CHANGES IN LEFT VENTRICULAR MITOCHONDRIA IN INTACT RABBITS DURING THE 24 HOUR PERIOD (DATA OF SCANNING ELECTRON MICROSCOPY)

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Mitochondria of the heart are known to undergo seasonal and circadian changes [3, 4]. However, by transmission electron microscopy it is impossible to assess the size of these organelles objectively, because their area which lies in the plane of the section may not reflect the true size of each particular mitochondrion. This paper describes a study of the myocardium of experimental animals at different times of the 24-h period by scanning electron microscopy and the results are compared with the characteristics of cardiac function at these same times.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male chinchilla rabbits weighing 2.5-3.5 kg. The experiments were carried out during the 24 hours of March 21, 1984, when the heliogeomagnetic situation was quiet, for this is known [5] to have a significant effect on cardiac function. At midnight, 6 a.m., noon, and 6 p.m., in acute experiments under hexobarbital anesthesia, the peak systolic intraventricular pressure under real conditions (VP_r) and during occlusion of the ascending aorta for 5 sec, when the intraventricular pressure reached a maximum (VP_{max}), were recorded in the experimental animals electromanometrically in the left ventricle. The test object for scanning electron microscopy consisted of papillary muscles of the left ventricle, treated by the usual methods [1]. A piece of papillary muscle was washed in Hanks' solution, then placed in 30% glycerol solution made up in Hanks' solution, frozen in liquid nitrogen, sheared, and transferred into a 2% solution of glutaric dialdehyde. The material was then dehydrated in acetone, dried by the method of passage through the critical point from liquid carbon dioxide (Balzers Union, Lichtenstein), sprayed with gold-palladium alloy by ionic bombardment, using a cold "Sputter" (Poliron, England). The specimens were examined in the ISI-60 scanning electron microscope with resolving power of 6 nm and magnification of 1,000-10,000. Electron micrographs were subjected to quantitative analysis. The volume of the mitochondria was calculated by the formula for an ellipsoid of rotation, for the overwhelming majority of mitochondria were closely similar in shape to this geometric figure.

All the numerical results were subjected to statistical analysis by Student's method. Differences between means were taken as significant at the $P \leqslant 0.05$ level. For correlation analysis, correlation was taken to be strong at $P \geqslant 0.7$.

EXPERIMENTAL RESULTS

At all times of the 24-h period a considerable number of mitochondria shaped like an ellipsoid of rotation, or less frequently a sphere, were observed in the left ventricular myocardium (Fig. 1a). However, quantitative and qualitative differences between the mitochondria were observed at different clock times. This applied principally to their volume (in μ^3): 0.57 ± 0.04 at midnight, 0.14 ± 0.025 at 6 a.m., 0.23 ± 0.02 at noon, and 1.75 ± 0.07 at 6 p.m. (the results differed significantly from each other at all times of the 24-h period).

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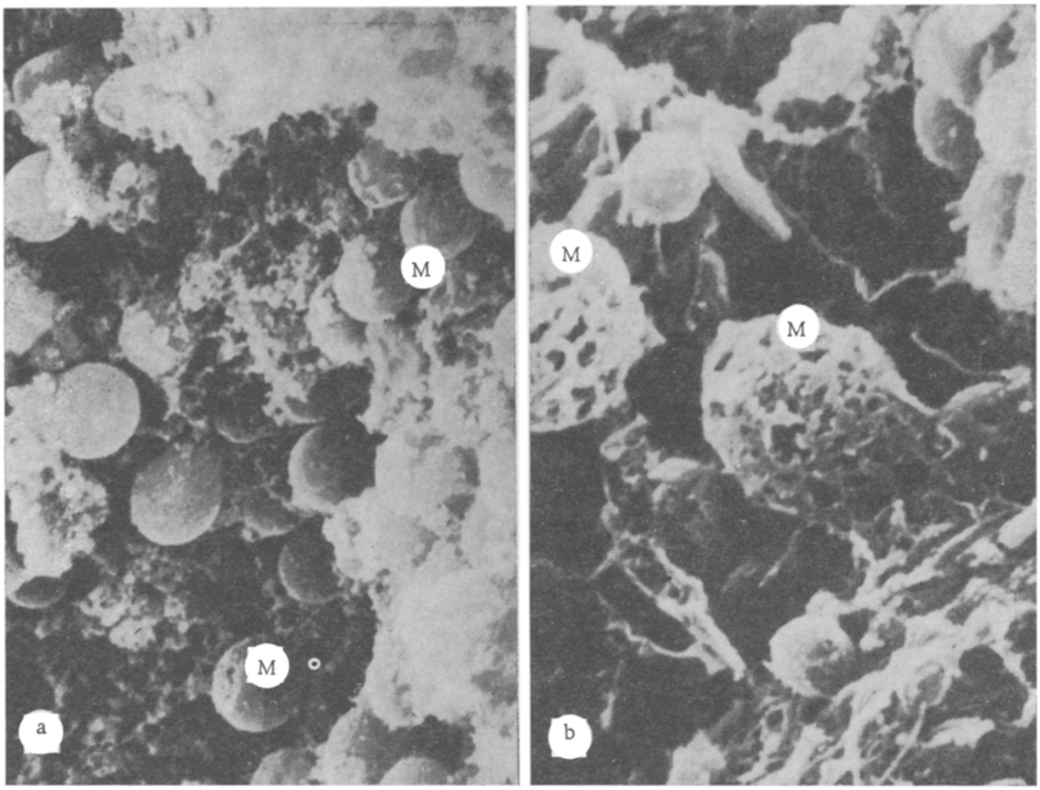

