SEEPAGE OF PLASMA INTO MYOCARDIAL CELLS IN ACUTE METABOLIC DISORDERS

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Using adrenalin-induced myocarditis in albino rats as the model, seepage of blood plasma into focal lesions of the myocardium was studied. Immunotopographic, histochemical, optical polarization, and phase-contrast methods of investigation were used. Plasma seepage into muscle cells was revealed in the late stages of contracture; it accompanied coagulation necrosis. Plasma seepage was absent in myocytolysis and primary colliquative necrosis.

Studies showing that in degenerative and necrobiotic processes seepage of blood plasma can take place into the heart muscle fibers have recently been published [1, 3, 4, 15].

Experiments [5-8, 10] have shown that necrobiotic and degenerative changes in muscle cells in metabolic disorders of the myocardium may differ in their mechanisms and morphological manifestations. The use of polarization and phase-contract microscopy enabled the authors cited to detect two types of injury to the myocardial cells; contracture, leading to coagulation necrosis, and myocytolysis, leading to colliquative necrosis. In both types of injury the process is reversible in the early stages, i.e., the muscle cells can recover completely.

The object of this investigation was to determine in what types and at what stages of metabolic disorders seepage of plasma takes place into the heart muscle cells, a factor of importance to the elucidation of the role of this process.

EXPERIMENTAL METHOD

Metabolic injury to the myocardial cells was obtained by means of the widely used experimental model of adrenalin myocarditis.

The occurrence of plasma seepage was determined by treatment of histological sections with rabbit γ-globulin against rat plasma, labeled with fluorescein isothiocyanate.

Experiments were carried out on 25 male albino rats weighing 250-300 g, which were given a single subcutaneous injection of adrenalin (6 mg/kg body weight). At intervals between 5 min and 4.5 h after injection of adrenalin 5 rats were sacrificed; the remaining animals were decapitated 30 min, and 1, 1.5, 3, 5, 6, 12, 24, and 48 h later. The hearts of two intact rats were used as controls.

The heart was removed from the thorax, frozen in liquid nitrogen, and cut into sections in a cryostat; the sections were fixed in alcohol and treated with antiplasma γ-globulin in a moist chamber at 37°. The PAS reaction after treatment with amylase, Adams's reaction for tryptophan, and staining with hematoxylin-eosin were carried out on parallel sections. All sections were examined under the microscope in ordinary light, polarized light, and phase contrast. Sections treated with labeled γ-globulin were examined in the light of luminescence excited by the blue-violet part of the spectrum. The light source was a type NVO-50 mercury vapor lamp, with a Schott BG-12 filter and an OG-1 cutoff filter.

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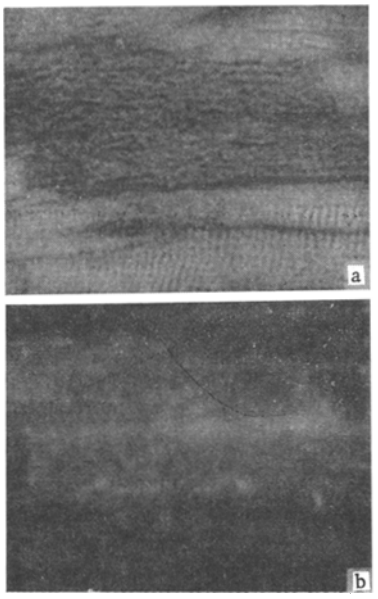

Fig. 1

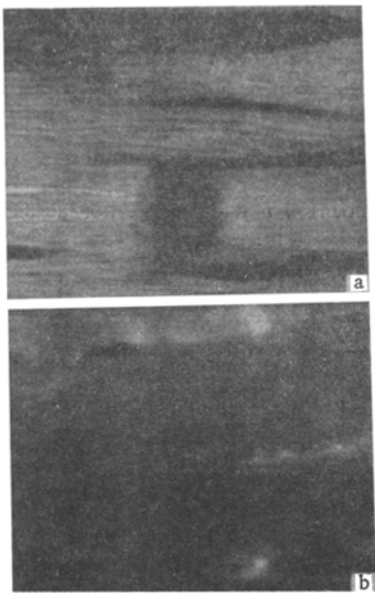

Fig. 2

Fig. 1. Contracture injuries of muscle fibers with coagulative necrosis of the anisotropic substance (section treated with labeled antiplasma γ-globulin). a) In polarized light; b) in luminescence light: luminescence of plasma seeping into injured fibers, 800×.

