STEREOLOGIC STUDY OF ABSOLUTE TOTAL VOLUMES OF STRUCTURAL COMPONENTS OF THE HYPERTROPHIED MYOCARDIUM

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Wistar rats weighing 200 g were adapted to high-altitude hypoxia in a pressure chamber for 8 h daily at an "altitude" of 8000 m. On the 40th day of the experiment the weight of the left ventricle was 41.5% greater than the control. The volume and surface densities of the structural components of the myocardium were determined stereologically at the light and electron-microscopic levels and the absolute total volumes and their surface areas were calculated for the ventricle as a whole. The total volume of myofibrils was shown to rise steadily during hypertrophy, whereas the total volume of mitochondria remained constant, although the total surface area of the mitochondira increased parallel to the volume of the muscular component. It is suggested that the controlling parameters during hypertrophy of the heart are the weight of the myofibrils and the surface area of the mitochondria.

KEY WORDS: stereology; hypertrophy of the myocardium; high-altitude hypoxia; mitochondria; myofibrils.

There have been many stereological studies of the myocardium during hypertrophy of the heart in recent years [3-5, 8-15]. However, the workers concerned as a rule have limited their attention to the study of relative indices of the volume and surface densities of the structures, calculated per unit volume of tissue. However, to understand the nature of the changes taking place during hypertrophy it is also interesting to examine changes in the absolute total volumes and surface area of the tissue and cell components calculated for the organ as a whole. The aim of the present investigation was to analyze such indices.

EXPERIMENTAL METHOD

Experiments were carried out on 17 male Wistar rats weighing initially 200 g, five of which served as the control; the rest were adapted for 8 h daily in a pressure chamber in accordance with the following program: from the 1st through the 8th days the "altitude" was increased daily by 1000 m, and after the 8th day the animals were kept at an "altitude" of 8000 M. Three rats were killed on the 9th day of the experiment, five on the 15th day, and four on the 40th day. Control animals were killed at the end of the experiment. The heart was stopped by cold [7] and fixed by perfusion through the aorta with a 4% solution of paraformaldehyde in 0.1M phosphate buffer, pH 7.4, under a pressure of 50 cm water. The right and left (with the ventricular septum) ventricles were weighed separately, after which ten pieces were excised from each ventricle, postfixed with 1% osmium tetroxide, embedded, and used for obtaining semithin and ultrathin sections.

The total weight of the fixed myocardium was determined by weighing a piece of tissue tied by a hair to the pan of torsion scales, in air and after immersion in water. The volume of the myocardial tissue of the ventricles (V_V) was calculated by dividing their weight by their specific gravity, which was the same in the experimental and control series (1.06 ± 0.006 g/cm^3). Using a stencil of dots and lines [1, 2], the bulk density (Bd) of the muscle fibers (Bd_f) and of the interstitial tissue, including small blood vessels, mainly capillaries (Bd_c) and of the connective tissue with all other structures (Bd_{ct}) was determined in percentages in semithin sections under the light microscope. Using dots, the bulk density (Bd^{if}) of the mitochondria (Bd^{if}_{mc}), myofibrils (Bd^{if}_{mf}) and of other structures (Bd^{if}_{os}), and using intersections of the lines, the surface density of the outer

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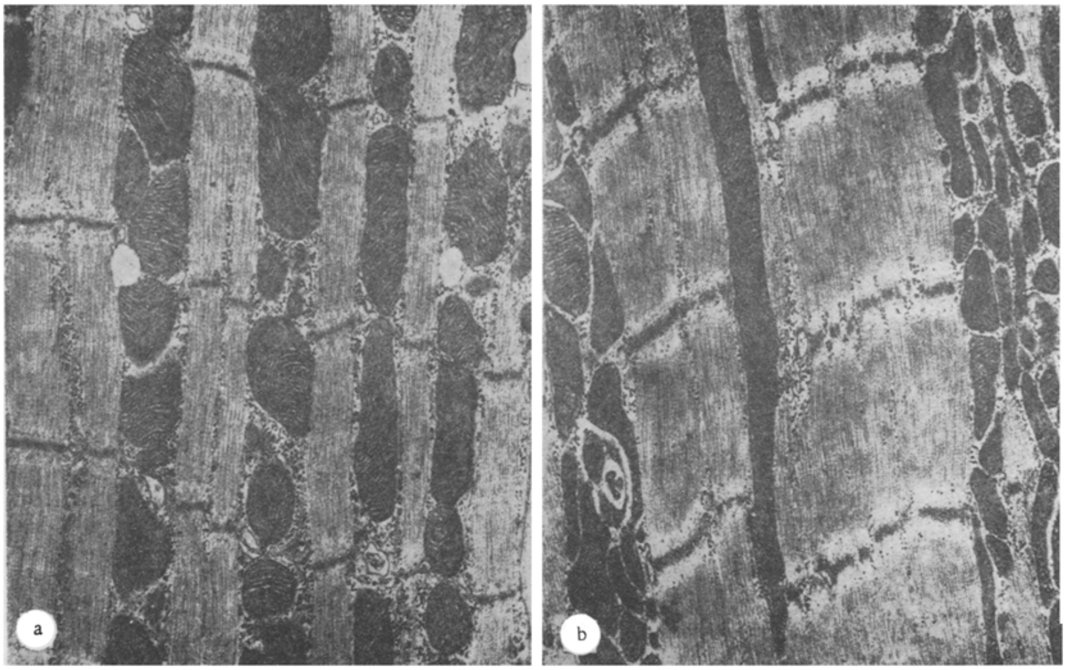

Fig. 1. Typical areas of myocardium of left ventricle of control (a) and experimental (b) animals (on 40th day). Experimental section shows thickening of myofibrils, widening of Z-bands, and smaller and more elongated mitochondria. 16,000×.

