animals' rate of growth with feeding at lib thus led to an increase in weight of the heart. The protein content of the individual cells increased considerably. In the period of cardiomyocyte reproduction in the ventricles, moreover, the heavy mice went through more polyploidizing mitoses, as reflected in accumulation of high-ploidy, chiefly multinuclear, cells. It will be interesting next to study whether the number of cardiomyocytes also increases.

LITERATURE CITED

1. V. Ya. Brodskii, N. N. Tsirekidze, M. E. Kogan, et al., Tsitologiya, No. 3, 260 (1983).
2. V. Ya. Brodskii, N. N. Tsirekidze, and A. M. Aref'eva, Tsitologiya, No. 4, 434 (1983).
3. P. P. Rumyantsev, Tsitologiya, No. 2, 132 (1978).
4. V. Ya. Brodskii (W. T. Brodsky), A. M. Aref'eva (A. M. Arefyeva), and I. V. Uryvaeva, Cell. Tissue Res., 210, 133 (1980).
5. W. Gräbner and P. Pfitzer, Arch. Pathol. Anat. Abt. B. Zellpathol., 15, 279 (1974).
6. K. Rakusan, Growth, 39, 463 (1975).
7. J. Tas, M. van der Ploeg, J. P. Mitchell, et al., J. Microsc., 119, 295 (1980).