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Myocardial Lesions in Hemorrhagic Hypotension

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Summary. The myocardial alterations were investigated in dogs after hemorrhagic hypotension. Development of metabolic acidosis with increased lactate concentration was observed in all cases. Focal hemorrhages and hypoxic changes developed in the subendocarial region. Typical so-called zonal lesions were demonstrable in the mural myocardium of both ventricles. The hypoxia, increased level of catecholamines, metabolic disturbances, and probably cardiotoxic products may play a role in the pathomechanism. The alterations which are potentially reversible are transformed into irreversible damage only in most severe cases. The necrosis is of hypercontraction type, and is associated with rapid calcification and mononuclear cell infiltration. The morphologic changes may explain the circulatory disturbances of cardiac origin and may represent the basis of development of the low cardiac output syndrome in hemorrhagic shock.

Key words: Hemorrhagic shock, myocardial alterations – Hypercontraction necrosis of myocardium, hemorrhagic shock

Zusammenfassung. Die Verfasser untersuchen myokardiale Läsionen, die bei Hunden im Falle hämorrhagischer Hypotonie entstanden sind. Bei allen Tieren wurde eine mit steigender Lactatkonzentration verbundene metabolische Acidosis registriert. In den subendokardialen Herzgebieten sind fokale Hämorrhagien und hypoxische Läsionen entstanden. In der wunden Muskulatur wurden typische sogenannte zonale Läsionen festgestellt. An ihrer Entstehung können die Hypoxie, die steigende Konzentration der Katecholamine, metabolische Schädigungen und vielleicht noch verschiedene kardiotoxische Materialien eine Rolle spielen. Die Läsion ist potentiell reversibel und geht nur in schweren Fällen in einen irreversiblen Prozeß über. In solchen Fällen bildet sich eine Zellnekrose vom hyperkontraktilen Typ aus, die mit schneller Verkalkung und mononuklearer Zellinfiltrierung verbunden

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ist. Die beobachteten mophologischen Veränderungen können die Entstehung des „low cardiac output"-Syndroms im hämorrhagischen Schock begründen.

Schlüsselwörter: Hämorrhagischer Schock, Myokardschäden - Hyperkontraktionsnekrose des Myokards - Myokardschäden, hämorrhagischer Schock

Although peripheral circulatory disorders play an important role in the development of circulatory disturbances caused by shock it has been known since the studies of Wiggers [44] that central circulatory disorder may also occur during hemorrhagic shock. It was demonstrated that the function of the right and the left ventricles decreases and myocardial insufficience may develop even in case of normal coronary perfusion. Kovách [23] demonstrated that during hemorrhagic hypotension the myocardial blood supply decreases by 60—70%. A very small number of morphologic studies deals with the myocardial changes observed during hemorrhagic shock and they are not mentioning the reversible or irreversible nature of these lesions [11, 13, 16, 17, 25—27, 36, 37, 41, 42].

The aim of the present study was to demonstrate the morphologic picture of myocardial lesions developing during hemorrhagic hypotension and the examination of the reversibility of these lesions.

Material and Method

Twenty-five mongrels of both sexes, weighing 12 to 20 kg were anesthetized with 0.1 g/kg of Chloralose. At the start of the expriment 300 U/kg Heparin were administered i.v. as anticoagulant. Five animals served as controls. Hypotension was induced by bleeding through the femoral artery and shed blood was stored at room temperature with 1000 U/100 ml Heparin. The blood pressure of the animals was adjusted to 40 mm Hg and the period of hypotension was maintained until the spontaneous withdrawal of 30% of the blood drawn off. At the end of the experiment the ECG changes were registered with standard leads at a paper speed of 50 cm/s.

The animals were divided into four groups:

I: The preparation was started at the end of the period of hypotension in six animals.
II: The blood drawn off was retransfused through the femoral artery to five animals and the preparation was carried out after 15 min.
III: In five animals the blood drawn off was retransfused through the femoral vein and the tissues were prepared after 15 min.
IV: In four animals the blood drawn off was retransfused in the vein and the preparation of animals was effectuated after 48 h.

At the beginning and at the end of the hypotensive phase, as well as at the end of the experiment (with the exception of the 48-h survival) the O_2 saturation of the arterial blood was determined with Kipp's hemoreflector, the values of pH, pCO_2, standard bicarbonate and buffer base with Astrup's system [1] and the lactate concentration according to Hohorst and Bergmeyer [17].