membrane of the mitochondria (Sd^{if}_{mc}), inside the muscle fibers were determined in mm^{-1} on ultrathin sections in the electron microscope. The bulk and surface density of the intracellular structures relative to the total myocardial tissue were next calculated by the equations:

$$Bd = \frac{Bd^{if} \cdot Bd_f}{100} \text{ and } Sd_{mc} = \frac{Sd^{if}_{mc} \cdot Bd_f}{100}.$$

The absolute total value of each component in the myocardial tissue of the left ventricle was calculated by the equation:

$$V = \frac{V_v \cdot Bd}{100}$$

and the absolute total surface area of the mitochondria by the equation:

$$S_{mc} = S_d \cdot V_v.$$

EXPERIMENTAL RESULTS

In both the experimental and control series different myocytes in the same section could differ significantly from one another in the number and size of the mitochondria, the thickness of the myofibrils, and other features, but thickened myofibrils with widened Z-bands and small mitochondria in increased numbers were more characteristic of the experimental animals (Fig. 1). The volume of tissue of the left ventricle, which was 543 ± 24 mm^3 in the control, was somewhat greater on the 9th day of the experiment, namely 567 ± 20 mm^3, and it increased significantly toward the 15th (753 ± 25 mm^3) and 40th (769 ± 54 mm^3) days. It will be clear from Fig. 2 that both the relative and, more especially, the absolute volumes of interstitial tissue were considerably increased in the experimental animals. In the earlier period (9-15 days) this was due chiefly to edema of the connective tissue, but later the edema subsided and the volume of the system of small vessels increased at the same time.

The differences between the relative and absolute indices were particularly marked when the most important functional structures of the myocardium – the myofibrils and mitochondria – were examined. The relative volume of the myofibrils in the experimental hearts showed a tendency to decrease a little, especially if calculated for the myocardial tissue as a whole (Fig. 2b). However, this decrease was attributable to an increase in the quantity of interstitial tissue and of the cytoplasmic matrix, which was most marked on the 15th day of the experiment. The absolute weight of the myofibrils in fact increased steadily (Fig. 2c).

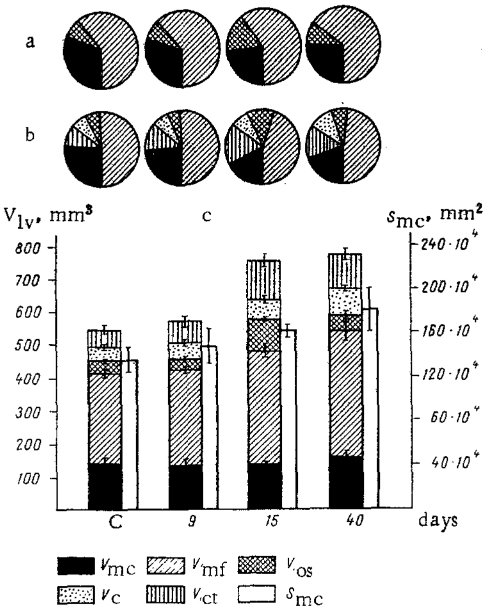

Fig. 2. Relative and absolute ratios of structural components of myocardium from left ventricle of rats in control (C) and on 9th, 15th, and 40th days of adaptation to high-altitude hypoxia. a) Relative volumes inside muscle fibers (Bd^{if}); b) relative volumes in total myocardial tissue (Bd); c) absolute volumes (V) of structural components (in mm^3) and total surface area (S_{mc}) of mitochondria (in $mm^2 \cdot 10^5$).

Changes in the mitochondria are particularly noteworthy, for it has been suggested that these organoids play a special role in adaptation of the myocardium [4, 6]. The relative volume of the mitochondria, it is held, ought to increase, but the results obtained by different workers have proved contradictory [3, 4, 8-15]. In the present experiments, just as in those of some other workers, the relative volume of the mitochondria of the myocardiocytes fell significantly (to 68% of the control). Calculation of the absolute values, however, showed that the total volume of the mitochondria in the myocardium remained unchanged within the limits of error. At the same time the total surface area of the mitochondria increased during hypertrophy of the ventricle parallel (coefficient of correlation 0.79) with the increase in weight of the myocardial muscle fibers (Fig. 2c). This indicates that the mitochondria, while preserving the same total volume, increase their surface area either through an increase in their number and a decrease in the size of the individual mitochondrion, or through changes in shape in the direction away from spherical. Visual observations show that the surface area is in fact increased in both these ways, but the first predominates.

This phenomenon is evidently of deep biological significance in myocardial adaptation, for the increase in surface area leads to increased efficiency of mitochondrial function while their total volume remains unchanged, with consequent economy in the requirement of structural materials for a rapid increase in weight of the myofibrils. There is reason to suppose that this state of affairs is common to the development of hypertrophy from different causes. In a recently published paper Anversa et al. [8] give the detailed results of a stereologic investigation of the rat myocardium during hypertrophy caused by renal hypertension, and by using these data we were able to calculate the absolute total indices. Unlike in the present experiments, these workers used rats weighing 90 g, i.e., in the period of intensive growth. Nevertheless, during an increase in weight of the ventricles by 30% the weight of the mitochondria increased by only 12%, and the increase in the weight of the myofibrils and in the surface area of the mitochondria corresponded almost exactly to the increase in weight of the ventricles.

The results show that the basic controlling parameters in the myocytes during the development of myocardial hypertrophy are evidently the weight of the myofibrils and the surface area of the mitochondria, whereas the weight of the mitochondria may increase much more slowly or, in adult animals, may even remain constant.

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