HISTOCHEMISTRY OF CARBOHYDRATE-CONTAINING BIOPOLYMERS OF THE MUCOSAL EPITHELIUM OF THE DOG STOMACH DURING DEVELOPMENT OF A HYPERSECRETORY SYNDROME

G. M. Mogil'naya and A. A. Fisher

UDC 616.33-008.811.1-092.9-07:616.33-018.73-008.9.-092.18-074

A histochemical study of the dynamics of the mucin content in the mucosal epithelium of the dog's stomach during development of a hypersecretory syndrome due to prolonged histamine stimulation revealed 3 consecutive stages: 1) an increase in the content of glycogen, neutral glycoproteins, sialomucins, and sulfomucins, 2) disappearance of glycogen and a decrease, followed by restoration of the level of carbohydrate-containing biopolymers and 3) a progressive fall in the level of carbohydrate-containing biopolymers with the development of erosions and ulcers. The most marked decrease in the surface epithelium affected the content of sulfomucins, while in the crypts the decrease was greatest in neutral glycoproteins and sialomucins. The reaction of the surface and crypt epithelium does not necessarily coincide in time.

There have been few histochemical studies of the gastric mucosa of dogs or other animals [1, 2, 6, 10, 13, 15, 20]. However, functional changes in the mucins in cells of a fundal glands in connection with their secretory activity have been described only in one paper [4], and the dynamics of the carbohydrate component in the mucosal epithelium has been completely ignored.

The object of the present investigation was to make a histochemical study of mucins of the mucosal epithelium of the stomach, which are carbohydrate-containing biopolymers, during the development of a hypersecretory syndrome in response to prolonged histamine stimulation.

EXPERIMENTAL METHOD*

Chronic experiments were carried out on four adult male mongrel dogs weighing from 14 to 18.9 kg with a Basow gastric fistula. Systematic intramuscular injections of a histamine-wax mixture [11] began after the study of the secretory activity of the stomach and the removal of biopsy material. The mixture was injected daily, in the evening, in a dose equivalent to 30 mg histamine base, for 11-61 days. Every three to six days, after starvation for 18 h the secretory activity of the stomach was investigated, and at the same time a biopsy specimen of the full thickness of the mucous membrane was taken through the fistula in the region of the middle third of the stomach. Altogether 32 gastric biopsies and 60 tests of the basal gastric secretion and the gastric secretion in response to maximal histamine stimulation were carried out; 183 samples of gastric juice were analyzed. Pieces of gastric mucosa were fixed in Hamperl's fluid [2] and the material was embedded in paraffin wax in the usual way. Polysaccharides were studied in sections stained by the PAS reaction, with alcian blue [18], and with basic brown [8, 9]. The control sections were treated with amylase, phenylhydrazine [19], and by mild acid hydrolysis [3].

*The experiments were carried out in the Department of Topographical Antamony, Stavropol' Medical Institute.

Departments of Histology and Propedeutics of Internal Diseases, Kuban Red Army Medical Institute, Krasnodar. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 117-120, June, 1972. Original article submitted December 7, 1971.

