ably caused sharp contractions of the cardiomyocyte protofibrils with the formation of contraction bands, and with detachment from the Z-membranes.

The results of these experiments thus show that 1 h after massive blood loss ultrastructural disturbances arise in the heart muscle, together with reduction of cAMP-dependent regulation of the Ca-transporting capacity of the cardiomyocyte sarcolemma. In this connection, modulation of the action potential of the cardiomyocytes by injections of the catalytic subunit is an interesting development in the abolition of the consequences of blood loss.

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ULTRASTRUCTURE OF SMOOTH MUSCLE CELLS OF THE FEMORAL ARTERY IN RATS EXPOSED TO VIBRATION

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KEY WORDS: smooth muscle; artery; vibration.

The earliest changes after exposure to vibration in man and animals are observed in the nervous and cardiovascular systems. The character and severity of the circulatory disturbance depend on the parameters of vibration: frequency, amplitude, and duration. Long-term exposure to mechanical oscillations with a frequency below 40 Hz leads to changes of different kinds: not only spastic, but frequently also spastic-atonic and atonic states of the arteries, whereas exposure to high-frequency vibration (40-100 Hz) leads to spasm of the small and large arteries and arterioles [2, 5, 7, 12].

Despite much progress in the study of the functional state of the vascular wall, virtually no attempt has been made to study the morphology of blood vessels in vibration pathology. Accordingly the aim of the present investigation was to analyze the ultrastructure of smooth-muscle cells (SMC) of the femoral artery of experimental animals exposed in the long term to general high-frequency vibration.

EXPERIMENTAL METHOD

The experimental group consisted of eight noninbred female albino rats weighing 140-160 g, which were exposed (3 h daily) for 10 weeks to the action of general vertical vibration with a frequency of 100 Hz and an amplitude of 0.5-0.8 mm. Eight rats of the corresponding sex and

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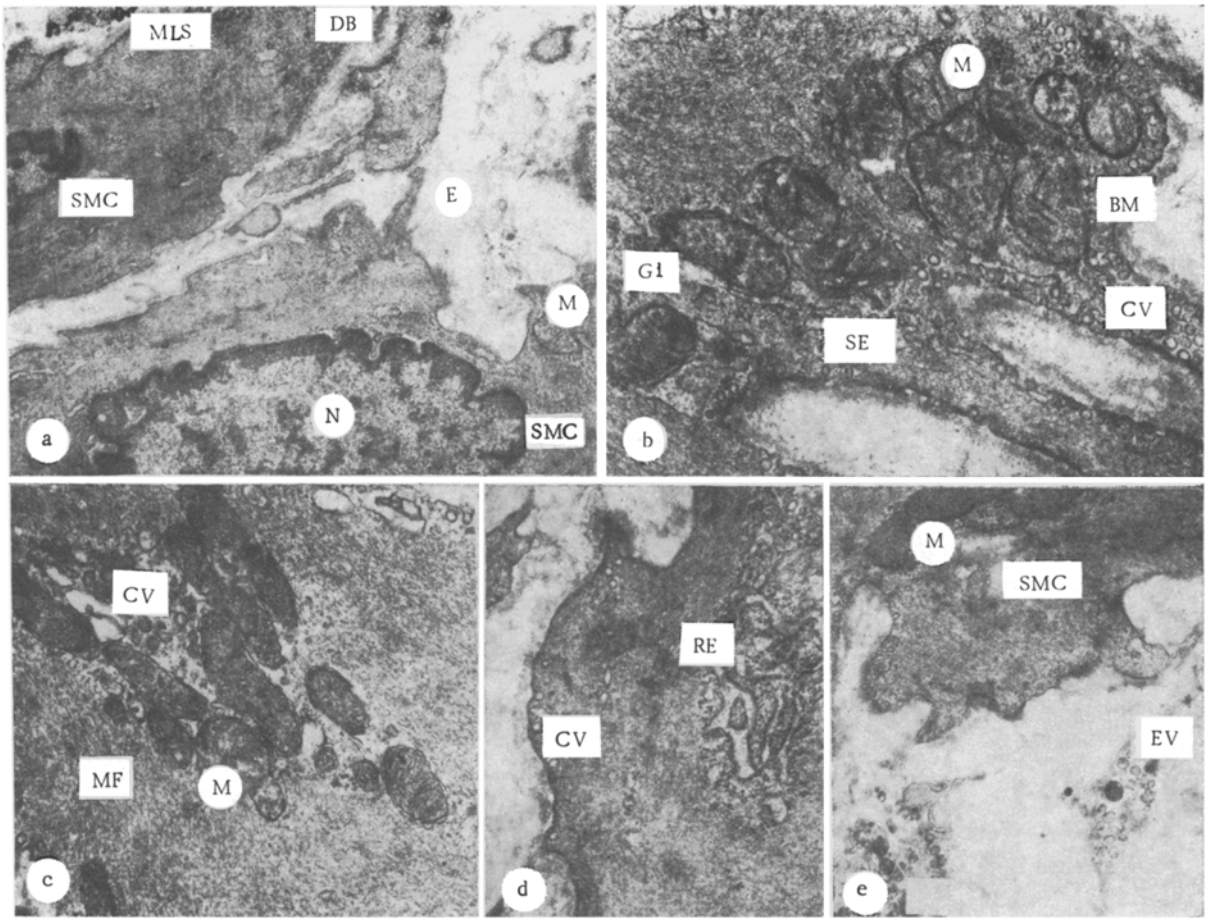


