ULTRASTRUCTURAL CHANGES IN THE MUSCLE CELLS DURING VENTRICULAR FIBRILLATION

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Specific changes in the muscle cells of the subepicardial and subendocardial layers of the myocardium arising during ventricular fibrillation were studied in experiments on rabbits. Parallel polarization and electron-microscopic investigations showed that hypercontractures of individual groups of sarcomeres are formed in the damaged cells along the course of the myofibrils. These "subsegmental contractures" are regarded as the result of discoordination between contractions of the sarcomeres within the muscle cell arising during fibrillation.

In previous publications describing the results of a study of the morphological manifestations of ventricular fibrillation the writers describe distinctive changes in the muscle cells of the subepicardial and subendocardial layers of the ventricular myocardium [3, 5, 7]. These changes were found by polarization and phase-contrast microscopy and consisted of disturbance of the cross-striation within the formation of disks and masses of anisotropic material of the myofibrils. In the phase-contrast microscope fine granules could be seen in the spaces between the masses. A combination of these changes with the specific localization of changes in the outer and inner layers of the myocardium and the constancy with which this group of findings appeared in fibrillation was considered to merit a pathological study as a possible diagnostic sign of the state of ventricular fibrillation.

This paper describes the results of parallel polarization and electronmicroscopic investigations of muscle cells damaged by fibrillation.

EXPERIMENTAL METHOD

Experiments were carried out on male chinchilla rabbits weighing 2.5-3.5 kg. All the animals were first anesthetized by intravenous injection of 5% Nembutal solution (25 mg/kg body weight). Ventricular fibrillation was induced by the action of the main electricity supply [5]. The course of the experiments was recorded as the ECG. Material for electron-microscopic investigation was taken from eight rabbits dying from ventricular fibrillation. Difficulties connected with the obtaining of specimens containing the damaged muscle cells to be investigated were overcome by the use of a method of formalin fixation and osmium prefixation [11] and by carrying out the polarization microscopy in stages. After complete cessation of the fibrillary contractions the heart was removed, washed with cold phosphate buffer, pH 7.2, and fixed in 10% formalin solution in the same buffer, containing 4.5% sucrose. After fixation for 18-20 h, sections were cut on a freezing microtome (using solid carbon dioxide) to a thickness of 30μ. For this purpose the left ventricle and the ventricular septum were excised so that the outer and inner layers of the myocardium were cut longitudinally. The frozen sections were rinsed three times with 7.5% sucrose and fixed with Caulfield's osmium fixative [10]. The sections were then dehydrated in alcohols of increasing concentration. They were taken one by one from the 70° alcohol on slides and examined under low power of the microscope in

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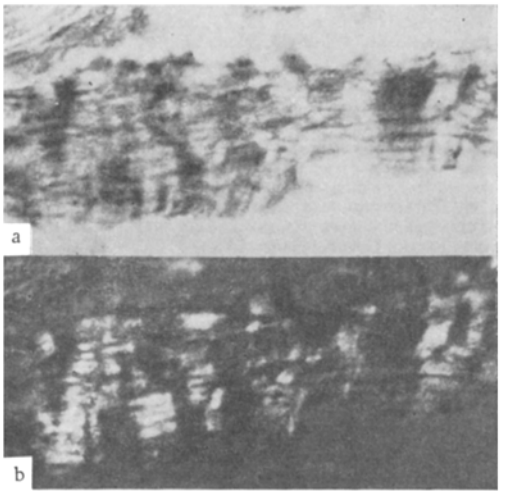

Fig. 1

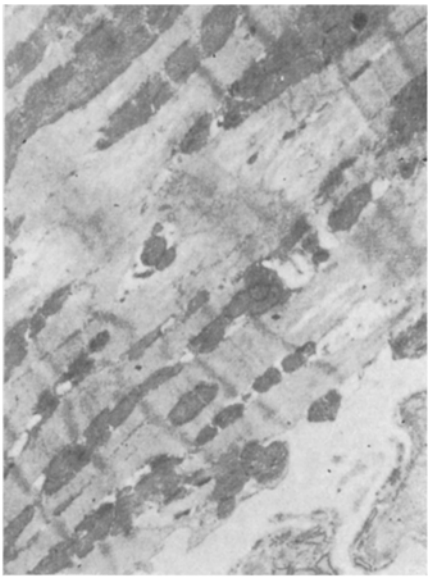

Fig. 2

Fig. 1. Myocardium of rabbit dying from ventricular fibrillation. Muscle fiber beneath epicardium. Reaction for succinate dehydrogenase: a) diformazan granules in damaged fiber arranged as patches and bands, 1250 ×; b) the same in polarized light. Areas without enzyme activity are masses of anisotropic material.

