# PROLIFERATIVE ACTIVITY OF EPITHELIUM OF THE SURFACE AND PITS OF THE GASTRIC MUCOSA AFTER ASPIRIN INJURY

G. V. Tsodikov, V. V. Klimenko, and S. N. Laz'kova

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The proliferative activity of the surface epithelium of the gastric mucosa and the epithelium of the gastric pits in albino mice was studied after injury by aspirin (200 mg/kg). An intraperitoneal injection of $^3$H-thymidine was given to all the animals 1 h before sacrifice. The mitotic index and index of labeled nuclei were counted on autoradiographs 3, 10, and 20 days after administration of aspirin. After administration of aspirin for 3 days the proliferative activity of the epithelium of the surface and pits of the gastric mucosa was unchanged compared with the control. After longer administration of aspirin (10 and 20 days) the number of erosions was not increased but the proliferative activity of the surface epithelium and epithelium of the pits was statistically significantly increased, on account of an increase in the number of proliferating cells in the pits and the neck of the glands, and also on account of widening of the zone of distribution of the precursor cells in the gastric glands, as far as the basal portions. These changes are a manifestation of a protective-adaptive reaction of the gastric mucosa in response to its injury by aspirin.

KEY WORDS: stomach; aspirin; proliferation; precursor cells.

Clinical observations on patients taking aspirin have shown that the gastric mucosa reacts differently to this drug: in some cases erosions or ulcers develop, whereas in others the mucosa remains intact. Since aspirin is taken by mouth, the surface epithelium, which performs a barrier function and is most frequently exposed to the action of environmental factors, must be exposed primarily to the harmful action of the drug. To understand the mechanism of the protective reaction of the gastric mucosa, definite help can be obtained by a study of the proliferative activity of the surface epithelium in response to its injury. Aspirin, which is an ulcerogenic substance, was used as the provocative agent [4, 5, 6].

## EXPERIMENTAL METHOD

Injury was produced by daily administration of a solution of aspirin in a dose of 200 mg/kg (0.2 ml per mouse) into the stomach of albino mice through a needle with rounded end to avoid injury to the esophagus. Administration continued for 3, 10, or 20 days. Considering the short life span of mice (under 2 years), administration of the drug for 20 days can be regarded as a chronic experiment. Altogether 28 male albino mice each weighing 20-25 g were used. Animals of group 1 were given a solution of aspirin (six mice for 3 days, five mice for 10 days, five mice for 20 days). Control groups consisted of three intact animals (group 2) and nine mice (group 3) receiving the solvent of aspirin, namely a 30% solution of dimethyl sulfoxide (DMSO). Tritiated thymidine with a specific activity of 1.4 Ci/mmole was injected in a dose of 1 $\mu$Ci/g body weight intraperitoneally into all the animals 1 h before sacrifice. After decapitation, the stomach was opened along the greater curvature. Pieces of mucosa were cut out along the line of incision, fixed in Carnoy's fluid, and embedded in paraffin wax. Slides with sections 5-6 $\mu$ thick were coated with type M emulsion and exposed for 24 days. After development, the sections were stained with hematoxylin-eosin and by the PAS reaction. The mitotic index (MI), and the index of DNA synthesis or index of labeled nuclei (ILN) were counted on the autoradiographs. The results were subjected to statistical analysis. The significance of differences for a small number of observations was determined by Student's t-test.

