REPAIR PROCESSES IN THE RAT STOMACH WITH INDOLENT EXPERIMENTAL ULCERS

T. B. Timashkevich
and I. G. Zaporozhchenko

UDC 616.33-002.44-092.9-07:616-003.93-07

A gastric ulcer was induced in rats by burning with acetic acid by Okabe's method. Repair processes in the gastric mucosa in the zone of the ulcer defect and adjacent fundal glands and also at a distance from the ulcer were studied by quantitative methods (determination of the cell composition of the glands, counting the daily number of DNA-synthesizing cells and the number of mitoses). On the 15th day after formation of the ulcer the level of proliferation in this zone was found to be much higher than in the control, whereas at a distance from the ulcer it was unchanged. On the 40th day regeneration took place with an even higher rise in the level of DNA synthesis and cell division. By this time the number of regenerating glands and the total number of cells forming them had increased. Similar changes were found up to 180 days close to the suture in glands of the resected rat stomach.

KEY WORDS: experimental gastric ulcer; regeneration; proliferation; DNA synthesis.

The rate, completeness, and methods of regeneration of superficial defects of the gastric mucosa have been studied in fair detail [2].

The object of this investigation was to study changes in proliferative activity in the mucous membrane of the fundus of the rat stomach in the region of an ulcer defect affecting the whole wall of the stomach, and also in regions of the mucosa at a distance from the site of injury, at different stages of the reparative period.

EXPERIMENTAL METHOD

An ulcer was produced in the fundus of the stomach by Okabe's method [6, 7] in experiments on 100 male Wistar rats weighing 160-180 g, by burning the wall of the gastric fundus from the side of the serous membrane with glacial acetic acid. The animals were killed at 10 a.m. 15 and 40 days after the operation. The stomachs of intact animals served as the control. All rats were given an intraperitoneal injection of thymidine-^{3}H before sacrifice in a dose of 0.3 μ Ci/g body weight five times in 24 h. The stomach was fixed in Carnoy's fluid, a strip was excised from the anterior wall of the stomach containing the ulcer, it was wound into a roll and embedded in paraffin wax. Sections 5-6 μ thick were stained by the method of Dominici and Kedrovskii, with hematoxylin-eosin, alcian blue, and Hale's reagent, and by the PAS reaction. Slides with sections for autoradiography were coated with type M emulsion in a dilution of 1 : 3; exposure lasted 5 weeks. Mitoses and labeled cells (the daily number) were counted in 100 longitudinally divided glands at each investigation (magnification 7 × 90 × 1.5). The mitotic index (MI) was expressed in pro mille and the index of labeled nuclei (ILN) in per cent. The total number of cells forming the gland of the newly formed epithelium, the marginal zone of the mucosa (adjacent to the ulcer), and remote from the ulcer was counted in each zone in 15 glands in each case. By using the same methods, changes in the daily number of DNA-synthesizing cells and the total number of cells were determined in the atypical glands of the gastric fundus in the region of the suture after resection of half of the fundal region in 36 noninbred mice. The numerical results were subjected to statistical analysis.

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Gastroenterology, Research Institute of Balneology and Physiotherapy, Pyatigorsk. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 226-229, February, 1980. Original article submitted June 11, 1979.

