EFFECT OF STROPHANTHIN ON THE DEVELOPMENT OF ACUTE MYOCARDIAL ISCHEMIA AS SHOWN BY LUMINESCENCE-HISTOCHEMICAL METHODS

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The morphological changes in the zone of ischemia, as revealed by the usual histological methods, have been fully, studied by many authors [9, 3, 17]. The histological changes produced by cardiac glycosides have also been studied [1, 5, 7, 10, 13].

However, no references could be found to the investigation of the histochemical changes in the zone of ischemia during experimental myocardial infarction developing following administration of strophanthin, nor has the pattern of the changes in the blood supply to the myocardium as revealed by luminescence microscopy yet been described. Because of the importance of these questions for the analysis of the action of strophanthin on the heart in the conditions of acute myocardial ischemia, the present investigation was undertaken.

EXPERIMENTAL METHOD

An experimental myocardial infarct was produced by ligating the descending branch of the left coronary artery through the open chest, under hexobarbital anesthesia, with artificial respiration. Experiments were carried out on 60 cats, in 35 of which the artery was ligated (controls), while in the other 25, after ligation of the artery, strophanthin was injected in a therapeutic dose of 0.2 cat unit/kg. The animals were sacrificed at various times after ligation of the artery: from 15 min to 48 h. The action of strophanthin was analyzed by a luminescence method of intravital definition of the boundaries of the zone of ischemia in the myocardium by means of uranin, injected in a dose of 30 mg/kg into the animal's femoral vein 5 min before extraction of the heart. The dynamics of uranin distribution were investigated from luminescence of the uranin fluorochrome in ultraviolet light.

For histological and histochemical analysis, the heart was fixed in Carnoy's fluid, absolute alcohol, and neutral formalin, and then embedded in paraffin wax by the usual method. Besides the ordinary histological stains, the following histochemical reactions were used: the PAS reaction and staining with toluidine blue to detect polysaccharides, the reactions of Brachet and Feulgen to detect nucleic acids, and control treatment of the sections with ribonuclease, lipase, and diastase.

EXPERIMENTAL RESULTS

One hour after ligation of the coronary artery the zone of ischemia occupied the whole of the anterior wall of the left ventricle and had partly spead to half the interventricular septum. Microscopic investigation revealed edema of the myocardial stroma, and here and there tiny hemorrhages were observed around the vessels. Glycogen had almost completely disappeared from the anterior wall of the left ventricle.

One hour after injection of strophanthin into animals with a myocardial infarct, the nonluminescent dark zone of ischemia was much smaller than in the control, and clearly demarcated from the epicardium and endocardium by the luminescent myocardium (Fig. 1). Histological investigation also revealed edema of the myocardial stroma, but it was less marked than in the control. The zone of disappearance of glycogen was much smaller than in the control, it was intramural in position and microfocal in character (Fig. 2).

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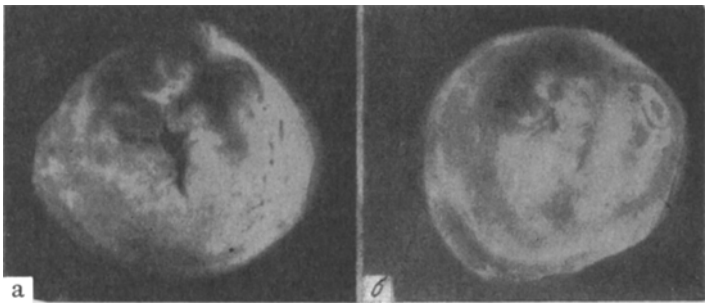

Fig. 1. Zone of ischemia of the myocardium (dark color) 1 h after ligation of the coronary artery. a) Control; b) after injection of strophanthin. Luminescence analysis.

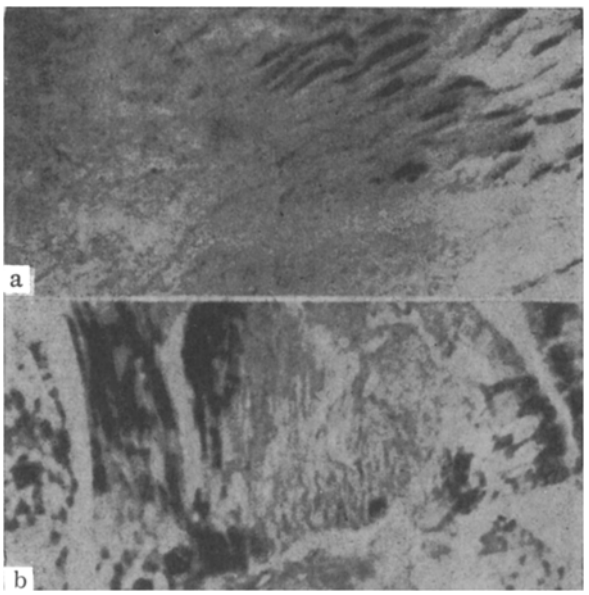

Fig. 2. Glycogen in the zone of ischemia in the myocardium 1 h after ligation of the coronary artery. a) Control; b) after injection of strophanthin. PAS reaction. 25 × 6.

