SCANNING ELECTRON MICROSCOPY OF CHRONIC EXPERIMENTAL GASTRIC ULCERS

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Scanning electron microscopy (SEM) has recently found wide application in the investigation of various biological objects [3], but the stomach has not yet been adequately studied by this method. There have been only isolated reports in which SEM of the normal gastric mucosa and of its changes in response to acute injury have been described [6-12].

EXPERIMENTAL METHOD

Experiments were carried out on 15 albino rats weighing 195-210 g in which chronic gastric ulcers were produced by the method of Okabe and Pfeiffer [14], by application of acetic acid to the serous membrane. The animals were killed 1 h, 10 days, and 3 and 7 months later. Under ether anesthesia laparotomy was performed and the stomach perfused through the aorta initially with phosphate buffer, pH 7.2, and later with 2.5% glutaraldehyde in the same buffer for 2 min. The stomach was filled with 2.5% glutaraldehyde and, after 2 h, it was washed in phosphate buffer, pH 7.2, for 5 min; pieces were then excised from the fundus of the stomach and from the floor and margins of the ulcer, and immersed in 2% osmium solution for 1.5 h, then washed twice or three times with the same buffer. Dehydration was carried out in 30, 50, 80, and 100% acetone for 1 h in each solution, and this was followed by freeze-drying in liquid nitrogen and by spraying with gold. Some of the material was broken in liquid nitrogen and the fracture surface was sprayed. Ordinary paraffin sections, glued to coverslips, also were sprayed with gold [5]. The material was studied in the HSM-2 Hitachi scanning electron microscope. Investigations were carried out in the electron microscopy laboratory (Head, Candidate of Technical Sciences B. N. Smirnov), All-Union Research Institute of Electrocarbon Products.

EXPERIMENTAL RESULTS

The surface of the gastric mucosa in the control animals was formed of closely packed swollen cells, creating a picture resembling paving with cobble stones [6]. The cells surrounded the orifices of the gastric pits concentrically and were round, oval, or slit-like in shape. Usually one pit opened into the orifice, but sometimes two pits shared a common orifice on the surface of the mucous membrane. A thin crest, lined with epithelial cells of slightly smaller volume than the surface mucocytes, was present in the depth of the orifice. If the block was tilted, the inner surface of the pits also could be seen. It was covered by flatter cells, laid one above the other, like tiles. Mucus could be seen in the form of small heaps and thin threads on the surface epithelium and in the lumen of the pits. Numerous short microvilli could be distinguished on the surface of the epithelium lining the rugae of the stomach. Meanwhile distinctive swellings (Fig. 1b), due to accumulation of mucus in the apical part of the cells, could also be seen. Pictures of this sort must be interpreted as the stage preceding extrusion of the secretion. Later the outer membrane ruptured and the secretion was set free. Under high power, granules of secretion could be seen beneath the torn outer membrane (Fig. 1c) in the apical part of the cells. Such pictures are rightly regarded as a reflection of microapocrine secretion, which has not previously been described in the epithelium of the stomach (which is characterized by merocrine secretion [4, 13]). To judge from the fact that signs of microapocrine secretion were comparatively rarely found, and that small pores on the surface of the cells (Fig. 1d) were a constant finding, it must be concluded that the basic type of liberation of secretion is merocrine.

On broken fragments of the mucous membrane fundal glands arranged perpendicularly to the surface and interwoven with a network of connective-tissue fibers and blood vessels could be seen. The densest network