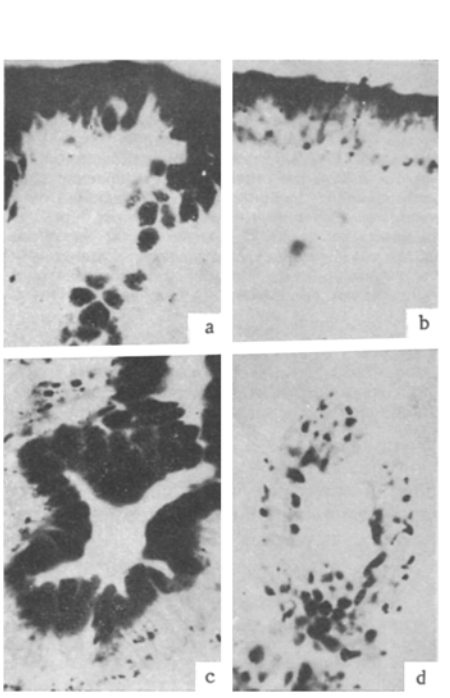

Fig. 1

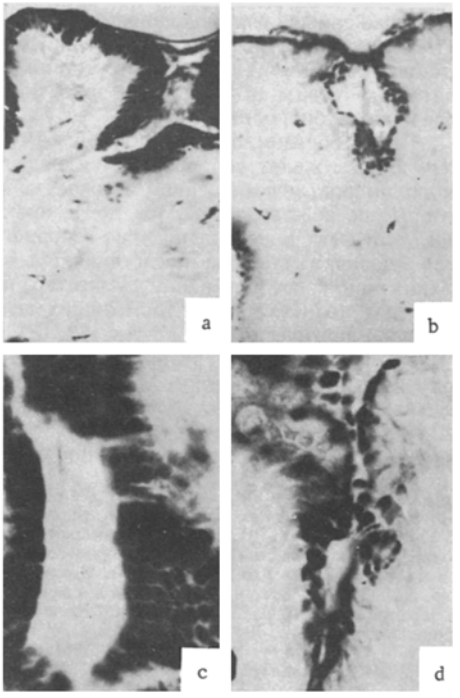

Fig. 2

Fig. 1. Decrease in content of neutral glycoproteins in mucosal epithelium of the dog's stomach during development of a hypersecretory syndrome (PAS-reaction, 800 ×); a, b) surface epithelium before and after administration of histamine-wax mixture for 11 days respectively; c, d) epithelium of gastric crypt before and after administration of histamine-wax mixture for 19 days respectively.

Fig. 2. Decrease in content of sulfomucins in mucosal epithelium of the dog's stomach during development of a hypersecretory syndrome (stained with basic brown at pH 1.0 by Shubich's method). a, b) surface epithelium before and after administration of histamine-wax mixture for 11 days respectively (280 ×); c, d) epithelium of gastric crypt before and after administration of histamine-wax mixture for 19 days respectively (800 ×).

EXPERIMENTAL RESULTS

Chronic histamine stimulation led to a marked increase in the secretion of gastric juice, which rose to a maximum and thereafter remained at about the same level or began to decrease despite continued administration of this mixture. The degree of increase in the individual components of the juice and the time taken by them to reach their secretory maximum varied from one dog to another. At the height of stimulation the hypersecretory syndrome, as shown by the results of the maximal histamine test, was reflected in the following indices. Compared with the initial level the total volume of secretion was increased by 67.5%, the volume of the acid component by 112%, the volume of the alkaline component by 78.8%, the volume of mucus by 76.4%, acid production by 95.5%, and pepsin production by 330.9%. The time taken for acid and pepsin secretion to reach their maximum varied from 6 to 59 days. It is easy to see that at the height of gastric hypersecretion the secretion of mucus and the volume of the alkaline component lagged behind the production of acid and pepsin.

The development of the hypersecretory syndrome was accompanied by structural changes in the gastric mucosa of two dogs. In the dog Mars, on the 28th day of administration of the histamine mixture, blood appeared in the stools, and the animal died two days later. Postmortem examination revealed multiple hemorrhage measuring 0.5 × 0.8 and 1 × 1 cm and numerous erosions measuring 0.3 × 0.8 cm in the mucus membrane of the fundus, body, and pylorus of the stomach. In another dog (Kruzhok) toxic manifestations and blood in the stools appeared on the 11th day after the beginning of administration of the histamine mixture, and accordingly no further histamine was given.

Histochemical examination of the gastric mucosa before administration of the histamine mixture showed that the cells of the mucosal epithelium contained glycogen, neutral glycoproteins giving a diastase-resistant, phenylhydrazine-sensitive PAS-reaction, acid glycoproteins of the sialomucin type, staining positively with alcian blue (pH 2.7) and sensitive to mild acid hydrolysis, and also sulfonated glycosaminoglycans (sulfomucins), with well marked basophilia (staining with alcian blue and basic brown at pH 1.0), partially sensitive to hyaluronate-liase. All these substances except glycogen, which was deposited as granules in the basal part of the epithelial cells, were located in the apical part of the cytoplasm.

Prolonged histamine stimulation induced significant changes in the production and secretion of carbohydrate-containing polymers by the mucosal epithelium. Changes were observed in the content of all secretory components: glycogen, neutral glycoproteins, sulfomucins, and sialomucins; the reactions of the surface and crypt epithelium could differ appreciably, and each of these components could vary independently of the others.

During histamine stimulation distinct morphological pictures corresponding to three successive stages could be distinguished. The first stage of stimulation was characterized by increased alcianophilia (pH 2.7) and basophilia (pH 1.0) and by an increase in the number of PAS-reactive granules in the basal part of the cells of the surface epithelium. The sensitivity of the PAS-reaction of these granules to diastase demonstrates their glycogen nature. It was impossible to detect changes in the content of neutral glycoproteins visually. The crypt epithelium reacted similarly to histamine stimulation. The times of development of changes in the surface and crypt epithelium did not necessarily coincide. In the first stage, for example, concurrently with the development of a hypersecretory syndrome, the content of glycogen, sulfomucins, and sialomucins increased in the cells of the mucosal epithelium.

The second stage included two periods: a) a decrease, followed by b) recovery of the level of carbohydrate-containing polymers in the cells of the mucosal epithelium. In the first period of the second stage the staining with basic brown and by the PAS-reaction continued in the surface epithelium, but only as a narrow band on the surface of the cells (Fig. 1a, b; Figs. 2a, b). The alcianophilia (pH 2.7) was characterized by a combined decrease in the area stained and in the intensity of the reaction. The epithelium of the gastric crypts behaved in a rather more stable manner. The decrease in alcianophilia and basophilia was less marked, and the PAS-reaction remained only in the apical surface of the cells (Fig. 1, c, d; Fig. 2c, d).