Fig. 2. Myocytolysis (section treated with labeled antiplasma γ-globulin). A) In polarized light, anisotropy has disappeared in focus of injury; luminescence of plasma in vessels, 800×.

Antisera against rat plasma were obtained by immunizing chinchilla rabbits. The antibody titers were determined by Boyden's indirect hemagglutination test [2]. The sera used had an antibody titer of not less than 1 : 1280. γ-globulin was isolated by the rivanol method [14] and labeled with fluorescein isothiocyanate by Coons's method [12, 13].

EXPERIMENTAL RESULTS

Two types of changes were found in the myocardium of the experimental animals: contracture injuries and myocytolysis, and the dynamics of their development and their morphology corresponded to those described previously [6].

In all sections treated with antiplasma γ-globulin bright luminescence of the plasma in the vessels was observed. In animals sacrificed 3 h after injection of adrenalin, specific luminescence appeared in individual muscle segments in sections treated with serum. When these same segments were examined in polarized light and phase contrast, as a rule a picture of coagulation necrosis of the anisotropic substance was found (Fig. 1). Corresponding segments gave a positive PAS reaction, and Adams's reaction for tryptophan was intensified. In early contracture changes, manifested only by increased anisotropy and preservation of cross striation, as a rule these reactions were negative. These segments likewise did not show specific luminescence on treatment with antiplasma γ-globulin, but they gave a weak increase in autoluminescence, unconnected with seepage of plasma and detectable only in the control sections untreated with γ-globulin. This phenomenon has been attributed by some workers [9] to an increase in the rigidity of the structures during contracture injuries (nonspecific autoluminescence, in contrast to luminescence of structures binding labeled γ-globulin, does not show up during the action of the exciting light).

Previous investigations [6, 10, 11] showed that coagulation necrosis of the anisotropic substance takes place in the irreversible stage of contracture injury and is, in fact, a necrobiotic change. In some cases seepage of plasma into segments with coagulation necrosis of the myofibrils was also observed in earlier stages – 1-2 h after injection of adrenalin. Cells with coagulation necrosis of the anisotropic substance and

revealing no evidence of plasma seepage were found at these same times. This shows that plasma seepage does not proceed necrobiotic changes in the cell, but is combined with them and is not the cause but the result of necrobiotic changes in the heart muscle cells.

In the later observations (from 6 h to 2 days), when a zone of infiltration is formed around the cells undergoing coagulation necrosis, and necrotic segments are broken up, signs of plasma seepage (specific luminescence on treatment with antiglobulin, PAS reaction) persist in the remnants of the necrotic segments until they undergo complete resorption. During the formation of granulomas at the site of the necrotic foci, diffuse plasma seepage into the granuloma territory is observed.

The second type of injury to the heart muscle cells – myocytolysis – is never accompanied by plasma seepage (Fig. 2). This also applies to cases when myocytolysis is progressive and the damaged cell undergoes colliquative necrosis.

The connection between plasma seepage and the contracture type of injury, and its absence at all stages of myocytolysis, including during colliquative necrosis, confirm the hypothesis [7] that during contracture changes it is the function of the cell membrane which is primarily affected, while myocytolysis and colliquative necrosis are associated with a lysosome effect and with preservation of the intact cell membrane. The hypothesis [4] that plasma seepage is typical of all forms of necrosis developing in areas of tissue bathed with blood is valid only for coagulation necrosis and not for necrosis of colliquative type (this does not refer to the so-called secondary colliquation, which is essentially not a type, but an outcome of necrosis).

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