0007-4888/80/8902-0218$07.50

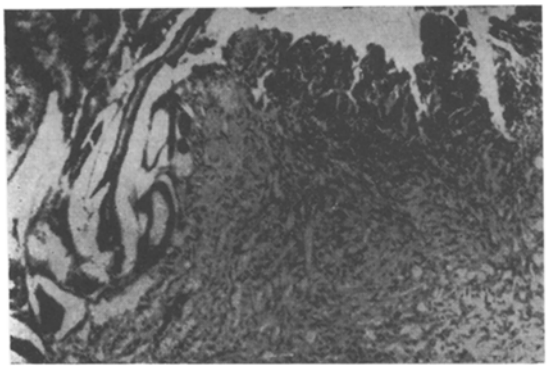

Fig. 1

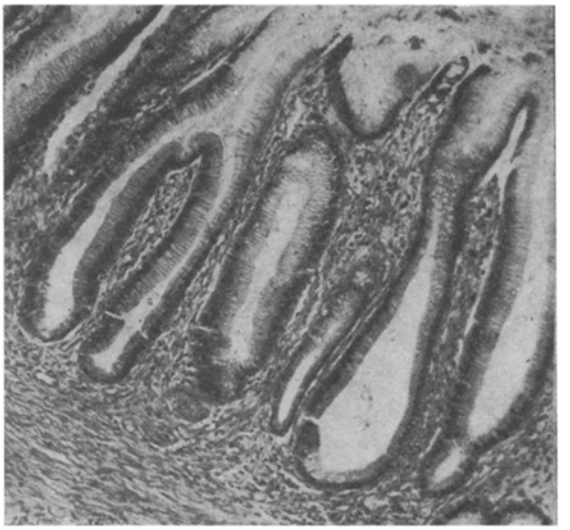

Fig. 2

Fig. 1. The floor of the ulcer consists of granulation tissue. At the edge of the defect regenerating glands can be seen (15 days after operation). Hematoxylin– eosin, 70×.

Fig. 2. Elongated glands of newly formed epithelium covering the defect (40 days after operation). Dominici–Kedrovskii method, 70×.

EXPERIMENTAL RESULTS

The pathological picture 15 and 40 days after production of the ulcer was basically similar to that described by other workers [1]. On the 15th day after the operation the floor of the ulcer consisted of necrotic masses, disintegrated leukocytes, and young granulation tissue with many newly formed blood vessels and collagen fibers (Fig. 1). Near the ulcer there was considerable infiltration, principally by leukocytes. Remote from the ulcer the mucosa of the fundus of the stomach on the whole preserved its structure, but small areas of hypertrophied glands with intensive secretion of mucoid, shortened glands, and also glands of pyloric type were observed. The level of proliferation and of secretion of acid and neutral mucopolysaccharides (MPS) was raised in these glands. Microerosions of the mucous membrane in a state of epithelization were noted. Acid MPS were detected in the basal segments of some glands, evidence of a change in the function of the peptic cells. On the 40th day after the operation the chronic gastric ulcers were partly epithelized and processes of sclerosis were actively developing in all layers of the stomach wall. The floor of the ulcer consisted of a layer of connective tissue, the outer part of which could be a zone of coarse fibrous necrosis. Most cells in the zone of infiltration consisted of lymphocytes, distributed among collagen fibers. The pits were shortened and widened in some places, the number of peptic cells was reduced in the groups of glands, and the basal portions were dilated and resembled cysts.

Repair of the ulcer took place in the same way as after various types of injury [1-9], i.e., by reconstruction of the glands adjacent to the ulcer, activation of proliferation in them, and the spread of newly formed epithelium from the margins of the defect. By the 15th day, mucous glands had formed in it. By the 40th day the thickness and area of the newly formed epithelium were increased. Glands formed in the region of the defect were of pseudopyloric type and of various shapes (Fig. 2). Mainly they consisted of cylindrical cells of epithelium of the surface pit type, but polymorphism of the covering epithelium could be observed, with a sharp increase in its content of acid and neutral MPS. Some intensively secreting glands were dilated to form cysts, packed with mucoid, but in cysts with flattened epithelium its secretory activity was sharply reduced.

We also analyzed the state of the epithelium, its secretory activity, and its proliferative activity in the region of the suture after resection of half of the gastric fundus in rats. These parameters showed similar changes (Fig. 3a). These changes were observed on both sides of the suture in approximately 20 glands. Near the suture atypical glands lined with cubical epithelium (less frequently high-prismatic) usually developed. In some cases cysts lined with flattened epithelium, in which the apical zone could be almost completely absent, were observed.

