in the blood serum are raised [4]. Activated macrophages also secrete colony-stimulating factor and monocytopoiesis-inducing factor [5, 6]. Accordingly the process of formation of foci of infiltration in the liver can be represented as follows. The injected zymosan first binds with the Kupffer cells and, in response to its assimilation they secrete factors disinhibiting monocytopoiesis. As a result the number of monocytes in the blood rises. Meanwhile secretion of lysosomal enzymes is stimulated and a high gradient of chemotaxic factors is formed around the stimulated macrophage. On the whole all these changes create the conditions for migration of monocytes from the blood into the liver and their "fixation" at the site of stimulated hepatic macrophages. The possibility cannot be ruled out that the reactive changes now observed in the liver soma play an important role in the formation of a qualitatively new state, that of tachyphylaxis.

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# STEREOLOGIC ANALYSIS OF MYOCARDIAL ULTRASTRUCTURE AFTER RESUSCITATION


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KEY WORDS: myocardium; stereology; postresuscitation period; electron microscopy.


The recovery period after clinical death is characterized by disturbance of the functions of many organs and systems of the body. One of the leading pathogenetic factors at the basis of postresuscitation sickness has been shown to be circulatory failure, largely due to a disturbance of myocardial contractility [1, 4, 7].

To study the causes of this phenomenon, an electron-microscopic investigation was made of heart muscle, using morphometric methods to assess ultrastructural changes.

## EXPERIMENTAL METHOD

Experiments were carried out on 59 dogs, 14 of which served as the controls. Clinical death for 5 min was produced by massive acute blood loss, and the animals were resuscitated by the method of Negovskii et al. [3]. All experiments were carried out under morphine-hexobarbital anesthesia. The myocardium of the left ventricle was taken for electron-microscopic investigation at the 5th minute of clinical death, during the first 5-10 min after the beginning of resuscitation, and 1.5, 6, and 12 h and 1, 3, 7, and 14 days later. Material was fixed in 25% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Araldite. Ultrathin sections were examined in the UÉMV-100K electron microscope. Besides the qualitative description of the myocardial ultrastructure, a detailed quantitative analysis of the electron micrographs was made by stereological methods [6]. The relative volumes (Vv), surface densities (Sv), and surface to volume ratios (S/V) were calculated


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TABLE 1. Results of Stereologic Analysis of Myocardial Ultrastructure in Postresuscitation Period (M ± m)