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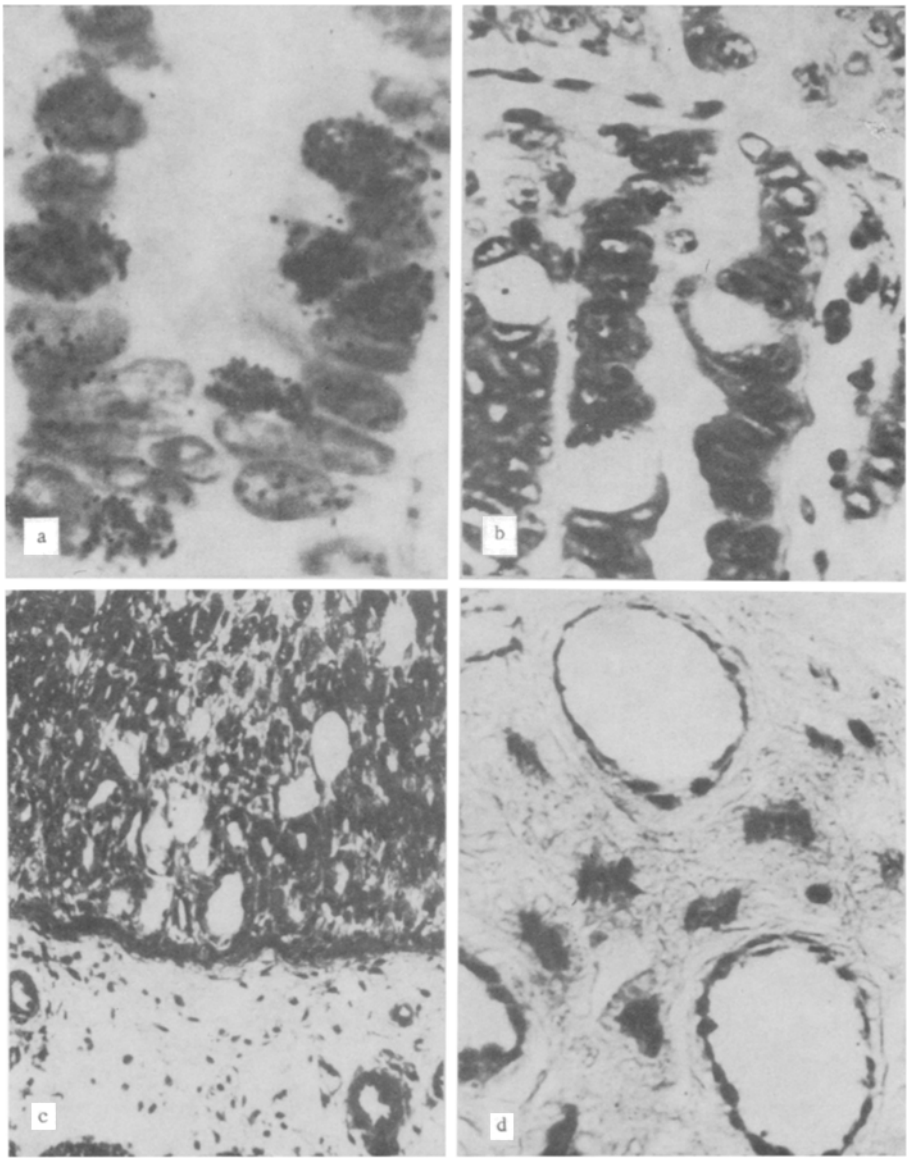


Fig. 1. Gastric mucosa of control and experimental animals. a) Radioactive label in nuclei of epithelium of pits and neck of gastric glands. Hematoxylin–eosin, 280×; b) vacuolar degeneration of epithelium of surface and pits, capillarectasia. Hematoxylin–eosin, 280×; c) focal atrophy of gastric glands, edema of submucosa. Microcysts of different sizes (administration of aspirin for 10 days). Hematoxylin–eosin, 160×; d) PAS-positive secretion in cytoplasm of cells lining microcysts (administration of aspirin for 10 days). PAS reaction, 200×.

## EXPERIMENTAL RESULTS

The radioactive label in the gastric mucosa of intact animals was localized above the nuclei of cells lining the neck and pits of the fundal glands (Fig. 1a). In the neck portion of the fundal glands stem cells were located [9, 12]. Since these cells are difficult to identify, all cells in the phase of DNA synthesis (S-phase) were called "precursor cells." Most of the dividing cells of the gastric glands of experimental animals (mice, rats, dogs) and also of man proliferate mainly on the surface of the mucosa, and are converted into surface epithelium. According to Zaporozhchenko (1973) [1], 85.6% of labeled cells belong to the epithelium of the surface and pits of the gastric glands in rats, accessory cells account for 12.8%, parietal cells for 0.7%, and chief cells for 0.9%. Labeled cells reach the surface at the mouth of the pits in the course of 1.5-6 days [10]. As the cells move toward the surface of the gastric mucosa they differentiate and begin to secrete neutral and