In the animals with experimental gastric ulcer, proliferating cells in the newly formed glands were located mainly in the basal portions, but they were also found in the body of the glands. The total number of

TABLE 1. Changes in Daily Number of DNA-Synthesizing Cells (in %) in Epithelium of Gastric Fundal Glands in Rats under Different Pathological Conditions

Time of investigation, days	Control (intact animals)	In the presence of indolent ulcers			After resection of stomach	
		chief glands remote from defect	glands of marginal zone	regenerating glands	atypical glands in region of defect	chief glands of resected stomach
15	8,94±0,18					
15	—	7,30±0,28	15,82±0,49	30,82±0,80	—	—
30	—	—	—	—	31,87±5,61	7,45±0,31
40	—	13,39±0,22	21,46±0,57	39,69±0,57	—	—
90	—	—	—	—	69,88±3,29	13,69±2,21
180	—	—	—	—	71,14±4,04	17,36±2,05

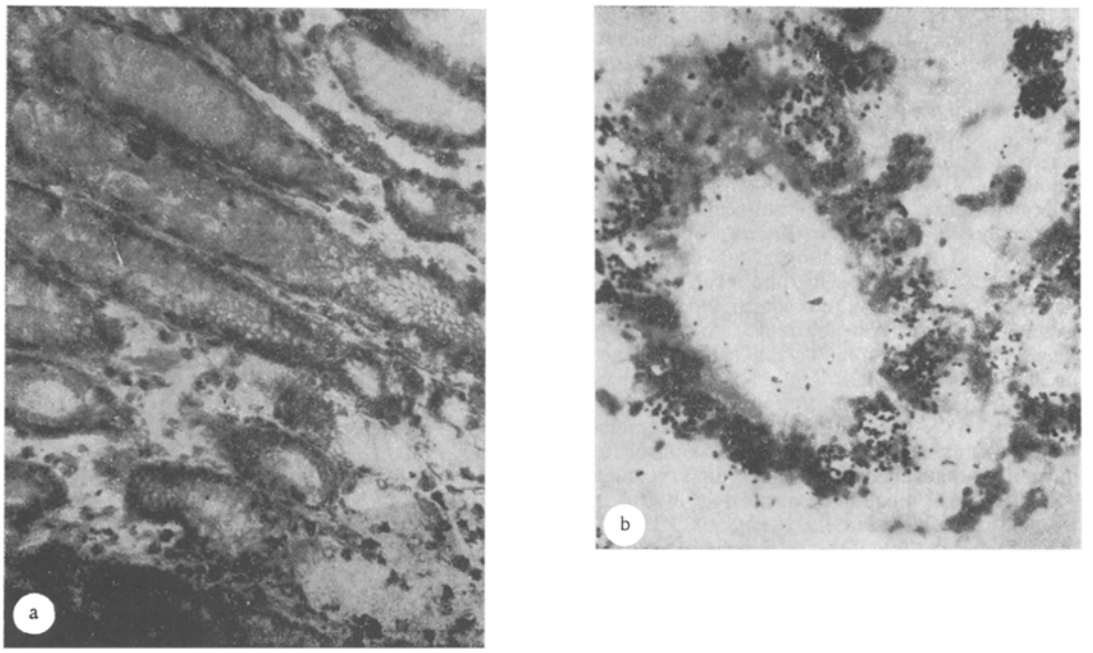

Fig. 3. Atypical glands near site of suture: a) general appearance; b) DNA synthesis in cells of atypical glands. Dominici–Kedrovskii method. Magnification: a) 280×, b) 630×.

cells forming a regenerating gland after 15 days of repair was 67.44 ± 0.92 (106.1 ± 1.82 in the control); the daily number of labeled nuclei was twice that in glands in the marginal zone (Table 1); the number of mitoses was significantly greater than in glands of the marginal zone at the same time (MI was 8.04 and 2.84‰ respectively, compared with 0.73‰ in the control). Remote from the site of injury the level of proliferation was not significantly different from normal (MI was 0.71‰). By the 40th day after formation of the ulcer, the number of cells in the newly formed glands had risen to 86.75 ± 1.05 and in the glands α the marginal zone to 98.96 ± 2.12. The rate of intensification of proliferation was similar (Table 1) in both zones (MI was 12.56 and 3.94‰ respectively). The changes in both parameters were practically identical, whereas at a distance from the ulcer mitotic activity was almost three times higher (2.02 ± 0.11) than at the previous time, ILN was increased by less than twice (Table 1). Budding of new chief glands was observed at this period at a distance from the ulcer.

