ULTRASTRUCTURAL CHANGES IN THE MUCOSA OF THE GALL BLADDER DURING TEMPORARY EXPERIMENTAL ISCHEMIA

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Temporary ischemia of the gall bladder was produced in rabbits by application of a silk ligature to the cystic artery. Histological examination revealed vascular disturbances, consisting of hyperemia, stasis of blood, and focal hemorrhages. Electron-microscopic investigation showed an increase in the number of dark epithelial cells, widening of the intercellular space, and loosening of the structure of the basement membrane with the formation of defects in it and invagination of epithelial cells into the submucosa. The most marked changes were found after occlusion of the cystic artery for 30 min on three occasions. The severity of the destructive changes depend not so much on the duration of ischemia as on the number of occlusions.

KEY WORDS: gall bladder; ischemia; epithelial cells; cystic artery.

An important role in the development of cholecystitis is ascribed at the present time to circulatory disorders. In medical practice, cholecystitis is found to occur in patients with cardiovascular diseases [4, 10]. Marked destructive changes have been observed [6, 7] in the wall of the gall bladder 1-14 days after ligation of the cystic artery. Meanwhile, initial changes in the mucosa, manifested as a rule at the ultrastructural level during hemodynamic disorders, still remain inadequately explained.

The object of this investigation was to study the ultrastructure of the wall of the gall bladder during experimental ischemia.

EXPERIMENTAL METHOD

The cystic artery was occluded temporarily in 30 rabbits by means of a silk ligature. Depending on the number and duration of the occlusions the animals were divided into five groups: group 1) four occlusions each of 10 min, group 2) four each of 20 min, group 3) three each of 30 min, group 4) two each of 40 min, and group 5) two each of 50 min. The interval between occlusions was always the same, 10 min. The animals were killed 30 min after the last occlusion. Pieces of tissue were cut from the wall of the fundus and neck of the gall bladder and fixed for histological examination in 10% neutral formalin and for electron-microscopic examination in 1% OsO_4 solution by Caulfield's method. Electron micrographs were obtained with the UÉM-100-V electron microscope under a magnification of 10,000-100,000 ×.

EXPERIMENTAL RESULTS

On histological examination of the wall of the gall bladder destructive changes were found in the mucosa together with vascular disorders of varying severity. After short periods of ischemia (group 1) slight hyperemia of the vessels and edema were observed. In the animals of group 2, partial desquamation of the epithelium was seen. The most marked changes were found in the rabbits of group 3: extensive desquamation of the epithelium of the mucosa of the gall bladder in the region of the fundus and partial desquamation

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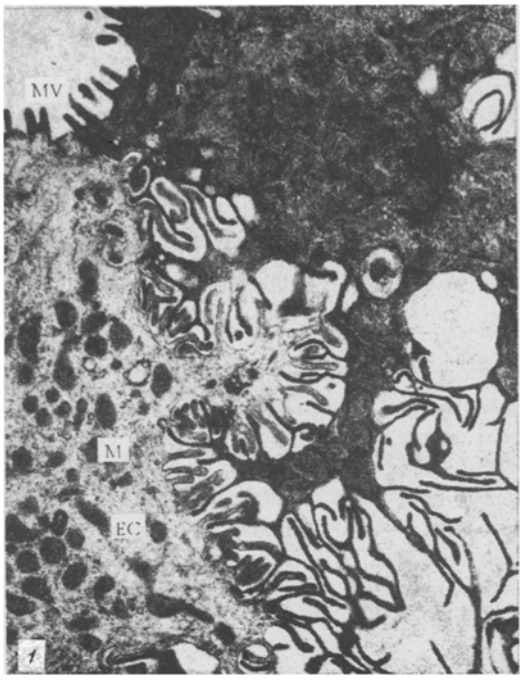

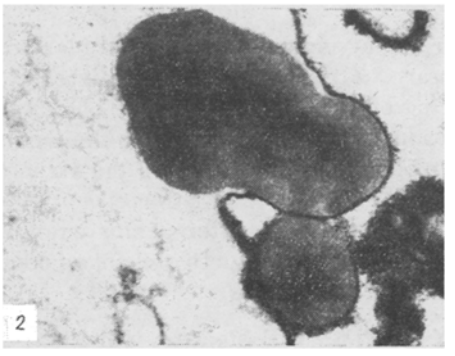

Fig. 1 Fig. 2

Fig. 1. Dark and pale epithelial cells of mucosa of gall bladder during tempory ischemia: EC) epithelial cell; MV) microvillus; M) mitochondrion; 20,000 ×.

Fig. 2. Myelin figures in cytoplasm of epithelial cells and in cavity of gall bladder 100,000 ×).