Fig. 1. Ultrastructure of myocytes in tunica media of femoral artery of control rats. a) SMC and elastic fibers; b) type I mitochondria; c) fragment of SMC with type II mitochondria; d) rough endoplasmic reticulum in perinuclear zone of SMC; e) accumulation of extracellular vesicles. N) Nucleus; M) mitochondrion; BM) basement membrane; E) elastic fiber; CV) cytoplasmic vesicles; SE) smooth endoplasmic reticulum; RE) rough endoplasmic reticulum; Mf) myofilaments; EV) extracellular vesicles; DB) dense bodies; Gl) glycogen; MLS) myelin-like structures. Magnification: a) 8640, b) 23,880, c) 20,400, d) 17,460, e) 14,400.

age served as the control. The animals were decapitated under ether anesthesia. The femoral arteries were removed and fixed in Karnovsky's fluid at room temperature, gradually cooled to 4°C, fixed for 3-4 h, washed with 0.1 M phosphate buffer, postfixed with 1% OsO_4 solution in Millonig's buffer, dehydrated, and embedded in Epon in the usual way [11]. Sections 40-60 nm thick, cut on the UMTP 5 ultramicrotome, were stained with uranyl acetate and lead citrate by Reynolds' method, and examined in the EMV-100B microscope. Electron micrographs were used to count the number of cristae and to measure the mitochondria by a gravimetric method [10] in an area of cytoplasm of the SMC equal to 400 μ^2 in both control and experimental groups. Distribution curves of the mitochondria by number of cristae and area of cross section in the intact and experimental animals were compared by the Smirnov–Kolmogorov test [3]. Morphometric data were used to calculate the coefficient of energy efficiency of the mitochondria (CEEM) [8]:

$$\text{CEEM} = \frac{\text{Total number of cristae of mitochondria in experiment}}{\text{Total number of cristae of mitochondria in control}} \times \frac{\text{total area of mitochondria in experiment}}{\text{total area of mitochondria in control}} \times 100\%.$$

EXPERIMENTAL RESULTS

The tunica media of the femoral arteries of intact animals consisted of long and frequently branching SMC, between which were many elastic fibers and also collagen fibrils and