| Experimental conditions | Vvmc % | Vvmf % | VvTs % | Svmc $\mu^{-1}$ | Svmf $\mu^{-1}$ | SvTs $\mu^{-1}$ | $\frac{Smc}{Vmc}$, $mm^{-1}$ | $\frac{Smf}{Vmf}$, $mm^{-1}$ | $\frac{STs}{VTs}$, $mm^{-1}$ | $\frac{Vmc}{Vmf}$ | $\frac{VTs}{Vmf}$ | $\frac{STs}{Vmf}$, $mm^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 24,82±0,91 | 66,96±1,50 | 1,68±0,12 | 1,136±0,046 | 0,818±0,036 | 0,201±0,023 | 4614±215 | 1221±45 | 12702±1635 | 0,37±0,02 | 0,025±0,002 | 298±27 |
| 5th minute of clinical death | 24,35±1,28 | 67,91±1,68 | 1,61±0,18 | 1,114±0,087 | 0,806±0,024 | 0,208±0,015 | 4676±171 | 1188±41 | 13462±1316 | 0,36±0,03 | 0,024±0,003 | 307±21 |
| Resuscitation measures | | | | | | | | | | | | |
| 5–10 min | 24,91±1,29 | 67,58±2,14 | 1,57±0,16 | 1,127±0,090 | 0,790±0,033 | 0,188±0,026 | 4566±289 | 1172±54 | 12965±2316 | 0,37±0,03 | 0,023±0,003 | 279±39 |
| 1½ h | 21,67±1,34 | 58,29±2,34 $P<0,05$ | 0,49±0,15 $P<0,001$ | 1,104±0,100 | 0,730±0,082 | 0,075±0,021 $P<0,001$ | 5098±285 | 1248±135 | 15794±2208 | 0,38±0,03 | 0,008±0,002 $P<0,001$ | 127±36 |
| 6 h | 20,81±0,97 $P<0,05$ | 66,53±1,53 | 1,23±0,07 $P<0,01$ | 0,939±0,048 $P<0,05$ | 0,880±0,064 | 0,163±0,025 | 4519±199 | 1325±88 | 13200±1563 | 0,31±0,02 | 0,018±0,002 $P<0,05$ | 247±38 |
| 12 h | 23,77±1,37 | 66,12±2,12 | 1,62±0,14 | 1,061±0,059 | 0,872±0,059 | 0,237±0,015 | 4480±345 | 1309±94 | 14832±1254 | 0,37±0,03 | 0,025±0,003 | 360±16 |
| 1 day | 23,38±0,99 | 62,01±1,84 | 1,17±0,14 $P<0,05$ | 1,107±0,092 | 0,820±0,060 | 0,141±0,010 $P<0,05$ | 4677±287 | 1326±106 | 12532±1968 | 0,38±0,03 | 0,019±0,002 | 228±20 |
| 3 days | 25,08±1,02 | 64,32±1,83 | 1,15±0,11 $P<0,01$ | 1,329±0,090 | 0,803±0,064 | 0,159±0,017 | 5300±299 | 1243±74 | 13972±1644 | 0,39±0,02 | 0,018±0,002 $P<0,05$ | 247±27 |
| 7 days | 24,12±1,30 | 65,15±0,58 | 1,04±0,19 $P<0,05$ | 1,253±0,136 | 0,749±0,061 | 0,151±0,017 | 5138±395 | 1152±99 | 16113±2263 | 0,37±0,02 | 0,016±0,003 $P<0,05$ | 233±28 |
| 14 days | 24,25±0,90 | 66,29±2,05 | 1,35±0,12 | 1,248±0,084 | 0,784±0,044 | 0,184±0,033 | 5139±295 | 1185±71 | 14038±2537 | 0,36±0,02 | 0,021±0,002 | 277±39 |

Legend. Where no P is given differences between means compared are not significant; mc) mitochondria, mf) myofibrils, Ts) T system.

for the mitochondria, myofibrils, and T systems in the cardiomyocytes, and the ratios of the volumes of the mitochondria and T systems to the volume of the myofibrils and the ratio of the surface area of tubules of the T system to the volume of the myofibrils also were calculated. In addition the relative volume of lipids in the myocardial cells was determined. The primary morphometric parameters were recorded by means of a test system of short segments making an angle of 19° with the axis of the myofibrils [2, 9, 11]. The results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

Stereologic analysis of electron micrographs of the myocardium showed that by 1.5 h after resuscitation marked disturbances of the ultrastructural organization of the cardiomyocytes had developed (Table 1). One of the most important pathological changes was massive destruction of tubules of the T system, as a result of which their relative volume in the myocardial cells fell from 1.68 ± 0.12% (in the control) to 0.49 ± 0.15% 1.5 h after resuscitation ($P < 0.001$), i.e., a more than threefold decrease. Although some of this decrease in bulk density could be attributed to tissue edema present at that period, the morphological picture observed was clear evidence of destruction of many tubules of the sarcotubular system. Excitation is known to be conducted along membranes of the T system into the depths of the muscle cell, and metabolites, especially calcium, are transported along the lumen of its tubules [8, 10]. Hence it can be understood why marked lysis of the T-systems and sarcoplasmic reticulum leads to a disturbance of conduction of the electrical signal from the outer membrane to the remaining cisterns of the sarcoplasmic reticulum, and causes damage to the calcium pump and, consequently, to electromechanical coupling.