In all the animals soon after the decrease in level of carbohydrate-containing polymers, the intensity of staining gradually recovered despite continued administration of the histamine and maintenance of the hypersecretion (the second period of the second stage). The period of recovery in the surface epithelium was characterized by an increase in the area of the PAS-reaction to its original level. The alcianophilia was only partially restored. Staining with basic brown was not restored. During this period it was possible to trace a clear difference between the functions of the surface and crypt epithelium. In the epithelium of the gastric crypts ability to stain with the PAS-reaction, with alcian blue (pH 2.7), and with basic brown was restored, indicating the accumulation of neutral glycoproteins, sulfomucins, and sialomucins. In the surface epithelium, on the other hand, recovery of the content of neutral glycoproteins and sialomucins took place in most cases without any appreciable increase in the sulfomucin level.

The third stage (exhaustion) was marked by a fresh and considerable decrease in the content of carbohydrate-containing biopolymers in the cells of the mucosal epithelium. No sulfomucins were found in the cells of the surface epithelium, the area occupied by PAS-positive substances was sharply reduced, and there was a decrease both in the intensity of the alcianophilia (pH 2.7), evidently because of a decrease in the volume concentration of sialomucins, and in the area of tissue stained. In the crypt epithelium the area of alcianophilia and of the PAS-reactive surface also was reduced, whereas staining with basic brown remained stable. In two animals the first stage was characterized by the development of erosions, bleeding from the gastric mucosa, and hemorrhages.

During the first three to four days after administration of histamine was discontinued the level of carbohydrate-containing biopolymers in the mucosal epithelium remained almost unchanged compared with the last days of stimulation, and sometimes the intensity of the PAS-reaction and basophilia actually decreased still further. After the 11th day the content of neutral glycoproteins and sialomucins usually recovered, but sulfomucins remained absent in the surface epithelium, while in the crypt epithelium all the carbohydrate-containing biopolymers were restored.

A prolonged increase in the secretory activity of the gastric glands is thus accompanied by a decrease in the level of carbohydrate-containing biopolymers and by disappearance of sulfomucins from the surface epithelium, to which they do not return after discontinuation of histamine stiumulation. This fact, together with the erosive and hemorrhagic injuries of the gastric mucosa, is important to the understanding of the local mechanisms of ulceration. A decrease in the synthesis of sulfonated polysaccharides, which inhibits the action of pepsin [5, 16], is known to be a cause of peptic ulcers in rats [12, 15]. Ludwig and Lipkin [17] state that the development of gastroduodenal ulcers in guinea pigs is also accompanied by a decrease in the content of PAS-positive substances in the cells of the surface epithelium of the gastric mucosa.

LITERATURE CITED

1. A. V. Kirillicheva and Z. F. Guseva, in: Collected Transactions of the Kazakh Republican Scientific Society of Anatomists, Histologists, and Embryologists [in Russian], No. 3, Alma-Ata (1968), p. 117.
2. G. I. Kutakh, Histological Investigation of Proteins, Nucleic Acids, and Polysaccharides of the Gastric Mucosa of the Dog. Condidate's Dissertation, Krasnodar (1964).
3. G. M. Mogil'naya, Arkh. Part., No. 3, 78 (1966).
4. A. A. Fisher and R. I. Polyak, Ter. Arkh. No. 8, 8 (1969).
5. G. V. Tsodikov, Vestn. Akad. Med., Nauk SSSR, No. 2, 90 (1965).
6. A. A. Shabadash, Problems in the Histochemical Investigation of Glycogen in the Normal Nervous System [in Russian], Moscow (1949).
7. M. G. Shubich, Byull. Éksperim. Biol. i Med., 46, No. 12, 110 (1958).
8. M. G. Shubich, Byull. Éksperim. Biol. i Med., No. 2, 116 (1961).
9. M. G. Shubich and G. I. Kutakh, Arkh. Anat., No. 11, 112 (1962).
10. C. F. Code and R. L. Varco, Proc. Soc. Exp. Biol. (New York), 44, 475 (1940).
11. I. Hakkinen, K. Hartiala, and H. Lang, Acta Physiol. Scand., 66, 333 (1966).
12. I. Hakkinen, R. Johansson, and M. Pantio, Gut, 9, 712 (1968).
13. R. Lambert, C. Andre, and F. Martin, Gastroenterology, 56, 200 (1969).
14. S. Lekey and S. Sheinfield, Gastroenterology, 27, 625 (1954).
15. W. M. Ludwig and M. Lipkin, Gastroenterology, 56, 895 (1969).
16. R. W. Mowry, J. Histochem. Cytochem., 4, 407 (1956).