The heart was resected with thoracal approach of anesthetized animals. In every case a histologic examination was carried out with HE, azane, PTAH, PAS reaction combined with diastase digestion, as well as with Kossa's reaction. On sections stained with H and E the variation of nuclear cubic capacity was measured. Under a projection enlargement of 2000 the longitudinal and transversal diameter of muscular cell nucleus was determined on 300 nuclei and after their classification with the help of monograms the curves of distribution was constructed [31]. In some animals the enzyme reaction of phosphorylase, SDH and LDH [35] was carried out on not fixed, freezed sections. In the same animals the myofibrillar proteins were separated after exposure with SDS-polyacrylamidegel electrophoretic method [43].

Table 1

	Control $\bar{x} \pm$ S.D.	Group I $\bar{x} \pm$ S.D.	Group II $\bar{x} \pm$ S.D.	Group III $\bar{x} \pm$ S.D.
pO_2 mm Hg	106.22 ± 14.60	128.27 ± 24.38$^+$	127.60 ± 32.28$^+$	145.25 ± 14.90
pCO_2 mm Hg	35.4 ± 2.34	22.6 ± 5.24	34.2 ± 7.01$^+$	24.3 ± 6.57
pH	7.29 ± 0.053	7.066 ± 0.119	7.033 ± 0.043	7.156 ± 0.060$^+$
Stand. bic. meg/l	17.17 ± 2.10	8.53 ± 2.22	9.52 ± 1.40	10.48 ± 1.51
Buffer base meg/l	36.30 ± 2.48	22.14 ± 3.85	25.20 ± 2.84	28.60 ± 3.06
Lactate mM/l	1.32 ± 0.97	7.29 ± 1.26	5.69 ± 2.38	6.28 ± 2.47

The deviation in every group (except for groups signed $^+$) is significant as compared to the control

In other animals the heart was fixed by perfusion with 3% of buffered glutaraldehyde after rinsing with physiological salt through a cannula inserted into the aorta. The blocks excised from various sections of the musculature were refixed in 1% of OsO_4 and embedded in Durcapan. After the selection of half-thick sections, the ultrathin sections were prepared on a Reichert ultramicrotome, contrasted with lead citrate and uranyl acetate. An electron microscope type JEM 100 B with an accelerating voltage of 80 kV was used.

Results

The Average Duration of the Hypotonic Period. 2.675 ± 0.242 h (between 2 h and 3 h 30 min). The blood chemical values are summarized in Table 1.

ECG Changes. In addition to considerable tachycardia (about 250/min) signs of myocardial ischemia and sympathetic preponderance in all animals.

Macroscopic Picture: Maintained muscular tension, plane hemorrhages of pin-head size—not in every animal—below the left ventricular endocardium on the septal surface and corresponding with the papillary muscles. The outline of the musculature is identifiable, scarlet, hemorrhage or necrosis is not visible within the irregular blood distribution.

Light Microscopic Picture. The subendocardiac hemorrhages are circumscribed, localized in the connective tissue around the vessels, but do not penetrate into the muscular fibers. In the periphery of hemorrhages intracellular edema occur in the myocardial cells.

In all cases, but with different extension, focal so-called zonal lesions developed in the myocardium and localized along the Eberth lines (Fig. 1). Around the intercalculated discs the muscular fibers are swollen, homogeneous, and in their cross situation cannot be demonstrated with a phase-contrast microscope. In the same area, the glycogen disappeared almost completely, the PTAH binding capacity of the fibers is distinct and around the discs there are

Fig. 1. Zonal lesions of the area of intercalated discs. The muscle fibers are slightly swollen and lost their cross-striation. PTAH 400×

some homogeneous zones staining more lightly. The dehydrogenase reactions disappeared in the damaged area. The phosphorylase reaction is also negative in correspondence to the alteration. It is typical that only the periphery of the Eberth lines shows a difference. The light microscopic picture is otherwise intact, also with respect to the damaged cells, cross striation and the enzyme reactions are maintained.

Following the arterial or venous retransfusion of the blood drawn off the light microscopic picture did not change and calcification did not take place.

