QUANTITATIVE CHANGES IN GASTRIC JUICE PROTEASES OF DOGS DURING GASTRIC ULCERATION

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UDC 616.33-002.44-07:616.33-008.831:577.152.344-074

Proteases in the gastric juice of normal dogs and cats with cinchophen-induced gastric ulcer were studied by disk electrophoresis, and ulceration in fasting animals was shown to be accompanied by considerable quantitative changes in the protease spectrum. A generalized reduction in the proteolytic fractions of the gastric juice in response to sham feeding of the dogs during ulcer formation was accompanied by a significant increase in fraction No. 6. Sham feeding of satiated dogs during ulcer formation revealed a significant increase in fractions Nos. 1 and 2. Changes in the protease spectrum of the gastric juice may be of ulcerogenic significance.

KEY WORDS: *gastric ulcer; proteases.*

A special feature of the proteases secreted in the digestive system is their high activity against exogenous substrates associated with evident "indifference" toward endogenous proteins. However, the development of acute pancreatitis following activation of proteases within the pancreas itself has been described [2]. Amirov and Fernandez-Costa [1] have shown that the gastric juice of dogs with gastric ulcer is far more aggressive against the gastric mucosa of rats than the gastric juice of healthy dogs. In patients with peptic ulcer an electrophoretic fraction of pepsin I, rarely found in healthy persons, is usually detected [6, 7, 13]. The proteolytic activity of the gastric juice, which normally has two pH maxima, develops a third during ulceration, a little toward the acid side [1, 6]. The successful treatment of gastric ulcer by pepsin inhibitors [8, 10] is also evidence of the possible role of the gastric proteases in the pathogenesis of ulceration.

The protease spectrum of the gastric juice of dogs was investigated in the acute period of ulcer formation, by the use of a highly sensitive disk-electrophoresis method.

EXPERIMENTAL METHOD

Experiments were carried out on three dogs with a Basov's gastric fistula. In the experiments of series I (34 normal dogs, 40 animals developing ulcers) the animals were deprived of food for 18-20 h before the experiment. Water was not restricted. The gastric juice was collected for 2 h under sham feeding conditions with raw meat (75 g). The experiments of series II (15 normal dogs, 38 developing ulcers) were carried out by the following scheme: The stomach was flushed out, the fistula closed, the dogs were fed with 75 g meat, and 2 h later the fistula was opened, the gastric contents washed out with warm water, and sham feeding carried out with a fresh portion of meat (75 g). Gastric ulcers were produced in the dogs by daily administration of 0.25 g/kg cinchophen, as an aqueous suspension, through the gastric fistula. The severity of the gastric lesion was judged from the appearance of latent or visible blood and also by visual examination through the lumen of the fistula.

The samples of gastric juice were kept at 2-4°C. Before the investigation the juice was concentrated 20 times on Sephadex G-25. For the electrophoretic fractionation [9] of

Central Scientific-Research Institute of Gastroenterology, Main Health Board, Moscow City Executive Committee. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 408-411, April, 1977. Original article submitted July 4, 1976.