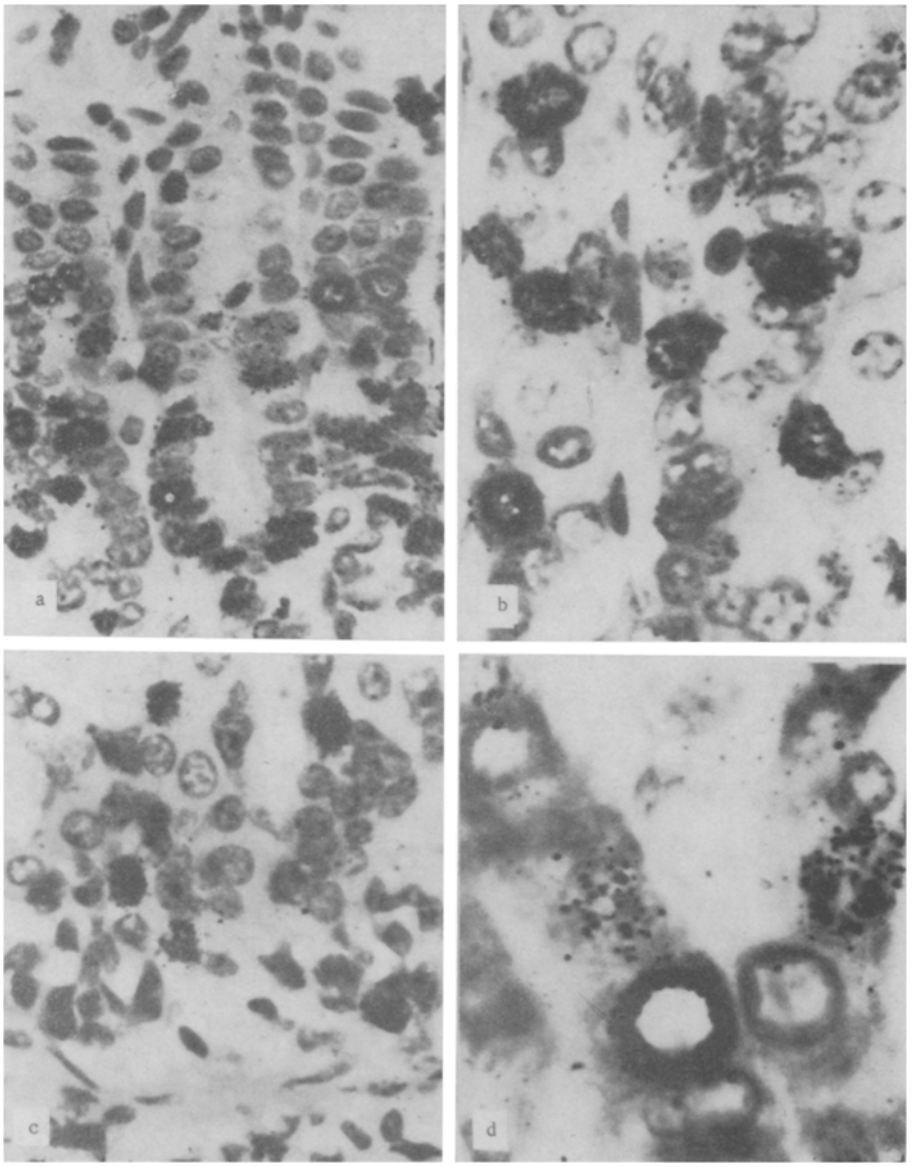

Fig. 2. Distribution of radioactive label in nuclei of gastric epithelium after administration of aspirin. a) Increased number of precursor cells in pits and neck of fundal glands. Hematoxylin–eosin, 280×; b) labeled cells in body (chief cells) of fundal glands. Hematoxylin–eosin, 400×; c) labeled cells reach basal portion of fundal glands, beneath which there is a layer of muscle fibers. Hematoxylin–eosin, 160×; d) disappearance of chromatin and radioactive label from center of nuclei of individual cells. Hematoxylin–eosin, 900×.

acid (in animals) mucopolysaccharides. Cells which have completed their life cycle are desquamated. In the pyloro-antral portion of the human stomach the mean time taken for labeled cells to migrate to the surface of the mucosa is 2-4 days. Grains of label above the nuclei of the parietal and chief cells appear in the mouse stomach 5-7 days after injection of $^{3}$H-thymidine (6-7% [10, 11]). By that time the label has almost completely disappeared from the surface epithelium and the epithelium of the pits and neck of the glands [11]. Since the mitotic activity of the epithelium is most marked in the neck portions of the fundal glands, the regenerative capacity of the gastric mucosa can best be judged by the study of the epithelium of these portions. Variants of the experiments and the results obtained are given in Table 1.