After a survival of 48 h focal necrosis of hypercontraction type may be found with mononuclear cell infiltration (Fig. 2). Calcification occurred in the necrotic cells in form of small granules. These cells do not give enzyme reaction whereas the reaction of the surrounding cells is normal. The damages are of focal type and may be demonstrated chiefly in the septal myocardium and in the left ventricular papillary musculature. However, in some animals they may be observed in all areas of the myocardium. The severity and extension of the histological picture shows a correlation with the duration of the hypotonic period and concentration of lactic acid. Disseminated damages affecting the entire heart are observed in cases of longest duration and highest lactate concentration.

At the end of hypotonic period and after the transfusion of the shed blood, the cubic capacity and distribution cell nuclei does not differ appreciably from that of controls. After 48 h the nuclei of cells showing alterations, but no necrosis, are enlarged and the curve of distribution is shifted toward higher values ($P<0.05$; Fig. 3).

An appreciable change in the distribution of myofibrillar proteins is not observed.

Fig. 2. Hypercontraction necrosis at the level of damaged cells after survival of 48 h. PTAH 400×

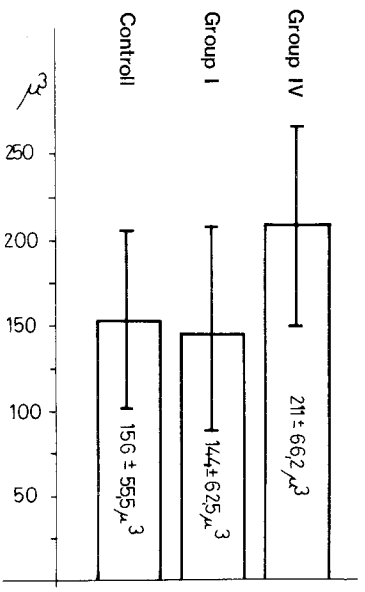

Fig. 3. The nuclear cubic capacity is slightly reduced after the hypotensic period ($t > 0.05$) and significantly increased after survival of 48 h ($t > 0.01$)

Electron Microscopic Examination

In certain cells hypoxic changes are noted as the reduction of glycogen content, the swelling of mitochondria and tubular structures. In some places the laminae of intercalated discs separate from each other and show a break of continuity through which glycogen passed into the intracellular space. However, the fibrillar

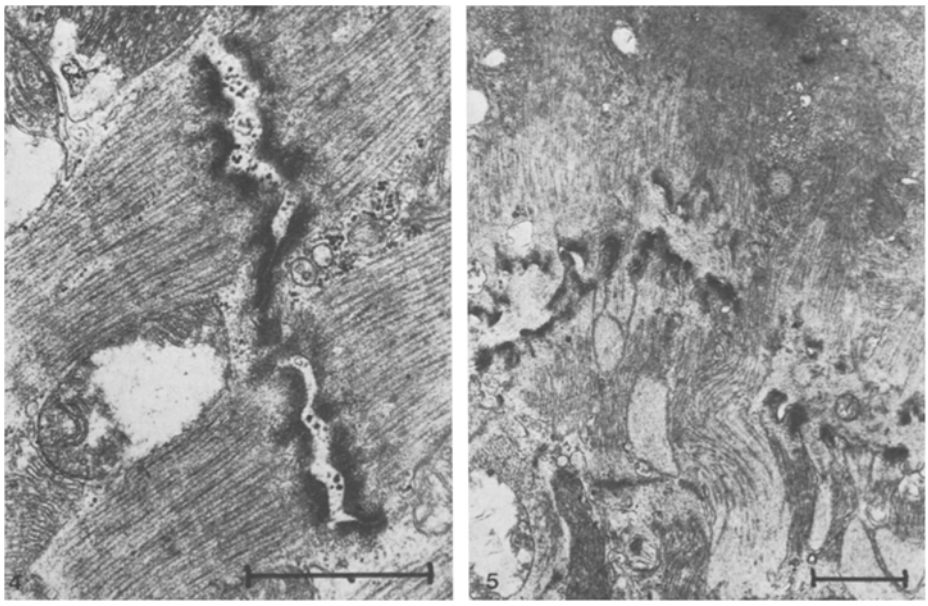

Fig. 4. Separation of the intercalated discs at the subendocardial area. Rupture of the membrane may be observed in some places, free glycogen granules within the intercellular space, hypoxic damages of mitochondria. The filamentar structure is intact. 36,000×

Fig. 5. Typical electron microscopic picture of the zonal lesions. 18,000×

structure is intact in these areas (Fig. 4). This type is mainly characteristic for cells with subendocardiac localization.