Fig. 1. Characteristics of left ventricular mitochondria (M) at 6 p.m. (a) and at midnight (b). Magnification: a) 2500; b) 10,000.

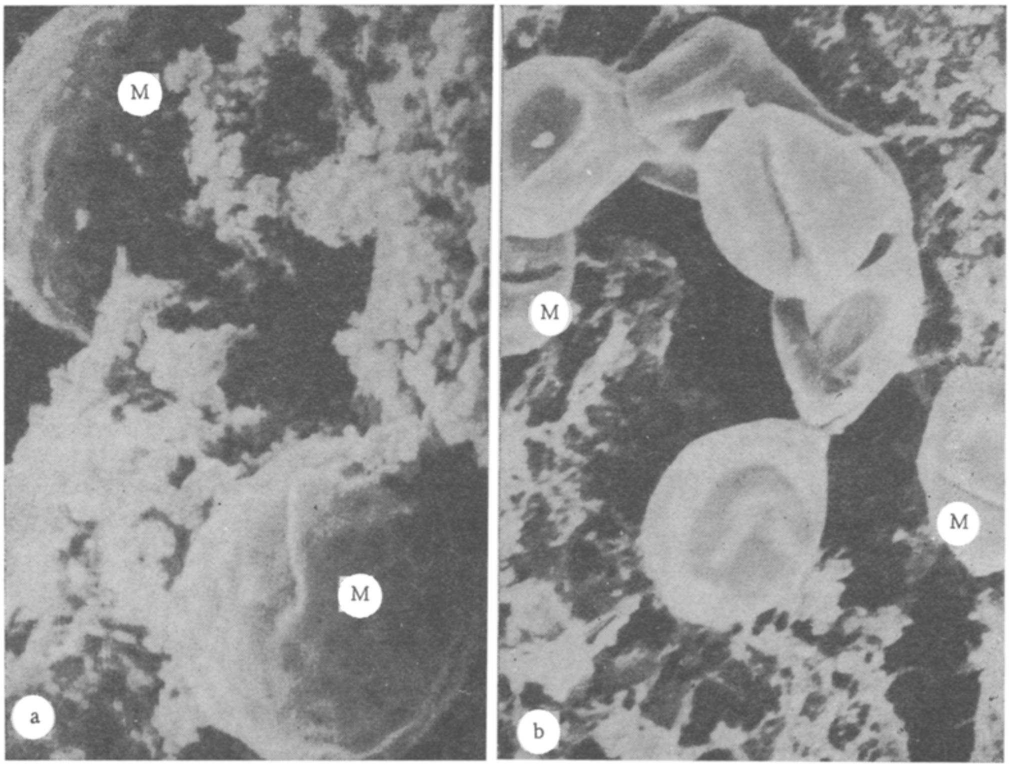

Fig. 2. Swelling and shrinking of left ventricular mitochondria (M) at 6 p.m. (a) and noon (b). Magnification 10,000.

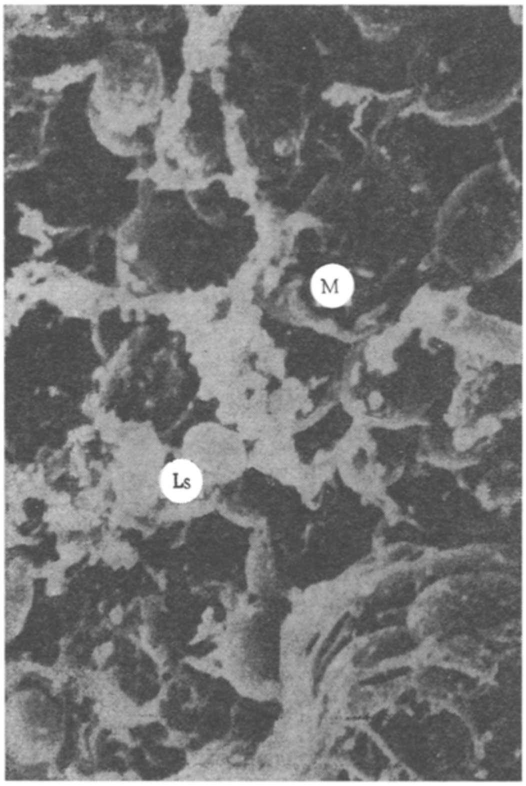

Fig. 3. Destruction of mitochondrion (M) by lysosomes (Ls). Left ventricular myocardium, midnight. Magnification 10,000.