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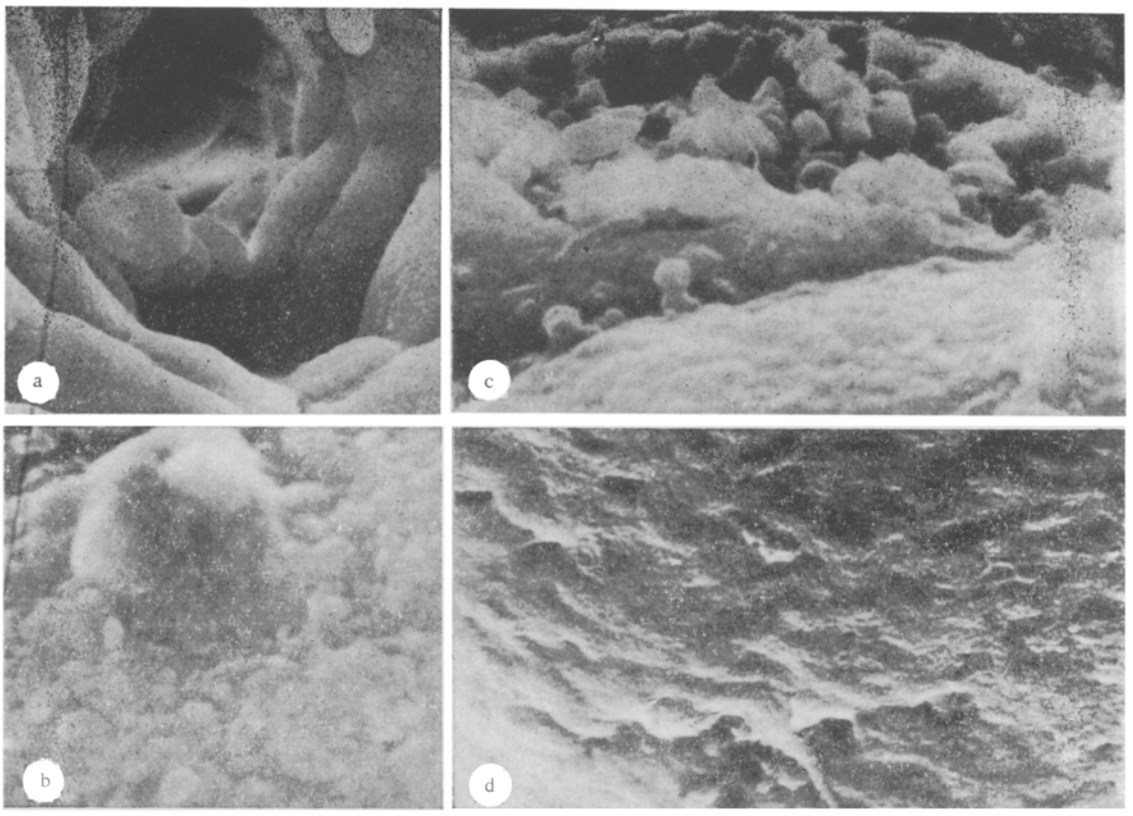

Fig. 1. Surface of normal gastric mucosa. a) Orifice of gastric pit: surface epithelium with clear cell boundaries, pile-like arrangement of mucocytes forming pits (3000×); b) microvilli and evagination of outer membrane of surface epithelium (20,000×); c) microapocrine secretion: outer membrane ruptured, granules of mucoid visible in apical part of cell (12,600×); d) micropores on surface of epithelium (21,100×).

of fibers and vessels was seen in the region of the neck of the glands (Fig. 2a), which they appeared to fix. Vessels were particularly numerous around the germinative cells located in the floor of the pits and around their neck. In this way their need for a rich blood supply was satisfied [11].

The surface of the mucosa 1 h after application of acid to the serous membrane of the stomach became uneven because of regions of hollows and evaginations. The cells were increased in volume, their microvilli were shortened, and they became almost indistinguishable. The intercellular spaces were greatly widened and in some places the cells were separated by wide spaces with uneven edges, crossed by individual cytoplasmic bridges (Fig. 2b). Widening of the intercellular spaces, although less marked, also is found in hypovolemic shock and after installation of acetylsalicyclic acid into the stomach [7]. The presence of such spaces disturbs the integrity of the mucous barrier, as a result of which conditions are created for the reverse diffusion of hydrogen ions. The surface of individual cells was destroyed and microerosions were formed (Fig. 2c). Breaking down of structural complexes of fundal gland cells could be seen on the fracture surfaces (Fig. 2d). At the periphery of the area of injury the fundal gland remained intact, but the intercellular junctions there were widened and individual cells were lost. The lumen of the glands appeared to be opened, its secretion could enter the tissue, and digestion of the mucous membrane within the tissue itself could arise. Confirmation of this mechanism was given by the distinctive wedge-shaped appearance of the necrosis (with the apex of the wedge facing the surface of the stomach), revealed by histological investigation [2], and reflecting the more intensive proteolysis in the depth of the mucosa. Histochemical study showed that the chief cells on the boundary with the zone of necrosis were secreting pepsinogen intensively. The necessary pH values to convert pepsinogen into pepsin are maintained by diffusion of H^+ through the intercellular spaces revealed by SEM.