The zonal lesions show a very typical structure and extend over the cell localized at one side of the intercalated discs, while the cell situated on the other side is relatively intact with the exception of invaginations, which will be described later.

In mild cases the course of the intercalated disc becomes undulated, some invagination appears in the non-specialized areas and protrudes into the neighboring cell. Both thin and thick filaments may be observed. Along the discus Z membrane has an irregular course in an extension of a few sarcomeres, then the regular structure of the filaments disappeares. Their course is not parallel and may form an angle of 90 deg. The proportion of thin and thick filaments seems to be maintained. There are no mitochondria in the affected area and glycogen is not demonstrable. The number of sarcoplasmic reticulum and the T tubules seem to be reduced, but they are not widened. The sarcolemma is intact (Fig. 5).

In areas with more severe alteration, the course of intercalculated discs is irregular, surrounded by very large invaginations penetrating deep into the adjacent cell. Within the invagination one may occasionally some mitochondria and tubular structure, as well as fibers consisting mainly of thin filaments (Fig. 7).

The course of specialized area of the intercalated discs is also irregular, the intramembraneous space is widened and around the discs thin filaments may be

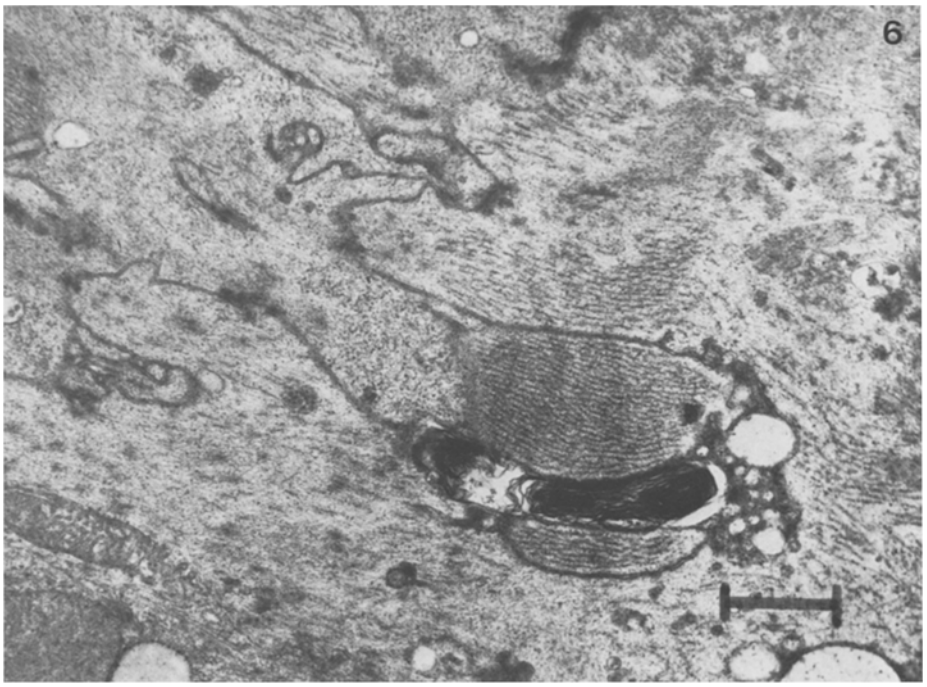

Fig. 6. Survival of 48 h. There are thin and thick filaments surrounded with tubular structures within the invaginations. 14,400×

observed. In the farther areas of the affected cells the structure of fibers is practically intact, only the widening and irregular course of the membranes Z may be observed in some places. The matrix of mitochondria is light and contains focal vacuoles, in which glycogen granules invaginate through the membrane fissure. The crists are elongated, have a concentric course around the vacuoles and fragmented in some places. Sometimes the tubules of the sarcoplasmic reticulum are widened and glycogen granules penetrated from outside. As a whole, the glycogen content appears to be increased. The nuclear structure is intact (Fig. 8). Following the arterial and venous retransfusion of blood and restoration of the circulation the changes are practically the same.

