SCANNING ELECTRON MICROSCOPIC STUDY OF TYPES OF MITOCHONDRIAL DESTRUCTION IN THE CARDIOMYOCYTE

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A clearly defined circadian rhythm of the volume, shape, and state of the outer mitochondrial membranes is observed in myocytes of the intact rabbit myocardium in the course of the 24-h period; the characteristics of the destructive changes, moreover, suggest the presence of several different types [2].

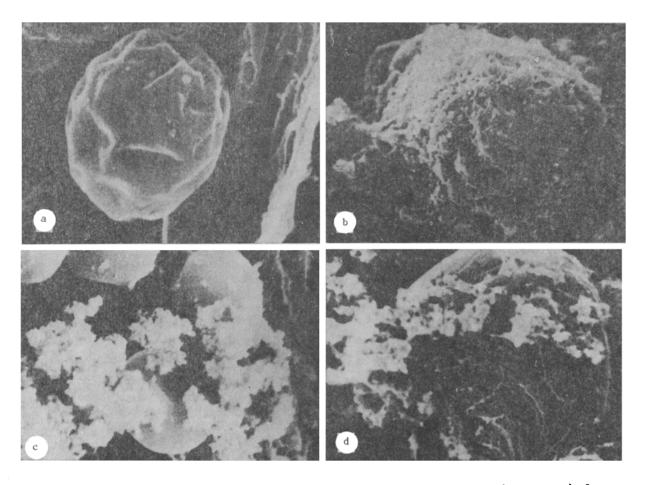


Fig. 1. Destruction of mitochondria by shedding of their outer membrane: a) formation of folds on surface of outer membrane  $(10,000\times)$ ; b) formation of amorphous lumps on surface of outer membrane  $(10,000\times)$ ; c) shedding of remnants of outer layer of outer membrane into hyaloplasm  $(5,000\times)$ ; d) complete destruction of outer membrane and exposure of filamentous framework of mitochondria  $(10,000\times)$ . Scanning electron micrographs of left ventricular myocardium of a rabbit.

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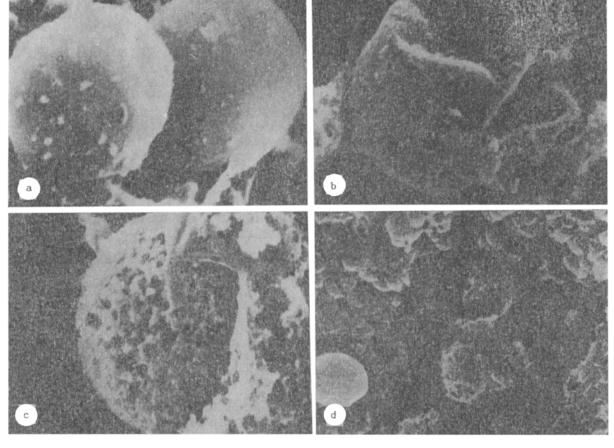


Fig. 2. Destruction of mitochondria by swelling and local ulceration of outer membrane: a) swelling of mitochondria (10,000×); b) rupture of membrane and deformation of mitochondria (10,000×); c) microfocal ulceration of outer mitochondrial with ulcerated membrane (2,000×); d) destruction of mitochondria with ulcerated membrane (2,000×). Scanning electron micrographs of left ventricle of rabbit heart.

For a more detailed study of these types, in the investigation described below scanning electron microscopy was used to analyze the state of myocardial mitochondria of intact animals and animals with ventricular fibrillation.

## METHODS

The hearts of 64 intact mature chinchilla rabbits weighing 2.5-3.5 kg were analyzed in a chronic biological experiment, carried out on March, June, September, and December 21, 1984, when in the course of a 24-h period, starting at 0 h, samples were taken every 3 h for morphological study (at each point of the experiment from 2 animals), and also the hearts of 9 animals 1, 3, and 5 min after the beginning of fibrillation induced by direct stimulation of the heart with a pulsed current (50 Hz, 50 V; 3 rabbits in each group) were analyzed. All the experiments were carried out under superficial hexobarbital anesthesia and with controlled respiration.

Regions of the papillary muscles of the left ventricle, treated by the usual methods [1], were chosen for scanning electron microscopy. The piece of papillary muscle was washed in Hanks' solution, frozen in liquid nitrogen, sheared, and immersed in a 2% solution of glutaric dialdehyde. The material was then dehydrated in acetone, dried by passage through the critical point from liquid carbon dioxide (Balzers Union, Liechtenstein), and sprayed with gold-palladium alloy, by ionic bombardment, using a cold "Sputter" (Poliron, England). The ISI-60 scanning electron microscope with a resolving power of 6 nm (magnification 1,000-10,000) was used.

## RESULTS

On scanning electron microscopy of the intact heart 3 main types of destruction of mitochondria in the cardiomyocyte were distinguished.

The most frequently observed type of mitochondrial destruction consisted of characteristic changes in the outer mitochondrial membrane. At first the membrane was gathered into folds (Fig. la), which later became granular in appearance, and as a result the surface of the mitochondria became covered by a large number of amorphous lumps (Fig. lb). This was followed by shedding of these conglomerates into the cytoplasm in the form of an amorphous mass (Fig. lc), and finally, the outer membrane was completely destroyed, exposing the distinctive filamentous framework of the mitochondria (Fig. ld).

The second type of destruction of the mitochondria was observed rather less frequently. At certain times of day sudden swelling of these organelles was observed, as shown by an increase in their volume and in the tension of the outer membrane (Fig. 2a), in which cracks frequently appeared. These mitochondria then broke up and lost their characteristic shape (Fig. 2b).

The third type of destruction of mitochondria in the cardiomyocyte was observed most rarely of all. It consisted of microfocal ulceration of their outer membrane (Fig. 2c) followed by gross destruction of the mitochondria (Fig. 2d).

Definite seasonal differences could be detected in the predominance of one or other type of mitochondrial destruction. For example, the third type of injury to these organelles was found more often in spring than in the other seasons, and in summer marked swelling followed by rupture of the outer membranes of the mitochondria was seen extremely rarely. There were also definite differences within the 24-h period specific for each season, evidence that the process of mitochondrial destruction possesses a circadian rhythm.

During fibrillation the same three types of destruction of the mitochondrial apparatus were observed, but the frequency and degree of mitochondrial destruction were much higher than in the intact cardiomyocyte; all three types of destruction, moreover, were found virtually equally often. The impression of more rapid "aging" of mitochondria under conditions of uncoordinated contraction of the myocardium is created, with the intensity of this process increasing in accordance with the duration of fibrillation.

## LITERATURE CITED

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