After 7-10 days an ulcer morphologically similar to a human gastric ulcer had formed [14]. At the edges of the ulcer regeneration of the mucosa was observed: a layer of flattened epithelium crept over the defect, forming crypts distally. SEM revealed an unusual structure of the surface in this area, in the form of cylindrical mounds with wide crater-like depressions (Fig. 3a). The surface mucocytes were reduced in volume and

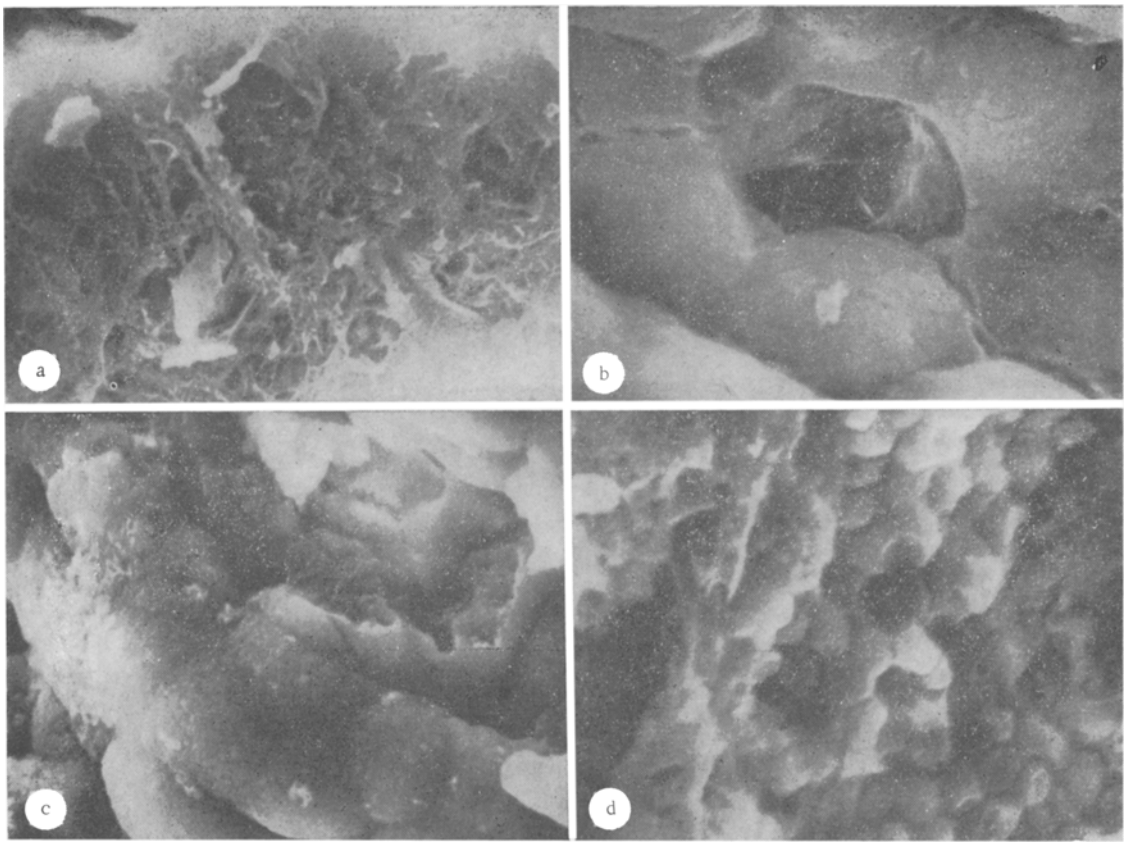

Fig. 2. SEM of gastric mucosa in experimental ulcer: a) control; vessels and connective-tissue fibers weaving around fundal glands (tissue fracture surface, 2000×); b) 1 h after application of acetic acid, swelling and beginning of breakdown of surface epithelium (6000×); c) formation of microerosion (6300×); d) breakdown of cells of fundal gland (tissue fracture surface, 6000×).