According to the alterations, two types of cells are formed after a survival of 48 h. The necrotic cells observed under the light microscope show the typical picture of necrosis associated with contraction bands; in the swollen mitochondria very dark amorphous substance is deposited, which—in accordance with its localization—corresponds to the small granular calcification observed under the light microscope. One may observe the irregular course of intercalated discs in the regenerating cells, but the lamellae are situated in a symmetrical distance from each other. Contractile elements consisting of thin and thick filaments surrounded by ramified tubular structures partly within the area of invagination and partly independently of it. There is no regular fibrillar structure along the intercalculated discs, the cytoplasma is filled with glycogen and

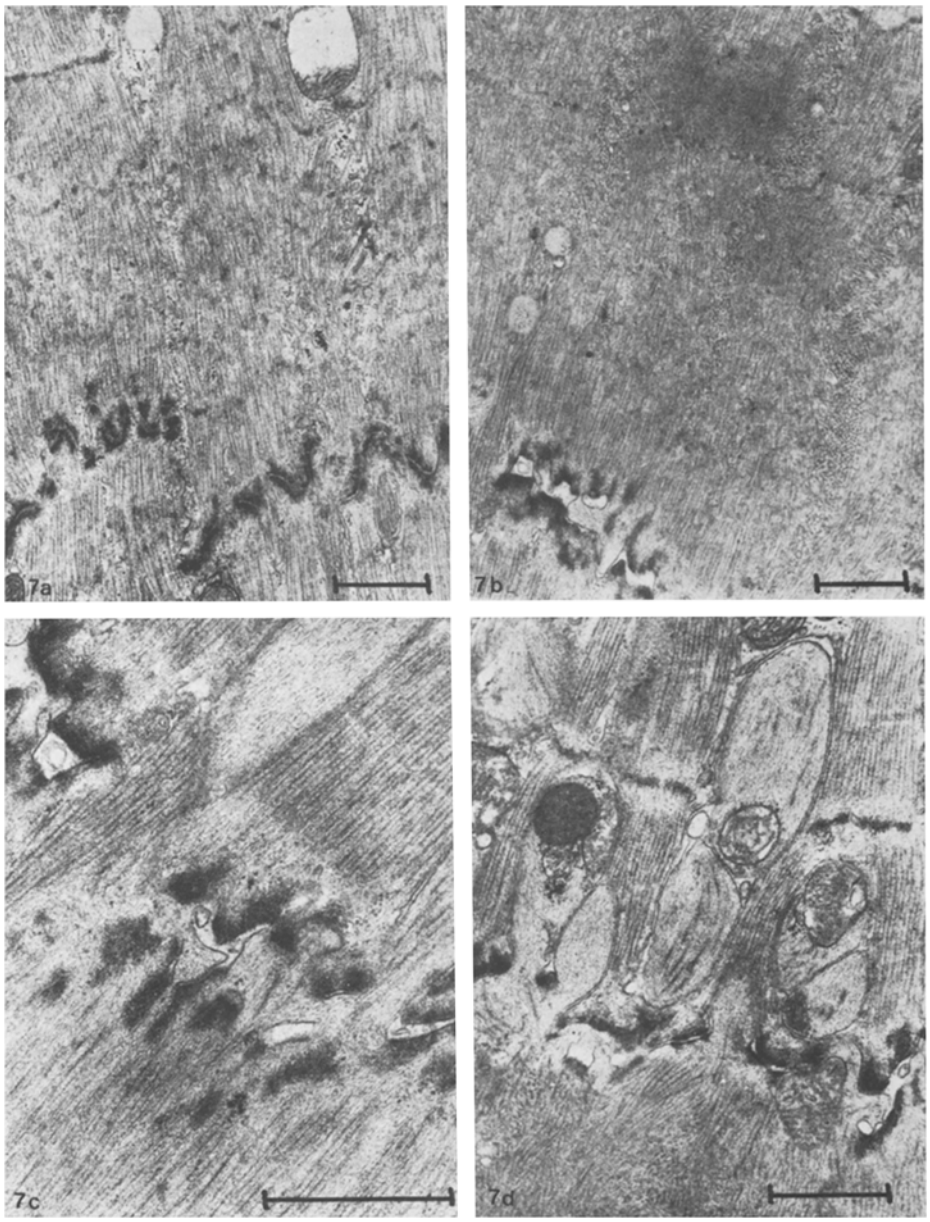

Fig. 7a–d. Formation of zonal lesions: **a** The mildest alteration is the undulating course of the intercalated discs. The course of the adjacent fibers becomes irregular, the cross-striation is not identificable. 18,000×. **b** The course of filaments is not parallel, there are no glycogen and mitochondria in the affected area. 18,000×. **c** The specialized area of the intercalated discs is irregular, the intermembraneous area is widened. 36,000×. **d** Thin filaments, tubular structures, and degenerated mitochondria are visible within the invagination. The myofibrillar structure is relatively intact in the adjacent cell. 24,000×

