CARBOHYDRATE-CONTAINING BIOPOLYMERS OF OXYNTIC AND CHIEF CELLS OF THE DOG GASTRIC MUCOSA AND THEIR CHANGES DURING PROLONGED HISTAMINE STIMULATION

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Histochemical study of the dynamics of carbohydrate-containing biopolymers of the oxyntic and chief cells during the development of histamine-induced hyperplasia and hypersecretion revealed three consecutive stages: I) an increase in the content of neutral mucopolysaccharides and glycogen in the oxyntic cells, an increase in the content of sulfonated polysaccharides, and the appearance of sialomucins in the chief cells; II) a decrease followed by recovery of the above carbohydrate-containing polymers in the oxyntic cells, and absence of change or an increase in the content of sulfonated polysaccharides and a decrease in the content of sialomucins in the chief cells; III) progressive exhaustion of the carbohydrate-containing polymers in the cells with the development of erosions and ulcers of the gastric mucosa.

The histochemical properties of the glandular epithelium of the gastric mucosa has been inadequately studied. According to some reports the cytoplasm of the oxyntic cells in dogs contains neutral mucopolysaccharide [10, 16], while pepsinogen granules in the chief cells are bound with sulfonated mucopolysaccharide [1, 7, 10, 16]. However, the role and functional changes of these carbohydrate-containing biopolymers (CCB) are unknown.

This paper describes the results of a histochemical investigation of the CCB in the oxyntic and chief cells of the dog gastric mucosa under normal conditions and during prolonged (for several days) histamine stimulation: changes in the histochemical properties of the cells were compared with their functional activity and number. These problems have not been examined before from this standpoint.

EXPERIMENTAL METHOD

Long-term experiments were carried out on four healthy adult mongrel male dogs weighing from 14 to 18.9 kg, with a Basow gastric fistula. Systematic intramuscular injections of a histamine-wax mixture, prepared by Gottschalk's method [9], began after a study of the secretory function of the stomach and the taking of biopsy material. The histamine-wax mixture in a dose equivalent to 30 mg histamine base, was injected daily in the evening for 11-61 days. Every 3-6 days the secretory activity of the stomach was investigated and a biopsy specimen, consisting of the whole thickness of the gastric mucosa, was obtained from the region of the middle third of the body of the stomach through the fistula. Altogether 32 gastric biopsies were performed.* Observations were made on the activity of the gastric glands for 1 h, after which histamine dihydrochloride was injected subcutaneously in a dose inducing maximum secretion of

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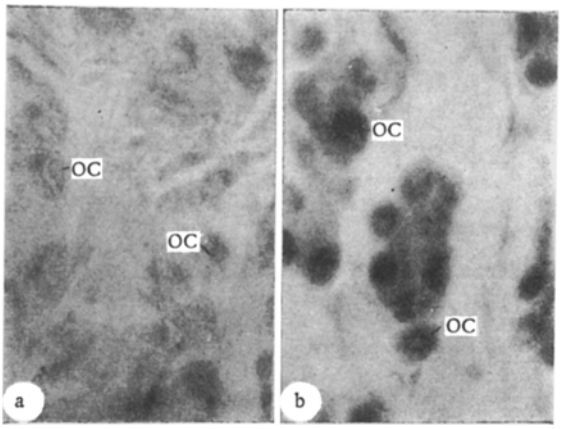

Fig. 1. Changes in PAS-reaction of oxyntic cells of dog gastric mucosa during prolonged histamine stimulation: a) control; b) increase in intensity of PAS-reaction (stage I), 280×.

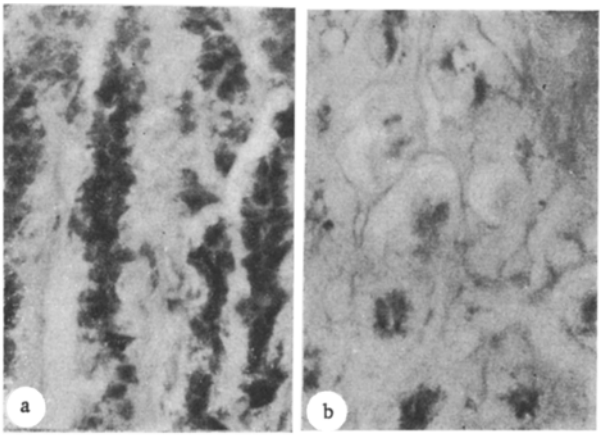

Fig. 2. Changes in basophilia of chief cells of dog gastric mucosa during prolonged histamine stimulation: a) control; b) sharp decrease in content of sulfonated polysaccharides in stage III. Alcian blue, pH 1.0, 280×.