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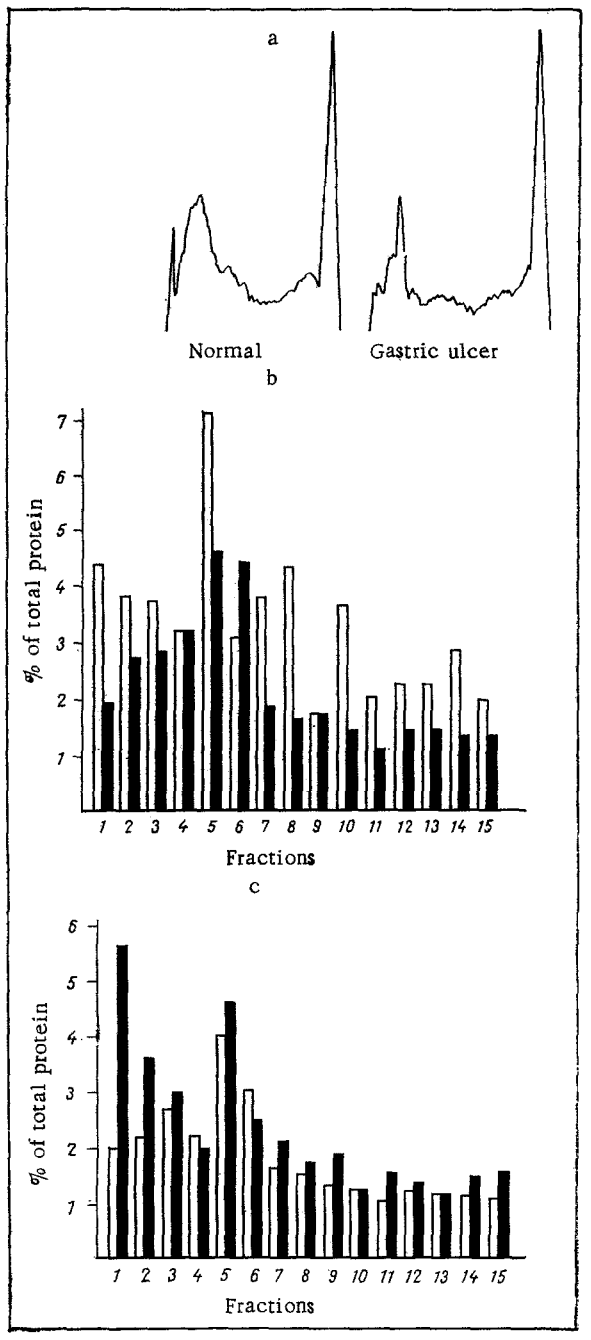

Fig. 1

the gastric juice proteases, 7.5% polyacrylamide gel, pH 8.9, was used. The concentrated samples of gastric juice were applied to the gel mixed with 40% sucrose in the ratio of 2:1. For the first 15 min the current used for electrophoresis was 2-3 mA, and this was later increased to 8 mA per tube. At the end of electrophoresis the gel columns were fixed in TCA, stained with Coomassie R-250, and examined on the ERL 65 (Carl Zeiss) densitometer. The protein content in the individual fractions was determined by differentiating the area of the peaks on the densitogram, and expressed as a percentage of the total area of the protein peaks. After electrophoresis the gels were incubated with hemoglobin in the presence of 0.2 M HCl, fixed, washed, and stained with Amido Black 10B. The zones of activity consisted of light bands against a general dark background. Coincidence between the zones of proteolytic activity and the protein fractions, revealed by staining with Coomassie, showed that the system of disk electrophoresis used did not cause irreversible denaturation of the acid proteases. This is in agreement with data showing the relatively high stability of pepsins in neutral and weakly alkaline media [11, 12]. The proteolytic activity of the gastric juice was determined by a modified hemoglobin method [3] and expressed in conventional extinction units.

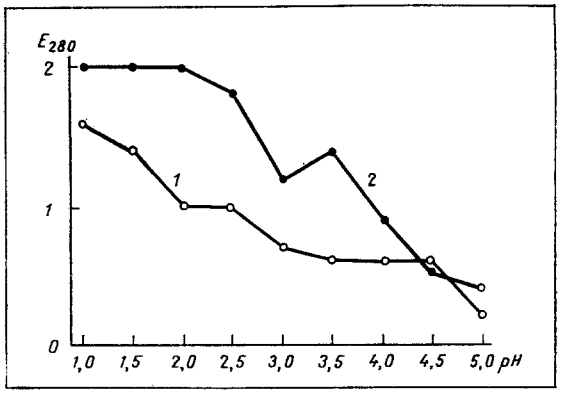

Fig. 2. The pH curves of activity of gastric juice of normal dogs (1) and of dogs with gastric ulcer (2).