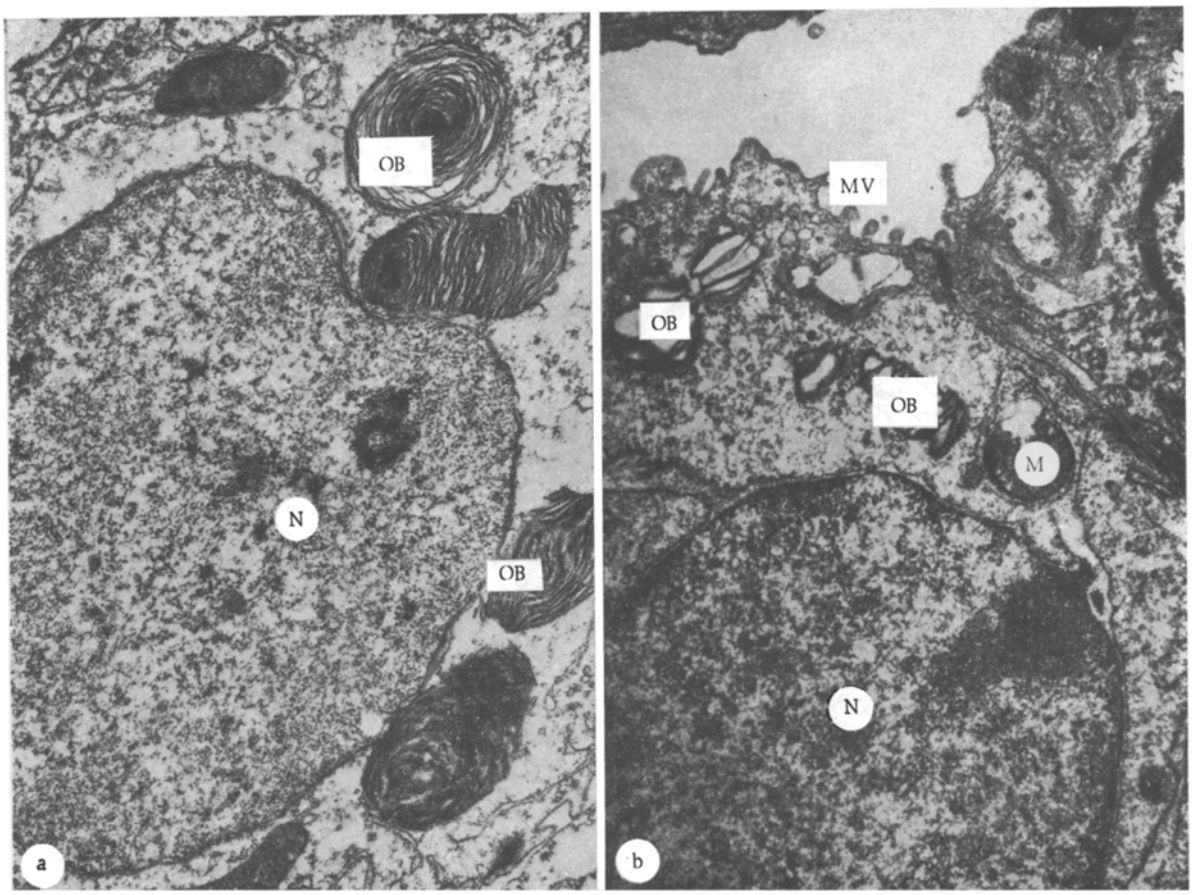
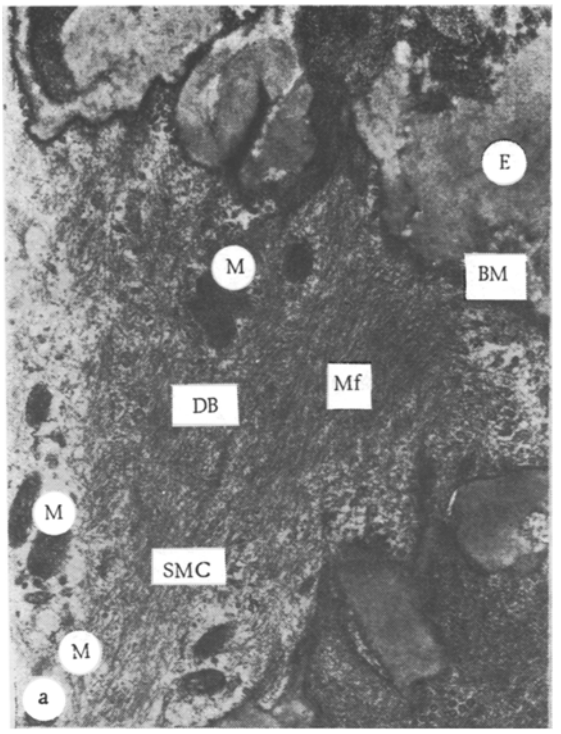
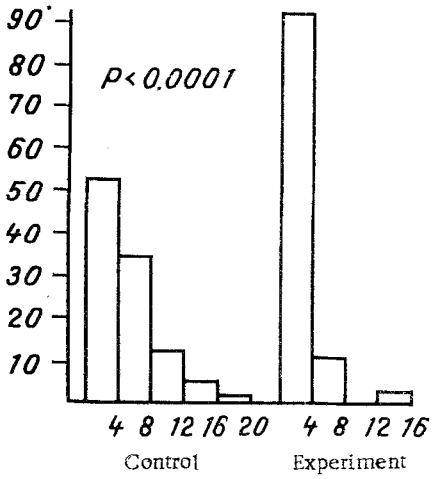