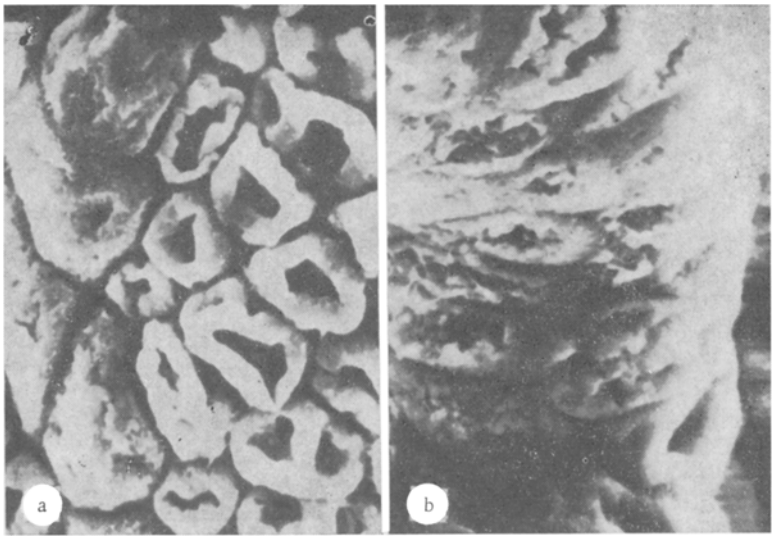

Fig. 3. SEM of gastric ulcers: a) 10 days after application of acid – cylindrical mounds and crater-like depressions on surface of mucous membrane at edge of ulcer (3000×); b) multiple erosions of epitheliocytes of newly formed mucosa (3000×).

their outer membrane was crinkled without definite microvilli, and with solitary pores. In other areas the newly formed mucosa resembled wide, twisting ridges. In these areas, at a distance of a few millimeters from the ulcer, the apical membrane of many epitheliocytes was destroyed (Fig. 3b). These defects were confined to individual cells and did not spread to their neighbors. Injuries of this type can be called apical "erosions" of the cells, and they evidently precede erosions of the mucous membrane penetrating into the tunica propria. Large areas of such erosions can rightly be regarded as an early sign of centripetal progression of the ulcer, as a result of which it recurs and does not heal in the course of several months. By contrast with the pores described above, the surface of the apical erosions of the cells was considerably larger, and their edges were always irregular, as it were eaten away, although the mechanism of their formation may be the same (apocrine secretion).

The formation of apical erosions has been regarded as dystrophy in situ, preceding desquamation of the cells [10].

On the fracture surface in the floor and edges of the ulcer numerous interweaving fibers and vessels of varied caliber could be seen. Occasional short deformed glands, interwoven by a denser network of collagen fibers and vessels than in the control, could be seen in the edges.

Later (3-7 months) the normal structure of the surface of the mucosa was not restored. The orifices of the pits were greatly widened to resemble craters, surrounded by elevated ridges of epitheliocytes, reduced in volume and almost completely without microvilli. Larger "craters" were seen into which 3 or 4 pits opened, separated by wide necks. The central parts of the healing ulcers were somewhat depressed, the mucous membrane hung over them, and from its edges spread out thin bands, with wavy outlines. On the surface of individual mucocytes apical erosions could still be seen even 7 months later, and this could be interpreted as an early sign of recurrence of the ulcer.

By SEM it is thus possible to study the nature of formation and healing of gastric ulcers, to detect the structure of the outer membranes of the regenerating epithelium, and to observe early changes in the cells preceding the formation of erosions.

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