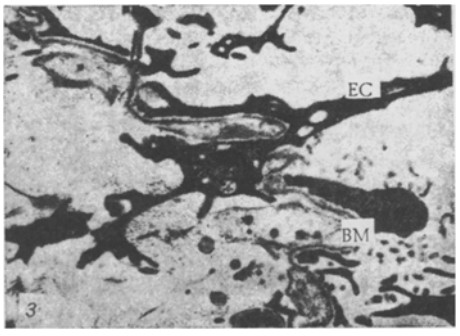

Fig. 3. Invagination of epithelial cells through defects in basement membrane: BM) basement membrane; EC epithelial cell; 20,000 ×.

in the region of its neck. In the animals of groups 4 and 5 the changes in the mucosa were less marked, but hemorrhagic foci were seen almost invariably.

The earliest and most constant features of electron-microscopic examination of the gall bladder wall were changes in the epithelial cells, which were similar in type after different periods of ischemia and differed only in their severity. In all groups the number of so-called dark cells was constantly increased by comparison with normal. The cytoplasm of these cells was extremely dense and osmiophilic and it contained many ribosomes and mitochondria. The mitochondria were enlarged and formed clusters in the perinuclear zone. The nuclei of the dark cells were reduced in size but had high electron density (Fig. 1).

In the pale epithelial cells the microvilli were lengthened to 2.5 μ and were deformed. Their cytoplasm after brief ischemia was osmiophobic; the organelles were arranged a considerable distance apart, indicating hydration of the cell. A noteworthy feature was variation in the degree of destruction of the mitochondria depending on the duration of ischemia and the frequency of occlusion of the cystic artery. With an increase in the duration of occlusion, the swelling and fragmentation of the mitochondria increased. However, with a decrease in the number of occlusions, despite a longer total duration of ischemia (100 min in group 5), these changes were less marked. The severest changes in the mitochondria were observed in group 3.

During ischemia of the gall bladder characteristic myelin figures were seen in the epithelial cells. Some were connected with mitochondria, others lay freely in the apical part of the cytoplasm. Some myelin figures were adjacent to the cytoplasmic surface membrane, causing it to bulge, or lay within the cavity of the gall bladder (Fig. 2).

Considerable widening of the intercellular space was a characteristic feature and sometimes the distance between the cells was 5-6 μ, evidence of marked hydration of the epithelial layer (Fig. 1). Electron-transparent material was contained in the widened intercellular space. The basement membrane of the epithelial layer had a more winding profile than normally. Loosening of its texture and an increase in its thickness to 500-600 nm, with ruptures and the formation of defects up to 1 μ in diameter were observed. Sometimes islands of cytoplasm of the epithelial cells penetrated into the submucosa through defects in the basement membrane. These cytoplasmic invaginations were observed as a rule where the capillaries of the submucosal layer were situated. The subbasal part of the epithelial cells differed from the suprabasal cytoplasm in its greater osmiophilia (Fig. 3).

During temporary occlusion of the cystic artery marked changes in the epithelial cells are thus observed in the wall of the gall bladder. The changes in the wall of the gall bladder at different times of ischemia indicate that the severity of the destructive lesions depends mainly on the number of stimulated spasms rather than on the duration of ischemia.

Early ischemia of the gall bladder is manifested as hydration of epithelial cells, which is evidently associated with hypoxia and increased vascular permeability and is expressed as increased translucency of the cytoplasm and swelling of the mitochondria. Similar changes have been observed in other cells in the early stages of myocardial ischemia [8] and in hypoxic hypoxia of the heart, lungs, pancreas [1, 11], and other organs. Destructive changes in the organelles are accompanied by disturbance of the normal function and a reduction in the concentrating power of the epithelial cells. Depression of the concentration function of the gall bladder during roentgenological investigation is observed in patients with cholecystitis [3] and in animals with experimental cholecystitis [5, 9]. Water, lipids, and other components of the gall bladder are known to be transported under normal conditions by epithelial cells from the cavity of the gall bladder into the blood vessels [2]. When the concentrating power is disturbed, fluid can accumulate in the cytoplasm of the epithelial cells, in the intercellular space, and in the subepithelial layer, increasing the hydration of the epithelium of the gall bladder wall. Considerable intercellular and subepithelial edema is accompanied by desquamation of the epithelium, as a result of which the way is laid open for invasion by microorganisms and for the toxic action of bile, pancreatic enzymes, and so on, so that the development of cholecystitis is promoted.

In some cases myelin figures were found in the cytoplasm of the epithelial cells. Myelin figures are considered to be formed by contact between phospholipids and an aqueous medium [12-14]. During hydration, favorable conditions may arise in the cytoplasm of the epithelial cells of the gall bladder for absorbed components of the bile (lipids, salts). Myelin figures, frequently located beneath the surface cytoplasmic membrane, frequently bud off and are liberated into the cavity of the gall bladder. Accumulations of myelin figures in the cavity of the gall bladder under certain conditions may provide the nucleus for stone formation and for the development of calculous cholecystitis.

Circulatory disorders, similar to those produced experimentally, can develop in patients as spasms or thromboses. The results of the investigation indicate that repeated disturbances of the circulation are one component in the complex pathogenesis of cholecystitis.

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