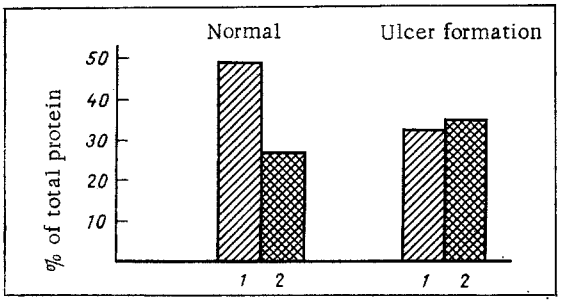

Fig. 3. Content of proteases in gastric juice of "fasting" (1) and "satiated" (2) dogs under normal conditions and during ulcer formation.

EXPERIMENTAL RESULTS

In the experiments with sham feeding of the "fasting" dogs proteolytic activity was exhibited by about 15 fractions of gastric juice located in the zone of "fast" proteins and accounting for 49.6% of all the protein added under normal conditions and 32.2% in the case of cinchophen ulcer.

Typical densitograms of fractions of gastric juice from normal dogs and dogs with gastric ulcer are given in Fig. 1a. The significant decrease in the content of proteases in the gastric juice during ulceration will be evident (Fig. 1b). This decrease ($P < 0,05$) affected all proteases except those contained in fractions Nos. 4 and 9. It is particularly important to note that the protein content in fraction No. 6 was actually considerably increased (by 43%). The sharp decrease in the protein content in the protease fractions was accompanied by a marked fall in the activity of the gastric juice during ulcer formation (by 34%). However, against the general background of a decrease in the activity of the juice, it was possible to discern some increase in proteolysis at pH 4,5 and a tendency toward the formation of a third peak of activity at pH 4,0-4,5 (Fig. 2).

In the experiments with sham feeding of the "satiated" dogs a statistically significant ($P < 0,05$) increase in the protein content was found during ulcer formation in fractions Nos. 1 and 2 of the gastric juice (Fig. 1c). The content of proteases, as a percentage of the total protein, during ulcer formation was almost identical in the fasting and satiated dogs (32,2 and 35%, respectively); moreover, it was only a little higher than the protease content in the gastric juice of the satiated normal dogs (26,3%), but distinctly higher than in the gastric juice of the fasting normal dogs (49,6%) (Fig. 3).

The results are evidence that the process of ulcer formation, inevitably associated with disturbance of the ionic transport of the gastric mucosa [5], disturbs the synthesis and accumulation of enzymes and this is reflected in the total content of proteases in the

gastric juices. With a decrease in the relative proportion of proteases in the gastric secretion, its proteolytic activity is correspondingly reduced. Considering the weakly alkaline reaction of most food products, this suggests that mainly proteases whose maximum of activity is shifted into the weakly acid region act in the initial stages of gastric digestion. The secretion of HCl and the amino acids liberated during proteolysis lead to acidification of the medium sufficient to activate other, more acidophilic proteases [4].

As the chief cells become depleted of their zymogen granules the synthesis of proteases for the second, humoral, phase of secretion is intensified. Their liberation can be stimulated by triggering the vagal mechanism of secretion again, as occurs during sham feeding of the "satiated" animals. In healthy "fasting" dogs a natural decrease in the secretion of proteases in the humoral phase compared with the conditioned-reflex phase was observed. During ulcer formation, however, the total content of proteases in the first and second phases was equalized in the "fasting" and "satiated" dogs on account of a decrease in enzyme secretion in the first phase and an increase in the second. The character of distribution of the proteases among the fractions in the second phase during ulcer formation differed significantly from that in the first phase. Considering the disturbance of the barrier of the gastric mucosa during ulcer formation, accompanied by increased H^+ transport in the opposite direction, it can be postulated that some proteases behave aggressively toward the tissues of the stomach. They must have their pH optimum in the weakly acid region, as is confirmed indirectly by the appearance of a third peak of activity of the gastric juice in the region of pH 4.5. Protease in fraction No. 6, which is considerably increased during ulcer formation, may play this role.

Ulcer formation in the stomach is thus evidently closely connected with a disturbance of the relative proportions of acid proteases of the gastric juice.

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