CHANGES IN MYOCARDIAL ULTRASTRUCTURE OF THE PERFUSED RABBIT HEART IN HYPOXIA

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Among the few published studies [4, 5] of the ultrastructure of the heart perfused by Langendorff's method there is no reference to quantitative analysis of ultrastructrural changes, so that it is impossible to utilize a structural-functional approach completely when evaluating the results.

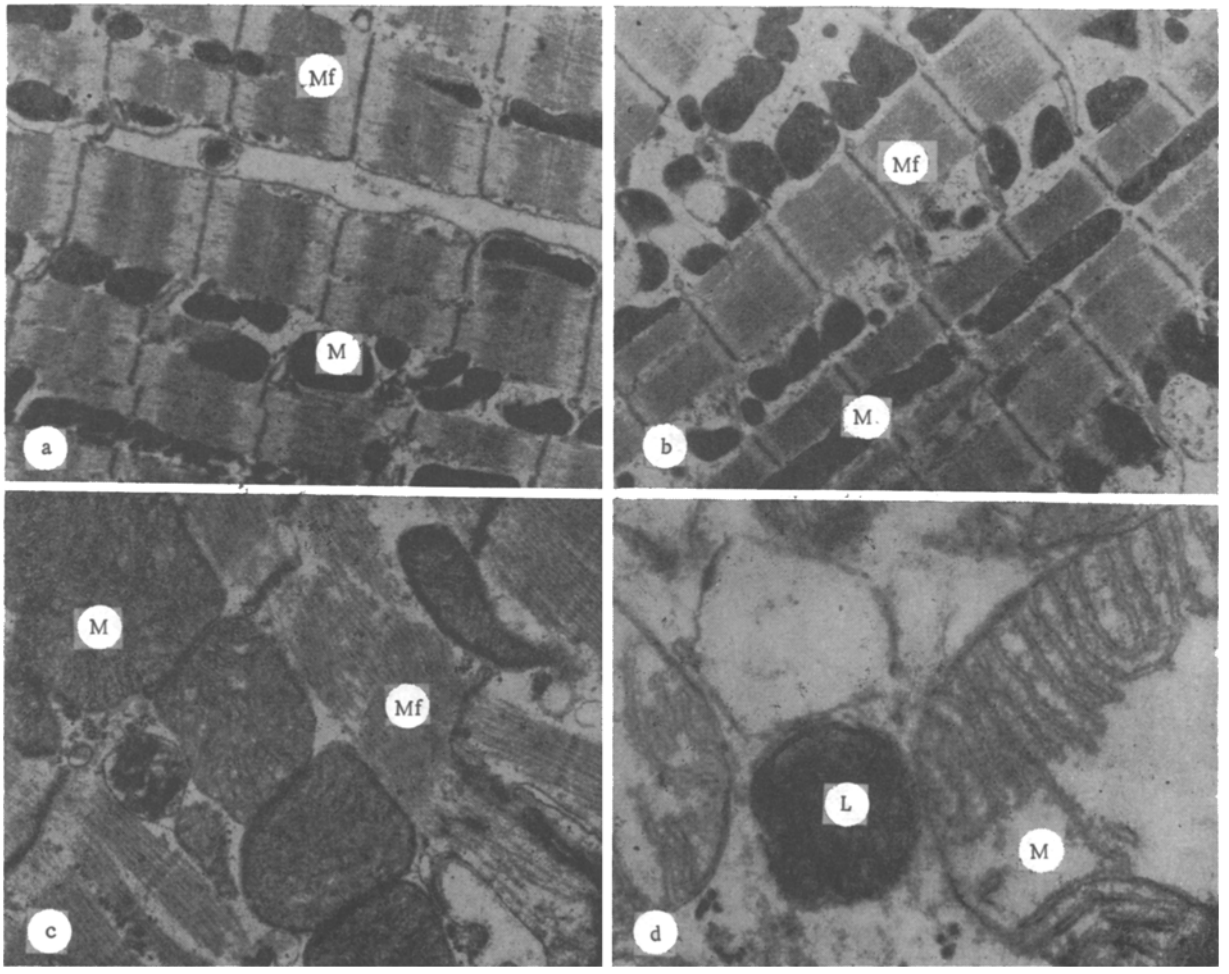

Fig. 1. Electron micrographs of left ventricle of intact rabbit (a) and of heart perfused by Langendorff's method: intact heart (b, c) and during hypoxic perfusion (d). Here and in Fig. 2: M) mitochondria, MF) myofibrils, L) lysosomes. Magnification: a, b) 6000, c) 22,000, d) 52,000 ×.