juice, and observations were continued for a further 1.5-2 h; 183 portions of gastric juice were analyzed. The pepsin concentration in the juice was determined by Pyatnitskii's method [3-7]. The basal and maximal production of acid and pepsin per hour were determined (expressed as meq/h and mg/h, respectively).

Pieces of gastric mucosa were fixed in Hamperl's fluid and then embedded in paraffin wax in the usual way. To count the cells a combined stain suggested by M. G. Shubich was used (Feulgen reaction – PAS reaction – active yellow-2K – alcian blue). The cells were counted with an ocular micrometer in a square of side 0.1 mm in the image of the MBR-1 microscope (objective 40×, ocular 15×). A correction was introduced to give the true number of cells [8]:

$$P=\frac{A\cdot M}{L+M},$$

where P is the true number of nuclei, M the thickness of the section (7 μ), A the number of nuclei counted, and L the mean diameter of the nucleus. The results were expressed as the number of cells per unit surface of gastric mucosa ($7\cdot10^{-4}$ mm^2).

Polysaccharides were studied in sections stained by the PAS reaction [5], with alcian blue [14], and with basic brown [6]. Control sections were treated with amylase, with phenylhydrazine [15], and by mild acid hydrolysis [2]. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Before chronic histamine stimulation began, the maximal acid production in the four experimental dogs averaged (x±m) 19.4±5.5 meq/h, the maximal pepsin production was 5.6±2.8 mg/h, the number of oxyntic cells 17.1±8.9, and the number of chief cells 72.9±36.2 per unit surface of gastric mucosa.

The cytoplasm of the oxyntic cells showed absence of basophilia and of all the stains used to demonstrate polysaccharides only the PAS-reaction was positive; usually the staining was weak and diffuse (Fig. 1a). In individual cases the cytoplasm of the oxyntic cells appeared as a mass of large vacuoles, in which the surface of the vacuoles and the small and large granules distributed irregularly throughout the cytoplasm gave a positive reaction. Treatment of the sections with diastase caused little if any change in the intensity of staining of the cytoplasm but sharply reduced the staining of the granules, indicating that they contain glycogen. The residual part of the PAS-reaction was due to the presence of neutral PAS-positive polysaccharide, as confirmed by the complete blocking of the PAS-reaction by phenylhydrazine.

In the apical part of the chief cells of the unstimulated dog stomach staining with hematoxylin-eosin revealed a large quantity of granular secretion. The chief cells were PAS-negative but showed alcianophilia (pH 2.7) and basophilia at pH 1.0 (alcian blue and basic brown) (Fig. 2a). Data relating to the mech-

anism of staining [6, 12] and the resistance of the basophilia to mild acid hydrolysis are evidence that the acid granules thus revealed consist of sulfonated polysaccharides. A partial decrease in the basophilia at pH 1.0 after treatment with hyaluronate liase indicates that these sulfo-groups belong to chondroitin-sulfate A or C, while the residual basophilia was connected with the presence of chondroitin-sulfate B. The alcianophilia (pH 2.7) of the chief cells, which was resistant to mild acid hydrolysis, was evidently due to the carboxyl groups of the sulfonated polysaccharides.

Daily administration of the histamine-wax mixture was accompanied by considerable changes in the morphological and functional properties of the oxyntic and chief cells. Their number and their acid production increased very rapidly to a maximum (which varied in the individual dogs), and then despite continued stimulation it remained at the same level or, more commonly, fell slightly and then fluctuated within limits close to the maximum. The maximum of the hyperplastic and hypersecretory changes in the oxyntic cells was observed 6-16 days after the beginning of administration of the histamine–wax mixture and its parameters were as follows: maximal acid production was increased on the average to 31.9±3.0 meq/h, i.e., it increased by 95.5%; the number of oxyntic cells increased to 50.4±2.9, i.e., by 288%. The number of chief cells reached its maximum at different times in different dogs (between the 6th-45th day of stimulation) and its value was 166.6±9.0, i.e., it was increased on the average by 190%. The pepsin secretion rose sharply: the maximal production of pepsin was increased to 18.4±5.8 mg/h, i.e., by 330.9%. However, the times when the maximum of the hyperplastic and hypersecretory changes was observed in the chief cells coincided: hypersecretion of pepsin was found 11-56 days after the beginning of histamine stimulation and 5-20 days after the number of chief cells reached its maximum.