Fig. 2. Subsegmental contractures in a damaged muscle cell of the subendocardial layer of the left ventricle. Stepwise arrangement of hypercontractures of several sarcomeres along the course of the myofibrils. Sarcomeres adjacent to zones of contraction are stretched, 14,000 ×.

polarized light. The group of muscle cells of the outer and inner layers, in which changes in the myofibrils characteristic of the state of fibrillation were found, were cut out with a razor. The excised areas of the sections were placed for 25 min in 96° alcohol, passed three times through absolute alcohol, and embedded in a mixture of methacrylates (1:6). Before the ultrathin sections were cut on a Tesla BS = 490A ultratome sections 1μ in thickness were obtained and examined and photographed in polarized light. The subsequent ultrathin sections, 600-900 Å in thickness, were stained by Reynolds' method [15] and examined in the Tesla BS = 513 electron microscope with an accelerating voltage of 80 kV. In addition, cryostat sections 5 μ in thickness were cut from material frozen in liquid nitrogen for the reaction for succinate dehydrogenase [9].

EXPERIMENTAL RESULTS

Deposition of diformazan granules in the spaces between the contracted segments of the myofibrils was observed in the injured cells of the subepicardial and subendocardial layers after the reaction for succinate dehydrogenase (Fig. 1).

The electron-microscopic study of changes in the muscle fibers in the myocardial layers described showed that the changes occurring during fibrillation affect mainly the myofibrils. These changes consisted of the formation of irregularly arranged pinhead thickening along the course of individual myofibrils. The ultrastructure of the sarcomeres in the thickenings was indistinguishable, and only alternate darker and lighter transverse bands could be seen (Fig. 2). According to some investigators [13, 14, 16] this picture corresponds to hypercontracture of the myofibrils. The dark, indistinct bands are formed as the result of hypercontractures at the level of the Z-bands, so that the number of locally contracted sarcomeres could be determined. The continuity of the myofibrils remained intact, and in polarized light the stretched sarcomeres adjacent to the areas of contractions gave a picture resembling "cloudy swelling" of the myofibrils. Sometimes cells were found in which some sarcomeres of all myofibrils were sharply contracted at about the same level, and under the light microscope this corresponds to the picture of alternation of areas of "condensation" and "rarefaction" [11].

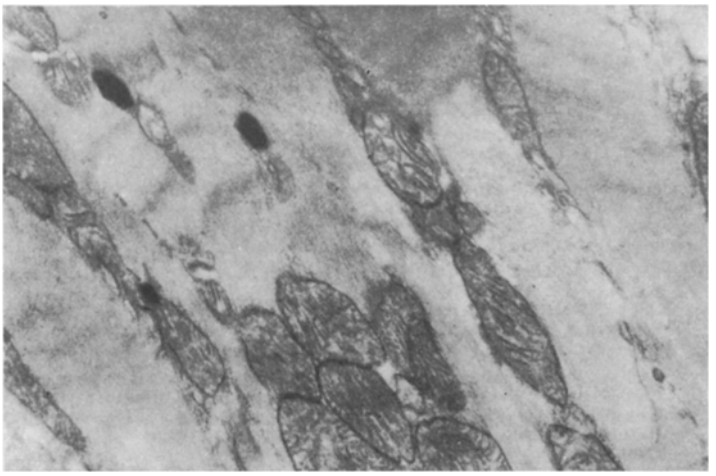

Fig. 3. The mitochondria are displaced in a cell with subsegmental contractures. Dark, indistinct bands are visible in the regions of hypercontractures at the site of the Z bands, 26,000 ×.

In cells with multiple areas of contraction of the myofibrils there were no significant changes in the mitochondria: the outer and inner membranes were clearly outlined in them, the matrix was of uniform density, although because of the formalin fixation used it appeared denser [4]. The localization of the mitochondria was altered in the damaged cells: they were displaced into the spaces between the areas of uncontracted myofibrils (Fig. 3). Comparison of this phenomenon with the results of the reaction for succinate dehydrogenase explains the localization of activity of this enzyme in the spaces between the "masses" of anistropic material.

The results of both polarization and electron microscopy thus show that the changes in the muscle cells of the subepicardial and subendocardial layers arising in ventricular fibrillation are based on contracture of individual sarcomeres of the myofibrils. In foci of metabolic injuries segmental contractures, i.e., contractures affecting the whole muscle segment (muscle cell), are more frequently observed [6, 8]. In fibrillation, however, the contractures are subsegmental in character: a sharp contraction of individual sarcomeres or groups of them is found, whereas other sarcomeres are in a state of moderate contraction or, on the contrary, are stretched. This picture can arise as the result of excitation of individual sarcomeres at different times or to different degrees. The possibility of isolated contractions of individual sarcomeres was demonstrated by the well known experiments of Huxley and Taylor [12]. The appearance of subsegmental contractures evidently indicates a disturbance of the spread of the excitation wave in the muscle cell. This suggests that the discoordination of the contractions characteristic of fibrillation may be manifested not only at the level of dissociation of contractions of individual muscle cells, but also within the cells themselves as discoordination of contractions of individual sarcomeres. The mechanisms of this phenomenon are not clear, like the mechanism of ventricular fibrillation itself. The reasons why injuries in the form of subsegmental contractures are observed mainly in the subepicardial and subendocardial layers likewise had not been established. The fact that these layers are exposed to the severest load may be significant in this connection. It must also be remembered that the subepicardium and subendocardium of the ventricles are in fact part of the same muscle. The bundles of this muscle form the longest pathway along which excitation can circulate in the myocardium and, as some investigators have pointed out [2], the adequate length of this pathway is considered to be one of the most important conditions in the pathogenesis of ventricular fibrillation.

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