The tendency for the relative volume of the mitochondria in the cardiomyocytes to decrease at that time could evidently be attributed to the presence of intracellular edema. The decrease in the bulk density of the myofibrils from 66.96 ± 1.50 to 58.29 ± 2.34% ($P < 0.05$) was evidently caused, first, by the intensive intracellular edema and, second, by their widespread microlysis. As a result of the marked fatty degeneration of the myocardium, many lipid inclusions accumulated in the muscle cells: Their bulk density was 3.27 ± 0.33%.

The relative volume of the mitochondria in the cardiomyocytes 6 h after resuscitation was down to 20.81 ± 0.97% ($P < 0.05$), evidently on account of their involvement in foci of degradation of the sarcoplasm and foci of necrosis, and also of partial myelinization. However, this decrease, provided that effective energy formation was present in the other mitochondria, could have no significant effect on the contractile function of the myocardium, for the volume of the mitochondria, calculated per unit volume of the myofibrils, did not differ significantly from the control (although it was appreciably reduced). The bulk density of the myofibrils increased and regained its intial level. Intracellular edema, although it persisted in some myocytes, was very slight. Lipid drops almost completely disappeared from the cells.

Partial recovery of the tubules of T systems took place: Their relative volumes in the cardiomyocytes was 2.5 times greater than 1.5 h after resuscitation, but as before it still remained significantly lower than the control level. Meanwhile the surface density of these tubules was increased so much that differences from the control were no longer statistically significant. In that way relative favorable conditions were created for conduction of the electrical impulse into the cell, despite the fact that part of the T system was still destroyed.

The tubules of the T system that still remained 12 h after clinical death were grossly dilated, and as a result their relative volume increased considerably and the unevenness of the distribution of these tubules in the cells became very clear. Its cause was probably a disturbance of the ionic balance in the extracellular fluid as a result of the outflow of potassium from the irreversibly damaged myocytes [5]. Stereologic analysis revealed an increase in the relative volume of the mitochondria (compared with their volume 6 h after resuscitation), evidently indicating their intenstive regeneration.

By 24 h after resuscitation the lumen of the tubules of the T system was reduced to its usual size, and as a result their bulk density again fell to 1.17 ± 0.14% ($P < 0.05$). After 3 days, because of intensification of intracellular regenerative processes, newly formed mitochondria appeared in the myocardial cells. This could account for the observed tendency for the surface–volume ratio of these organelles to increase.

By the end of the 2nd week of the postresuscitation period the ultrastructure of the heart muscle was largely restored to normal. As a result of intensive repair processes gradual regeneration of the T system was observed and its relative volume in the cardiomyocytes increased to 1.35 ± 0.12%, and the differences from the control were no longer significant.

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# ULTRASTRUCTURAL LOCALIZATION OF α-FETOPROTEIN SYNTHESIS IN THE REGENERATING MOUSE LIVER


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After partial hepatectomy or the action of various hepatotoxins a sharp but temporary rise in the α-fetoprotein (AFP) level is observed in the animals' blood, and cells containing AFP are found in liver sections at these times [1]. Cells containing AFP have been shown by the ordinary immunomorphologic methods to be typical differentiated hepatocytes. In adult mice after the action of hepatotoxins as a rule these cells are few in number and they are located mainly on the boundary with the injured tissue [4, 6, 7]. These data suggest that during regeneration of the mouse liver temporary derepression of AFP synthesis takes place in mature differentiated hepatocytes [1]. However, only an immunoenzymic technique of antigen localization at the electron-microscopic level would enable the cells synthesizing this protein to be confidently differentiated from cells passively accumulating it as a result of toxic injury. Such a technique was used previously to detect AFP-synthesizing cells in human and mouse hepatomas, in human fetal liver, and in the liver of animals during chemical carcinogenesis [9, 10, 14, 15].

In the investigation described below an electron-microscopic immunoperoxidase technique [11] was used to identify and characterize AFP-synthesizing cells during regeneration of the mouse liver.


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