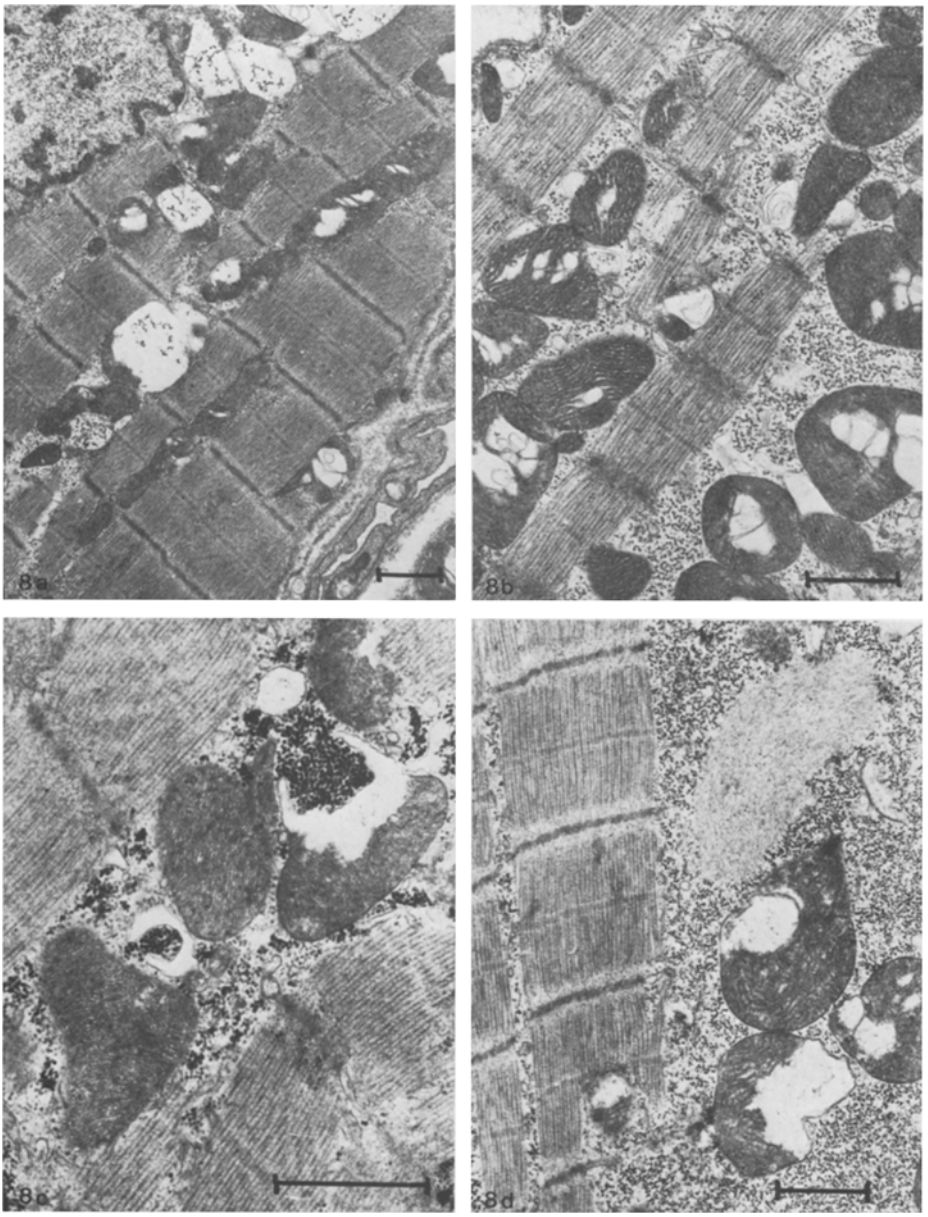

Fig. 8a—d. Alterations of the damaged cells. **a** The filameter structure is intact except for the widening of Z membranes. 12,000×. **b** Increased glycogen conten, vacuolization of mitochondria, and tubular structures. 18,000×. **c** Penetration of glycogen into the mitochondria and tubular structures. 32,000×. **d** Accumulation of non-specialized filamentous material. 18,000×

polyribosome. Further on the fibrous structure is maintained, but its course—especially with respect to the Z bands—is irregular. The mitochondria are dark, the crists increased and arranged parallel to each other. Lipid drops may be observed in their surrounding. The nuclear structure is intact, on the nuclear membrane break of continuity and numerous polyribosomes may be observed (Fig. 6).

Discussion

Since the studies of Wiggers [44] it has been known that after hemorrhagic hypotension myocardial damage my occur, which is signaled by the decrease of arterial tension, increase of left auricular pressure, and the development of low cardiac output syndrome. Opinions vary with respect to the significance of the damage. According to the clinical examinations of Shoemacher [38a], the direct or indirect signs of myocardiac affection may be demonstrated in 40% of patients in the state of shock. Morphologic studies were carried out only on a small material and mostly under experimental conditions. In experiments Haeckel et al. [4, 11, 13, 25, 26, 27, 36, 37] found that after hypotension lasting for several hours subendocardial hemorrhages take place surrounded by necrosis and in the myocardium focal, so-called zonal lesions develop. The change is signaled by the disappearance of striation, as well as by the disorders of the myofibrillar structure and the intercalated discs. They supposed that the subendocardial hemorrhages are caused by oxygen lack, whereas the development of zonal lesions may be prevented with beta-receptor blockers and thus, it seems to be a catecholamine effect. The results were substantiated by Japan authors [20, 21], however, Hiott [16] who administered isoproterenol in combined experiments did not think it likely that the catecholamines play a causal role in the development of zonal lesions.

The blood chemical values observed in the present experimental series correspond with the data published in the literature in every respect and indicate the development of metabolic acidosis associated with the accumulation of lactate. The reversion to the initial state is very slow and after the retransfusion of the blood drawn off one may not register significantly different values as compared to the controls.