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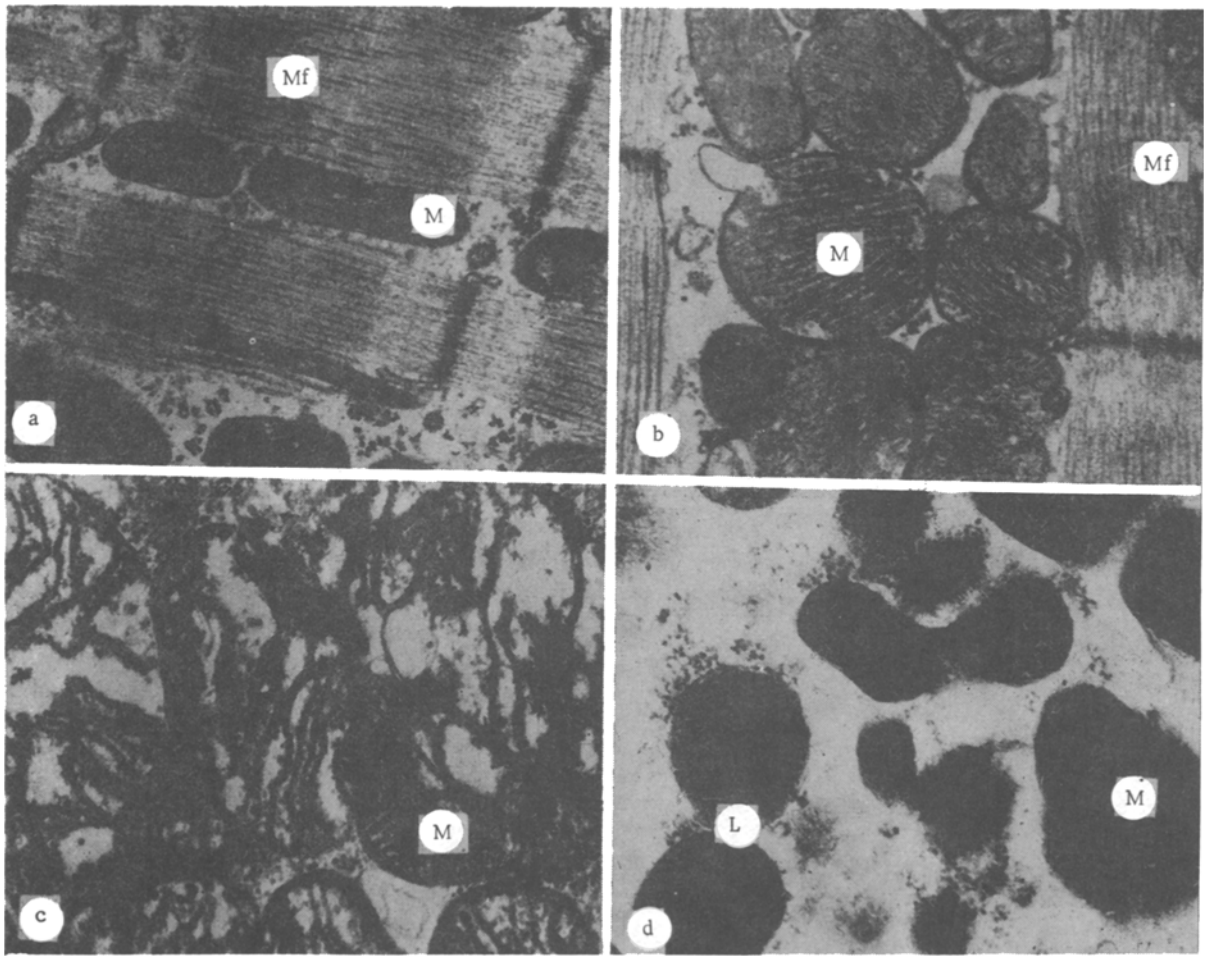

Fig. 2. Electron micrographs of "intact" zone of left ventricle of heart isolated by Langendorff's method, with local hypoxia (22,000 ×). Explanation in text.