On the basis of the histochemical results, the reactions of the oxyntic and chief cells to chronic histamine stimulation can be divided into three consecutive stages. In stage I the intensity of staining of the cytoplasm of the oxyntic cells by the PAS method is increased (Fig. 1b), and it can be concluded from the result of treatment with diastase and phenylhydrazine that glycogen and neutral PAS-positive polysaccharides have accumulated. The chief cells are characterized by a relative increase in the content of chondroitin-sulfates and by the appearance of sialomucins. Basophilia at pH 1.0 is found not only in the apical part of the cells, but the whole cytoplasm is filled with numerous darkly stained secretory granules. Sensitivity of the basophilia to hyaluronate liase indicates the presence of sulfonated polysaccharides of the chondroitin-sulfate A or C type. The very weak basophilia remaining in the sections after treatment with hyaluronate liase indicates an increase in the content of chondroitin-sulfates A or C and a decrease in the content of chondroitin-sulfate B. The appearance of alcianophilia (pH 2.7), sensitive to mild acid hydrolysis, reflects accumulation of sialomucins. The changes described are observed during the first 3-26 days of administration of the histamine-wax mixture and they coincide with the time of development of hyperplasia of the chief cells. Pepsin secretion is either unchanged at this time or, more frequently, is reduced.

Stage II corresponds to a state of relatively stable hyperplasia of the oxyntic and chief cells, hypersecretion of acid, and normal or reduced secretion of pepsin. In this stage the content of chondroitin-sulfate A or C and of chondroitin-sulfate B in the chief cells is either the same as or greater than in stage I, while the content of sialomucins is reduced. Changes in the CCB content in the oxyntic cells occur in two periods: a fall and subsequent recovery. In the first period of stage II the PAS-reaction disappears, indicating disappearance of glycogen and neutral polysaccharides, but the CCB content is quickly restored (after 3-9 days) during continuing histamine administration.

In stage III (also against the background of relatively stable hyperplasia of the oxyntic and chief cells), the CCB falls sharply or even disappears (Fig. 2b). This is accompanied by vacuolation of the apical cytoplasm of the chief cells, which is particularly marked in the proximal zones of the fundal glands, and by an increase in pepsin secretion, which rises to a maximum. It is important to note that in the two animals in which the chief cells completely lost their basophilia at pH 1.0 and their alcianophilia (pH 2.7), erosions and ulcers developed during this period.

Recovery of the CCB of the oxyntic cells was not observed until the 3rd-11th day after the end of administration of the histamine-wax mixture. By the 11th day the basophilia (pH 1.0) and alcianophilia (pH 2.7) of the chief cells was gradually restored, indicating partial recovery of the content of chondroitin-sulfates.

The histochemical dynamics of the CCB level as described above agrees with the biochemical observations: under the influence of histamine the biosynthesis of polysaccharides and hexosamines in the rat mucous membrane at first increases, but then falls again during continued stimulation [11].

Comparison of the changes in the number of oxyntic cells, the level of acid secretion, and the CCB content in the cells shows that although the presence of CCB is not an absolutely necessary component for acid production, these compounds play a definite role in the complex mechanism of the hyperplastic and hypersecretory effects of histamine. It is a particularly interesting fact that the accumulation of CCB, especially of sulfonated polysaccharides of the chondroitin-sulfate type, in the chief cells is accompanied by a marked decrease in pepsin secretion (stage I), while an increased content of the enzyme in the gastric juice is observed only when the content of these CCB falls sharply or disappears completely (stage III). Bearing in mind the known antipeptic and antiulcerative action of natural and synthetic sulfonated polysaccharides [4, 13], it can be considered that the sulfonated CCB play the role of regulators of pepsin activity in the chief cells and their secretion, while disappearance of these CCB is probably one cause of the development of erosions and ulcers of the gastric mucosa.

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