Six hours after ligation the zone of ischemia occupied the anterior wall of the left ventricle, but in contrast to the preceding period, it showed a tendency to diminish in size, with the appearance of a brighter luminescent band on the sides facing the endocardium and epicardium. The zone of ischemia itself began to luminesce weakly. Histological investigation of transverse histotopographical sections showed muscle fibers in the region of the anterior wall of the left ventricle which stained more intensively by the usual methods with eosin, fuchsin, and picric acid. In histochemical reactions these muscle fibers showed weak pyroninophilia and a PAS-positive reaction. This effect was not removed by preliminary treatment with the appropriate anzymes. In Feulgen's reaction for DNA, vacuolation of the nuclei was observed in the degenerating muscle fibers.

At the same period, after injection of strophanthin, the zone of ischemia in the transverse section was visible as a small, oval lesion, in the region of the anterior wall of the left ventricle, giving weak luminescence. It was much smaller than in the control and consisted of tiny dark-colored spots. On histological investigation, signs of changes in the staining properties of the cytoplasm appeared in certain fibers; staining with eosin revealed eosinophilia, Brachet's reaction—pyroninophilia, and staining for glycogen—PAS-positive material. In contrast to the controls, only in single muscle fibers were changes observed in the staining properties. Leukocytes appeared around these muscle fibers.

Luminescence analysis 24 h after ligation showed that the zone of ischemia, which had been dark during the first first few hours after ligation, now gave off a bright luminescence, in sharp contrast to the weak luminescence of the background of the remaining myocardium, and it occupied the whole anterior wall of the left ventricle. In histotopographical sections, muscle fibers were clearly seen in this zone which exhibited pyroninophilia and a PAS- positive reaction. The nuclei of the muscle fibers were pycnotic, and in some places could not be detected. No RNA could be detected in the nuclei of the muscle fibers. At this time well marked edema of the stroma was observed, here and there with perivascular hemorrhages and definite metachromasia (staining with toluidine blue). On impregnation with silver by Foot's method, thickening and, in some places, fragmentation of the argyrophilic fibers were seen in the zone of ischemia. Glycogen remained only in the muscle layer, immediately next to the endocardium.

After injection of strophanthin, examination in ultraviolet light at the same period showed that the zone of ischemia was bounded by a well defined luminescent barrier and the luminescence of the zone

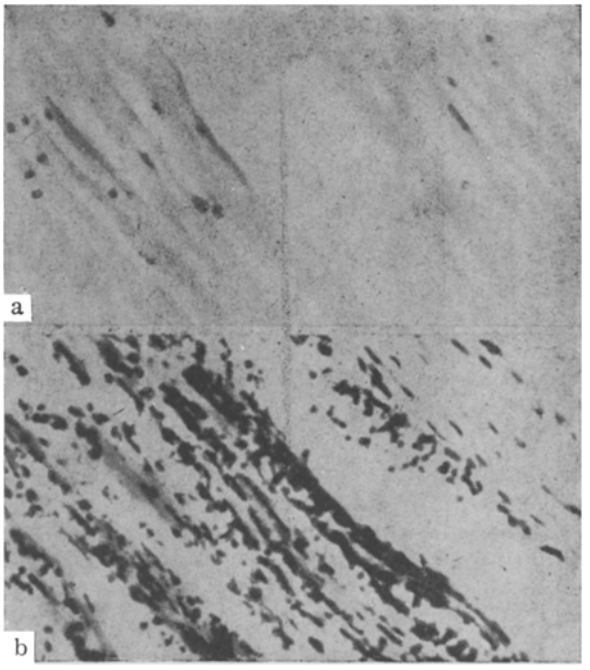

Fig. 3. Necrosis of muscle fibers 24 h after ligation of the coronary artery. a) Control; b) after injection of strophanthin. Stained with toluidine blue. 10 × 6.

itself was weaker than in the control. In histotopographical sections stained by the ordinary methods, small scattered areas of necrotic muscle fibers could be seen beneath the epicardium and also in the central part of the anterior wall of the left ventricle. Characteristically, the conducting system retained granules of glycogen, but deeper to it was a zone of muscle fibers without glycogen, in which only isolated groups of muscle fibers with a marked PAS-positive reaction could be seen (Fig. 3). Examination after 48 h revealed the same as 24 h afer ligation, but they were more marked. In histotopographical sections the necrotic muscle fibers in the control were seen to occupy the whole anterior wall of the left ventricle, but in the experiments with injection of strophanthin the foci of necrosis in the anterior wall of the left ventricle were microfocal in character.

The results obtained are in agreement with those described by other authors [6, 8, 11, 14].

The decrease in size of the zone of ischemia of the necrotic foci under the influence of strophanthin may evidently be attributed to improvement in the blood supply to the myocardium [2, 4, 5, 15] and to the restoration of its normal nutrition.

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