The aim of this investigation was to study the ultrastructure of the perfused heart during local and total hypoxia.

EXPERIMENTAL METHOD

Fifteen rabbits weighing 2.5-3.5 kg were used. There were three series of experiments: control, with acute local hypoxia, and with total hypoxia of the heart. In the first two series the Tyrode solution used for perfusion was oxygenated, but in the third series it was saturated with nitrogen. The coronary vessel was ligated before extirpation of the heart. The duration of perfusion in all series was 20 min. A piece of papillary muscle from the left ventricle was excised for the investigation (from the "intact" zone in the case of local hypoxia). The material was fixed with glutaraldehyde and OsO_4 and embedded in Araldite by the usual method. Ultrathin sections were cut on a Reichert-Jung Ultracut ultramicrotome and examined in the Tesla-540 electron microscope under a magnification of 6000-66,000 times. Thirty electron micrographs from each series of experiments were subjected to quantitative analysis [1]. A T1-58 minicomputer was used for statistical analysis.

EXPERIMENTAL RESULTS

The electron-microscopic investigation showed that the ultrastructure of the heart when perfused by Langendorff's method differs only negligibly from that of the heart *in situ* (Fig. 1a-c). In the case of total myocardial hypoxia signs of most severe extracellular and intracellular edema and most severe swelling of the mitochondria coupled with gross destruction of these organelles were found (Fig. 1d). In the myocardium there were very many lysosomes, mainly primary, with a distinct membrane. In local ischemia marked extracellular and intracellular edema was observed. The sarcolemma was loose in structure and homogenized in some places. Marked heterogeneity of the mitochondria was characteristic (Fig. 2). Three types

TABLE 1. Results of Quantitative Analysis of Electron Micrographs of Left Ventricular Myocardium of Rabbit Heart Perfused by Langendorff's Method, under Normal (compared with the heart *in situ*) and Certain Pathological Conditions

Parameter	Heart in situ (normal)	Heart isolated by Langendorff's method		
		intact myocardium	hypoxic perfusion	local hypoxia
Number of mitochondria per electron micrograph	7,0±0,32	7,1±0,7	4,9±0,3**	7,6±0,7
Number of cristae per mitochondrion	6,0±0,18	5,8±0,4	5,2±0,37	5,3±0,36
Area of one mitochondrion, μ^2	0,79±0,02	1,05±0,06*	1,34±0,08**	0,6±0,04
Number of cristae per electron micrograph	45±2,1	42±4,2	25±2**	40±4,7
Total area of mitochondria per electron micrograph, μ^2	5,89±0,22	7,51±0,78*	6,4±0,6	4,44±0,44**
$CEEM_{mc}$	5,48±0,34 (71,2%)	7,69±0,77* (100%)	7,49±0,74 (98,7%)	3,7±0,51** (48,1%)
$CEEM_{em}$	42±2,2 (77,5%)	54,2±5,9 (100%)	34,2±3,6** (63,1%)	26,1±4,2** (41,0%)

Legend. *P ⩽ 0.05 compared with heart *in situ*, **P ⩽ 0.05 compared with intact isolated heart.

TABLE 2. Results of Quantitative Evaluation of Degree of Ultrastructural Heterogeneity of Intact Zone of Left Ventricle of Rabbit's Heart Isolated by Langendorff's Method, in the presence of Acute Local Hypoxia

Parameter	Electron micrograph as a whole	Mean area of mitochondria, μ^2		
		0,6	0,6—1,05	1,05
Number of mitochondria per electron micrograph	7,6±0,7	3,9±0,55*	1,4±0,23*	1,5±0,25*
Number of cristae per mitochondrion	5,3±0,36	3,2±0,23*	5,8±0,68	7,9±0,68*
Area of one mitochondrion, μ^2	0,6±0,04	0,26±0,01*	0,78±0,02*	1,57±0,06*
Number of cristae per electron micrograph	40±4,7	12,4±2*	8,8±1,9*	11,5±2*
Total area of mitochondria per electron micrograph, μ^2	4,44±0,44	1,0±0,13*	1,18±0,19*	2,35±0,04*
$CEEM_{mc}$	3,7±0,51	1,0±0,11*	4,6±0,56	12,2±1,14*
$CEEM_{em}$	26,1±4,2 (100%)	3,4±0,61* (13%)	6,97±1,53* (26,7%)	17,75±3,1 (68,1%)