Changes in proliferation in the glands of the resected stomach were similar (Table 1). Whereas in cells of the atypical epithelium of the glands in the region of the suture 30 days after resection of the stomach proliferation was increased by 2.3 times, after 90-180 days it was increased by 4.7 times compared with that in intact animals of the same age. At a distance from the site of resection the number of DNA-synthesizing cells was unchanged after 30 days, but after 90 days it was almost doubled. In both these cases the reaction of the mucous membrane at a distance from the defect was "delayed." The chronic course of the ulcer, a development of gastritis, and also the increased functional load on the mucous membrane of the resected stomach are perhaps constant pathogenic stimuli acting on the system of compensatory and reparative mechanisms.

Prolonged maintenance of a high level of proliferation in the epithelium of the atypical glands in the region of the suture (Fig. 3b) may be one cause of the development of subsequent pathological changes.

LITERATURE CITED

1. L. I. Aruin, B. Z. Chikunova, and I. E. Trubitsyna, in: Current Problems in Gastroenterology [in Russian], Vol. 2, No. 9, 97 (1976).
2. T. B. Timashkevich, Ways and Mechanisms of Regeneration of the Digestive Tract in Vertebrates [in Russian], Moscow (1978).
3. H. M. Adair, J. Anat. (London), 125, 401 (1978).
4. T. E. Hunt, Anat. Rec., 131, 193 (1958).
5. E. Myhre, Arch. Path., 69, 314 (1960).
6. S. Okabe and C. Pfeiffer, Fed. Proc., 29, 255 (1970).
7. S. Okabe and C. Pfeiffer, in: Peptic Ulcer, Philadelphia (1971), p. 13.
8. S. P. Townsend, Am. J. Anat., 109, 133 (1961).
9. A. W. Williams, Br. J. Surg., 48, 564 (1961).

COURSE OF STRUCTURAL CHANGES IN THE MICROCIRCULATION OF GROWING AND ATRETIC OVARIAN FOLLICLES

O. V. Volkova,* L. M. Mel'nikova, M. I. Pekarskii, and T. M. Povalii

UDC 611.651.15+611.653:611.161.

Synchronized populations of growing and atretic vesicular follicles were obtained in the ovaries of prepubertal mice by injection of serum gonadotrophin. Light-optical and ultrastructural analysis by morphometric methods revealed definite correlation between the size of the follicles and the degree of their vascularization during growth and atresia. The results suggest that the microcirculation plays the leading role in the initiation of atresia.

KEY WORDS: ovarian follicles; atresia; microcirculation.

The ovarian follicular blood vessels are known to appear in the course of formation of the specialized theca folliculi, and the density of the network of vessels increases with growth of the follicle [2]. The hypothesis that the follicular vessels contribute to the development of atresia of the follicles [3, 5] has not yet been confirmed.

In this investigation the pattern of vascularization of the theca interna at different stages of development of vesicular follicles and the dynamics of ultrastructural changes in the vessels of the microcirculation during induced growth and atresia of the follicles were studied.

EXPERIMENTAL METHOD

Stimulation of synchronized growth and atresia of ovarian follicles in prepubertal animals was used as the model. Experiments were carried out on noninbred prepubertal female albino mice weighing 7-9 g, which were given a subcutaneous injection of 5 i.u. pregnant mare's serum (PMS). Control animals received an injection of physiological saline. Animals of this age were chosen because of data in the literature showing that the ovaries of prepubertal mice can respond adequately to injection of PMS, and the dose of gonadotrophin used corresponded to that used by other investigators [4, 7].

For the light-optical and electron-microscopic investigations 22 experimental and six control animals were used.

*Corresponding Member, Academy of Medical Sciences of the USSR.

Department of Histology and Embryology of the Pediatric Faculty, and Laboratory of Electron Microscopy of the Research Institute, N. I. Pirogov Second Moscow Medical Institute, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 229-232, February, 1980. Original article submitted April 5, 1979.

0007-4888/80/8902-0221$07.50