It will be clear from Table 1 that MI and ILN in intact animals ($8.3 \pm 0.3‰$; $10.1 \pm 1.0\%$), after administration of 30% DMSO for 3 days ($8.7 \pm 0.3‰$; $10.4 \pm 1.1\%$), and after administration of aspirin solution ($7.0 \pm 0.9‰$; $12.1 \pm 0.8\%$) did not differ significantly, although histologically at this time the largest number of acute

TABLE 1. Variants of Experiments and Results of Investigation of MI and ILN of Surface Epithelium and Epithelium of Pits of Gastric Mucosa

| Group of mice | Duration of experiment, days | Total number of animals | Change in stomach | Number of animals | MI, ‰ | ILN, % |
|---|---|---|---|---|---|---|
| | | | | | M ± m | |
| 1 | 3 | 6 | Erosions | 2 | 7,0±0,9 | 12,1±0,8 |
| | | | Hemorrhages | 2 | | |
| 2 | | 3 | — | | 8,3±0,3 | 10,1±1,0 |
| 3 | 3 | 3 | Increased infiltration with histiocytes | 1 | 8,7±0,3 | 10,4±1,1 |
| 3 | 10 | 3 | — | | 8,3±0,9 | 9,9±1,1 |
| 1 | 10 | 5 | Erosions | 1 | 13,2±2,8 | 19.3±3,1 |
| | | | Focal atrophy of mucosa | 3 | | |
| 1 | 20 | 5 | Erosions | 1 | 13,0±2,1 | 16,3±1,7 |
| | | | Focal atrophy mucosa | 4 | | |
| 3 | 20 | 3 | — | | 7,7±0,7 | 9,8±1,5 |

injuries to the stomach in the form of erosions, acute degenerative changes of the surface epithelium, disturbances of the microcirculation and, macroscopically, foci of hemorrhage [3] could be demonstrated. After administration of aspirin for 10 days MI reached a value of 13.2 ± 2.8‰, and ILN 19.3 ± 3.1%; after 20 days of the experiment the corresponding values of these indices were 13.0 ± 2.1‰ and 16.3 ± 1.7% (the difference between MI and ILN in the experimental and control groups was significant; t = 3). The increase in MI and ILN on the 10th and 20th days of the experiment indicated an increase in proliferative activity of the surface epithelium and the epithelium of the pits. Since during the first 3 days of aspirin administration MI and ILN did not differ from their values in the control group, their increase toward the 10th and 20th days of the experiment must be regarded as a reaction of the mucosa to injury due to aspirin. On the 10th day of the experiment the highly differentiated epithelium (parietal and chief cells) was replaced by flat, cubical epithelium. The lumen of the glands became dilated. These changes were particularly marked on the 20th day of the experiment, when widespread damage to the fundal glands took place, leading ultimately to focal atrophy of the gastric mucosa. Large vacuoles appeared in the cytoplasm of the gastric epithelium, and degenerative and necrobiotic changes developed (Fig. 1b), and were followed by replacement with flattened epithelium. Such glands had the appearance of microcysts, and because of thinning of their walls, some of them resembled vessels with a dilated lumen (Fig. 1c). The presence of PAS-positive secretion in the cytoplasm of the cells lining these glands enabled them to be differentiated from vessels (Fig. 1d). Despite the increase in disturbed regenerative processes, the number of acute destructive lesions, in the form of erosions, was no greater than on the 3rd day of the experiment. This "adaptation" was due not only to the increasing detoxicating power of the mucosa, as Hietanen [8] considers, but also to the more rapid reparative regeneration of the gastric epithelium. During the study of this reaction in response to injury of the mucosa two phases could be distinguished: I (until the 10th day of the experiment) was characterized by an increase in the number of precursor cells in the pit and neck regions of the glands, often spreading to the upper third of the body (the chief portion) of the fundal glands (Fig. 2a); II (until the 20th day of the experiment) was characterized by widening of the zone of proliferating cells along the fundal glands as far as the basal portions (Fig. 2b, c). Changes of this sort probably contribute not only to the more rapid replacement of lost cells, but also to the more rapid access of proliferating epithelium to the place where its precursor has died. The growth of atrophic processes in the gastric mucosa under these experimental conditions is evidence that the rate of regeneration of the epithelium does not correspond to the rate of its death. Irrespective of the duration of the experiment, aspirin was found to have a harmful effect on the nuclei of the surface epithelium and epithelium of the pits. Acute degenerative changes appeared even in cells in the phase of DNA synthesis (Fig. 2d). These changes corresponded to disappearance of chromatin from the center of the nuclei and a distribution of radioactive label only at the periphery of the nuclei. The changes discovered are possibly part of the morphological basis for its teratogenic action, which has been described in women taking aspirin in the later stage of pregnancy [5, 7] and also of chromosomal aberrations in patients with rheumatic fever being treated with this drug [2].