Legend. *P ⩽ 0.05 compared with electron micrograph as a whole. $CEEM_{mc}$ given only in absolute values, because this parameter was not coparable in certain groups with the analogous parameter for the electron micrograph as a whole, but simple characterized the degree of heterogeneity of the mitochondria. Values of $CEEM_{em}$ for the three isolated groups, added together is a little in excess of 100% because of statistical scatter of the results.

of mitochondria were noted: 1) small, with a clearly defined, intact outer membrane and with dense cristae and a dark matrix (Fig. 2a); 2) moderately swollen with mainly whole cristae (Fig. 2b); 3) very swollen, with a partially or completely eluted matrix (Fig. 2c). There were many lysosomes, mainly primary, and the membranes of some lysosomes were destroyed (Fig. 2d).

The results of quantitative analysis (Table 1) revealed only moderate swelling of the mitochondria in the heart perfused by Langendorff's method, by contrast with the heart *in situ*. During hypoxic perfusion swelling of the mitochondria was more marked and accompanied by a reduction in the number of cristae; together with a decrease in the total number of mitochondria, this is evidence of their sharply increased hyperfunction without signs of regeneration.

In local hypoxia a decrease in the mean area of one mitochondrion was found, and consequently, the coefficient of energetic efficiency of the mitochondria ($CEEM_{mc}$) and the coefficient of energetic efficiency of the electron micrograph ($CEEM_{em}$) also were reduced. The high degree of heterogeneity of the mitochondria in local hypoxia enabled them to be divided into three groups (Table 2): 1) with a mean area of under 0.6 μ^2, i.e., smaller than on average in local hypoxia, 2) with a mean area of 0.6-1.05 μ^2 (from the mean size of mitochondria of the "intact" zone during local hypoxia to the mean size of mitochondria of the intact perfused heart); 3) with a mean area larger than in the intact perfused heart. Mitochondria of group 3 contained the largest number of cristae.

Comparison of these data showed that mitochondria of group 3 had the highest energetic efficiency.

Besides the above-mentioned three groups of mitochondria, individual organelles were found with an area of over 4.0 μ^2. During statistical anlaysis these mitochondria were excluded from counting as "escaping" variants, for the area exceeded three times the standard deviation. $CEEM_{em}$ characteristic of these mitochondria was only 12% of the mean value of $CEEM_{em}$, i.e., it was actually less than $CEEM_{em}$ in the mitochondria of group 1. Correlation analysis showed strong and significant correlation between the area of the mitochondria and $CEEM_{em}$ and between $CEEM_{mc}$ and $CEEM_{em}$ (ρ = +0.986 and +0.997 respectively).

It can accordingly be concluded that excessively swollen mitochondria do not play a role in the supplying of the cell with energy and should not be taken in account when bioenergetic processes in the cardiomyocyte are assessed, in agreement with data obtained previously on the heart *in situ* [3].

The results show that a unique mitochondrial "conveyor" exists in the "intact" zone of the infarcted isolated heart. Most mitochondria in the myocardium are in a state of functional rest, and constitute a reserve for the relatively small number of these organelles which are in a state of hyperfunction and which supply the cardiomyocyte with energy. On destruction of the hyperfunctioning mitochondria, the next group to take its turn becomes swollen and the bioenergetics of the heart is maintained at the necessary level. This "conveyor" possesses a profound adaptive significance, for in this case a principle of alternating activity of functional structures applies, so that they can restore their trophoplastic potential actually during work [2]. In total myocardial hypoxia this principle is not observed, evidently because of the drastic fall in the partial pressure of oxygen in all parts of the myocardium, which converts all mitochondria into a state of hyperfunction immediately. This inevitably leads to destruction of the mitochondria, as reflected in the decrease in their number (Table 1), and to rapid death of the heart as a whole.

Since the results described above were obtained on the isolated heart, the mechanism presented can be characterized as purely myocardial and unconnected with neurohumoral influences.

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