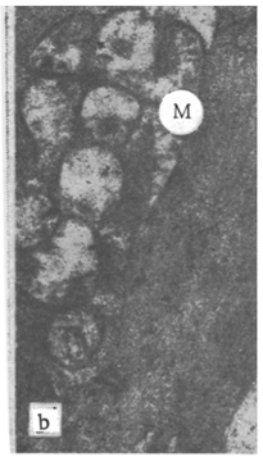
Fig. 2. Ultrastructure of myocytes in tunica media of femoral artery of experimental rats. a, b) Pale SMC with different degrees of myofilament disintegration; c) dark SMC with concentration of destroyed mitochondria; d) destruction of myofilaments, clearing of cytoplasm in subsarcolemmal zone of SMC. IEM) Inner elastic membrane; Mt) microtubules. Magnification: a, b) 11,760, c) 8640, d) 17,460. Remainder of legend as to Fig. 1.

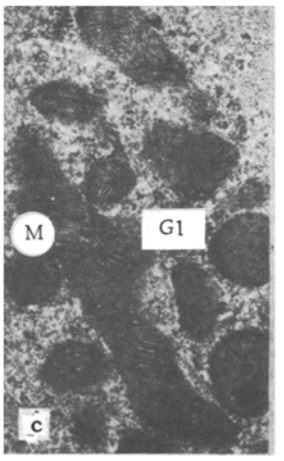
racemose collections of extracellular vesicles. The bodies and processes of the myocytes were covered by a loose basement membrane. The structure of SMC (Fig. 1) as a whole agreed with that given in the literature [6, 13, 14], but the method of fixation used did not preserve the thick myofilaments, and the contractile apparatus of the cells had the appearance of bundles of thin myofilaments and dense bodies. The heterogeneity of the mitochondria of the myocytes in the normal femoral artery was noteworthy: some mitochondria (type I) were oval, or sometimes irregular in shape, with a pale matrix and with cristae pointing in different directions, others (type II) had an electron-dense matrix and curved cristae. Usually the mitochondria of each type in a muscle cell formed homogeneous concentrations near the nuclei and at the periphery; the perinuclear zone, moreover, was more frequently the site of pale mitochondria, whereas both pale and dark types were found in the processes (Fig. 1b, c).