In contrast to other studies, subendocardial hemorrhages were observed only occasionally. According to Hackel et al. [13] these are of hypoxic origin. Because their occurrence cannot be demonstrated regularly and these changes may be observed also in other disease entities—mainly in those associated with arrhythmia [5, 8]—the non-specific etiology seems to be more likely.

Diffuse mitochondrial alterations, separation rupture of the laminae of intercalated discs, local focal loss of staining in addition to maintained myofibrillar structure are characteristic for the damage of subendocardial musculature. On the other hand, this picture is indicative of hypoxic origin [7, 12, 18] and corresponds to the statement of former authors [13, 20, 26].

The presence of zonal lesions, considered to be typical, was demonstrable in every case. However, the morphological picture has not been entirely identical

with that published in the literature. This may be attributed partly to the different animal species used for the experiments, and partly to the fact that the preparation was carried out immediately after the conclusion of intervention, while others used isolated cardiac perfusion after the period of hypotension and this was followed by the electron microscopic study [4, 11, 13, 26, 36]. The difference in the experimental arrangement may serve as an explanation for the fact that we did not observe hypercontraction bands after the acute phase [18]. The pathomechanism is not fully cleared. The localization and some of the ECG changes are indicative of hypoxic origin. However, the increased glycogen content, the phosphorylase reaction maintained in the damaged cells, as well as the type of mitochondrial alterations go against this. Also, the unchanged state observed after the restoration of circulation renders the purely hypoxic origin unlikely. Another possible explanation is the effect of catecholamines accumulated with one order of magnitude during the hypotonic period [21]. This may also be supported by the sympathicotonic signs of ECG alterations. With adrenergic beta-receptor blockers Hackel et al. [13] could inhibit the development of lesions, but the blood chemical values were not registered. Thus, it is possible that by acting on the metabolic disturbances the adrenergic beta-receptor blockers are preventing the development of alterations not directly, but indirectly. Also, the morphologic picture is not the same as that usually seen after the administration of catecholamines. Likewise, the experiments of Hiott [16] with the combination of hemorrhagic hypotonia and isoproterenol go against the exclusiveness of this effect.