The quantitative analysis thus showed that at 6 p.m. the mitochondria were greatly swollen, whereas at 6 a.m. they reached their smallest size.

Besides quantitative differences, qualitative differences between mitochondria at different times also were found. At midnight somewhat larger mitochondria with an uneven, "eroded" surface (Fig. 1b), evidence of destruction of the outer mitochondrial membrane, were particularly numerous. At 6 p.m., when the average volume of the mitochondria was maximal, many greatly swollen spherical mitochondria were observed (Fig. 2a). As a rule these mitochondria were surrounded by bands of amorphous pale material, which appeared to flow out of defects in the mitochondrial membranes. It can be tentatively suggested that in this case the matrix of the mitochondria, which resembles an amorphous dense mass, or coagulates on emerging into the cytoplasm, was being washed out.

A feature characteristic of virtually all times of the investigation, but most marked at noon, is illustrated in Fig. 2b. At this time, many flattened, deformed mitochondria of a considerable size were found and were considered to be mitochondrial membranes from which all the matrix had been expelled.

At all times of the investigation, but in particular at midnight, many contacts between mitochondria and lysosomes were seen (Fig. 3); the lysosomes were evidently destroying the outer mitochondrial membranes, in full agreement with the phenomenon discovered previously by transmission electron microscopy [2].

The results of myocardial function testing at midnight, 6 a.m., noon, and 6 p.m. revealed no significant differences in VP_r and VP_{max} of the left ventricle, although there was a marked tendency for both parameters to fall at midnight. Correlation analysis revealed the following interesting phenomenon. In intact animals strong correlation was present between the different parameters of cardiac contractility, which evidently reflects physiological synchronization of activity of different parts of the heart, determining activity of the heart as a whole. However, the character of these correlations changed during the 24-h period and correlation between certain parameters at certain times could be very weak. For instance, the coefficient of correlation (r) between VP_r and VP_{max} of the left ventricle had the following values during the 24-h period: +0.24 at midnight, −0.22 at 6 a.m., +0.4 at noon, and +0.96 at 6 p.m. Consequently, at 6 p.m. (when the mitochondria reached their largest size) this correlation became strong, positive, and significant. Moreover, strong correlation ($r = +0.88$) was found between the volume of the mitochondria and the degree of correlation between VP_r and VP_{max} of the left ventricle. In other words, the more normal the volume of the mitochondria, the stronger the correlation between the real and maximal attainable function of the left ventricular myocardium, i.e., the more synchronized the working of the myocytes of the left ventricle. Since swelling of the mitochondria leads to increased energy production by them, it can be postulated that an excess of this energy is utilized for processes determining this synchronization. According to this point of view it can be concluded that at 6 p.m. the heart muscle functions optimally compared with other times of the 24-h period studied.

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REACTIVE MOBILITY OF DENDRITES

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Normal or increased peristaltic activity of the intestine may provide an adequate mechanical factor to which neurons of the autonomic nervous system can react. It was in fact shown some time ago by neurohistological methods that the nervous system of hollow organs capable of mechanical activity is characterized by a special kind of change in the dendrites, known as "overflows of neuroplasm" [3, 6, 7]. The concept of overflows of neuroplasm is in harmony with data on the proximal-distal flow of neuroplasm and it may be regarded as a reactive state, although it is often found in completely normal animals [5, 10, 14]. The frequency of this phenomenon is known to be increased under experimental conditions [2, 4, 6]. Many neurons with changes of this kind have been found in experiments with gravitational overloads. On the basis of fixed preparations, the dynamics and mechanisms of this phenomenon can only be conjectured, and this process has not hitherto been studied in living objects.

Accordingly, in the investigation described below, an attempt was made to compare neurohistologic data on the nonspecific reactive phenomenon of overflows of neuroplasm with intravital observations on reactive mobility of dendrites of surviving neurons or nerve cells in tissue culture.

EXPERIMENTAL METHOD

Neurons of the large intestine of 12 rabbits and five cats, exposed to functionally tolerable overloads of up to 8g according to a special graph, were studied. The direction of the overloads was: head—pelvis, pelvis—head, chest—spine, spine—chest. The material (total preparations of intestine) was processes by the Gomori or Bielschowsky—Gros methods.

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