SMC with different morphology were found in the tunica media of the femoral arteries of animals exposed to vibration. Besides myocytes with a near-normal structure, pale and dark cells could be seen in the tunica media. Pale SMC were swollen, with many branches resembling broad paddles, and with a dense plasma membrane. Their cytoplasm was filled with fragments of destroyed myofilaments, among which microtubules, cytoplasmic vesicles, and mitochondria were distinctly visible (Fig. 2a, b). Only some cells were involved in the destructive process, and destruction of the myofilaments took place particularly often in the subsarcolemmal zone. Myocytes with local disintegration of the myofilaments and with clearing of the cytoplasm in the cell body or in its processes also were found (Fig. 2d; Fig. 3a). The plasma membrane of these cells was torn in some areas and the space between SMC was filled with a loose, structureless mass formed from remnants of organelles and myofilaments. Long-term ex-









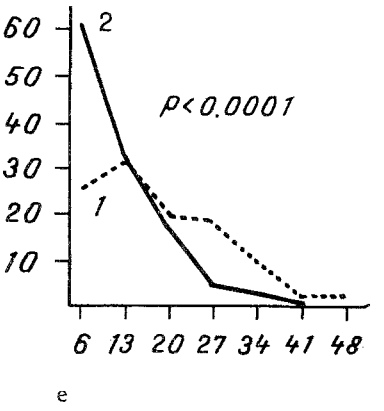


Fig. 3. Changes in structure of mitochondria (M) of SMC following exposure to vibration. a) Mitochondria with electron-dense matrix and ruptured membranes; b) swelling of mitochondria, fragmentation and destruction of cristae; c) mitochondria with osmiophilic matrix, many cristae, and rupture of inner and outer membranes. Reduction in number of cristae (d) and area of section of mitochondria (e) following exposure to vibration. Magnification: a) 17,460, b) 20,400, c) 26,250. Remainder of legend as to Fig. 1.

posure to vibration led to amalgamation of the intercellular matrix with the extracellular vesicles, characteristic of the blood vessel wall of healthy young animals with active morphogenetic processes, connected with growth of the body, and animals with modification of the physiological parameters of the blood flow under experimental conditions [4].

Among the myocytes of the femoarl arteries in the rats exposed to vibration, individual dark cells were found whose high electron density was evidently due to melting of the myofilaments and coagulation necrosis of their cytoplasm. Nuclei, concentrations of mitochondria at different stages of destruction, and small vacuoles could be seen in the dark cells. Cytoplasmic vesicles, the endoplasmic reticulum, glycogen granules, and other organelles were absent, and the basement and plasma membranes could not be distinguished over the greater part of the cell surface (Fig. 2c).

Particularly marked changes following exposure to vibration were observed in the mitochondria of SMC. Both in pale SMC and in cells with no signs of injury to the myofilaments, the mitochondria had an osmiophilic matrix, partly or completely destroyed inner and outer membranes, and a few cristae (Fig. 2a; Fig. 3a). Mitochondria of dark SMC were very swollen, with a translucent or faint matrix, fragmented cristae, and destroyed outer and inner membranes (Fig. 3b). According to the morphometric data CEEM of the experimental animals was down to 10% because of the appearance of numerous small forms and because of reduction of the number of cristae (Fig. 3d, e). It must be mentioned in particular that cigar-shaped, branched, and round mitochondria with an electron-dense matrix and with many curved cristae were found in the SMC of the experimental rats (Fig 3c). They differed from normal mitochondria in the partial destruction of their outer membranes, but nevertheless their state was closely similar to the condensed configuration of these organelles that corresponds to active ATP production [1]. The appearance of such mitochondria was probably connected with partial compensation of the energy deficit of the cell arising on account of disturbance of oxidative phosphorylation processes [9] and of the structural integrity of the membranes following exposure to vibration.

Analysis of the results of this investigation shows that following exposure to general high-frequency vibration changes are observed in the ultrastructure of cells in the tunica media of the femoral arteries of rats. The SMC swell, myofilaments and plasma and mitochondrial membranes are ruptured and destroyed, and the energy supply to the cells falls. Individual myocytes undergo coagulation necrosis, and the number of extracellular vesicles in the intercellular substance falls considerably. The phenomena described may be the result of the direct harmful action of mechanical oscillations, and may also reflect disturbances of the trophic influence of the nervous system due to damage to the central and peripheral nervous system in vibration sickness.

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