The general effect of metabolic disturbances due to shock is more probable. During the last years several cardiotoxic products were isolated in cases of shock of various origin (myocardial depressing factor: Lefer [23a] passively transferable lethal factor: Nagler et al. [30]), the presence of which may explain the unusual histologic picture. The significance of plasmic alterations caused by metabolic disturbance is supported by the fact that, after restoration of circulation and practically unchanged blood chemical values, there are no appreciable alterations demonstrable within the area of focal damage. Another characteristic is that the severity and extension of lesions showed a connection primarily with the lactate concentration in addition to the duration of the hypotonic phase.

According to data published in the literature, there is a connection between the isolated lesions of the intercalated discs and changes occurring in the intracellular calcium concentration [29]. The trend of pH toward acidity may itself decrease the quantity of ionized calcium. The increase of lactate and ionic H concentration may also draw away the ionized calcium from the points of linkage [12], and the elevated catecholamine level may contribute to the upset of balanced calcium metabolism [10]. The pathologic differences in local calcium metabolism may explain the damages of the adjacent myofilamentar structure, because the decrease of calcium concentration may provoke a reversible disaggregation of the myofilaments [6, 7]. Thus, the changes taking place in iso-ionia may be able to produce damages localized on the intercalated discs and in their surroundings. However, this does not explain why changes are occurring only in cells present on one side of the Eberth line.

Unger et al. [42] found that the proportion of thin and thick filaments is maintained within the invaginations of intercalated disc. In the present case,

however, a relative local decrease of thick filaments was demonstrable. A similar difference may be noted in cases of congenital cardiomyopathy, experimental coarctation of the aorta, as well as after the administration of isoproterenol or glucose-insulin-potassium [3, 6, 7, 14, 15, 40]. According to some authors this is a sign of new myofilamentar synthesis [32, 33, 40], while others consider it as being the transformation of contractile elements into more primitive form, a phenomenon of degeneration [7]. The half-life of myofilamentar proteins is between 5 and 7 days [28, 45], and this makes the formation of new fibers masses within few hours improbable. This problem cannot be solved on the basis of electron microscopic examination alone. The measurement of the cubic capacity of the cell nucleus showed that at the end of the hypotonic phase there is no appreciable change and, thus, the significant increasing of synthetic processes cannot be supported. The same results were obtained with the isolation of myofilamentar proteins, which showed an identical distribution as the controls. In the present case the local decrease of thick filaments may be considered as a degenerative phenomenon.

Accumulation of glycogen is known to occur in myocardial hypertrophy [19, 24], in the periphery of cardiac infarction [22] and this may be explained by the fact that fibers, which are incapable of contraction may nevertheless synthetize a large amount of glycogen. Most likely the breakdown and synthesis of glycogen in the myocardium is regulated directly by the changes in the P concentration. Also, the decrease of high energy phosphates may contribute to increasing of synthesis or at least to the inhibition of breakdown [31]. Initially, the metabolic disturbance due to shock may lead to increased glyconeogenesis [9], which in addition to damages already present may explain the elevation of glycogen content observed. However, the intramitochondrial appearance is only virtual, the granules pass into the matrix through the ruptures of the membrane.

Although the pathomechanism is not cleared in its details, the morphologic changes present are able to explain the circulatory disturbances of cardiac origin. Because of the disoriented myofilamentar structure the affected cells are obviously unable to exert a contractile force and this may represent the basis of the development of the low cardiac output syndrome.

A certain proportion of the cells is irreversibly damaged. Necrosis is of the hypercontraction type, associated with rapid calcification and mononuclear cell infiltration. This corresponds to the course of coagulation myocytolysis as described by Baroldi [2]. Other cells, however, are regenerated and a sign of this is an increased cubic capacity of the nucleus in addition to the histological picture. But during the acute phase there are no signs on the basis of which one may draw the line of demarcation between the reversible and irreversible damage. Most likely, there is a potentially reversible change which is transformed into irreversible damage only in most severe cases.

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