The results of these experiments thus indicate that the growth of proliferative activity of the surface epithelium and epithelium of the pits is due to an increase in the number of proliferating cells in the neck and

pits of the glands, followed by widening of the zone of the precursor cells along the gastric glands, as far as the basal portions. These changes must be regarded as a protective and adaptive response to damage to the gastric epithelium by aspirin.

## LITERATURE CITED


1. I. G. Zaporozhchenko, "Effect of peroral administration of radon waters on physiological and reparative regeneration in the mucosa of the gastric fundus in rats," Author's Abstract of Candidate's Dissertation, Moscow (1973).
2. V. A. Nasonova, N. M. Karaseva, V. B. Khvatov, et al., Ter. Arkh., No. 3, 26 (1972).
3. G. V. Tsodikov and V. V. Klimenko, in: Current Problems in Gastroenterology [in Russian], No. 9, Moscow (1976), p. 86.
4. B. Billington, Med. J. Aust., 2, 485 (1964).
5. W. Bleyer and R. Breckenridge, J. Am. Med. Assoc., 213, 2049 (1970).
6. J. Duggan, Gut, 17, 378 (1976).
7. E. Ryring, J. Grosfeld, and P. Connelly, Ann. Surg., 177, 307 (1973).
8. E. Hietanen, Am. J. Dig. Dis., 20, 31 (1975).
9. F. Liavag, Acta Path. Microbiol. Scand., 72, 43 (1968).
10. M. Lipkin, P. Sherlock, and B. Bell, Gastroenterology, 45, 721 (1963).
11. H. Ragins, F. Wincze, S. Lin, et al., Anat. Rec., 162, 99 (1968).


# FUNCTIONAL MORPHOLOGY OF THE SUBMAXILLARY SALIVARY GLANDS DURING AGE INVOLUTION IN ALBINO RATS


M. G. Rybakova



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A quantitative study was made of the morphological and functional characteristics of different parts of the submaxillary salivary glands of albino rats in different stages of involution of the neuroendocrine system. During aging the salivary gland function of rats is preserved, and just as in young animals, clear cyclic fluctuations are observed depending on the phases of the estrous cycle, although the basal level of proteins and mucopolysaccharides is reduced, to correspond on average to the minimal found in young animals. Meanwhile activation of the enzymes responsible for energy and transport processes in the cell is observed and relations between the enzymes are altered. The results prove that the salivary glands are connected with the endocrine system and they confirm the view that in early age involution what occurs is not so much a change of function as a disturbance of relations between the different indices of functional activity of the organ.



KEY WORDS: salivary glands; enzymes; involution; estrous cycle.


Age involution of the salivary glands is an interesting problem. This is because of the diversity of their function (the saliva contains enzymes responsible for starting the digestive process in the mouth, it has protective properties due to the presence of lysozyme and IgA [2]); there is also evidence of an internal secretory function of the salivary glands and of their connections with other endocrine glands, including the gonads [9].

There are as yet few data on age changes in the structural and histochemical indices of salivary gland function in animals of different species and in man. It has been shown that during age involution the type of secretion in all salivary glands varies, usually to the mucous type, giving greater protective properties [10, 14], many intercalated ducts are formed, and acid phosphatase and succinate dehydrogenase activity are reduced, whereas the number of terminal portions undergoing lipomatous changes is increased [15]. There is also evidence that with age proteolytic enzyme activity in the salivary glands of rats increases [1].


Department of Pathological Anatomy, I. P. Pavlov First Leningrad Medical Institute.


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