Fixation stress in intact rats, incidentally, was accompanied by extensive venoconstriction [4], whereas the same stress 6 days after ligation of the bile duct was characterized by signs of extensive venodilatation. Obturation of the biliary tract thus qualitatively changed the response of the capacitive function of the circulation to fixation stress. Instead of a stage of its intensification, with extensive venoconstriction, in response to fixation of intact animals, a stage of its exhaustion was observed, with signs of extensive venodilatation [2] in rats fixed after obturation of the biliary tract for 6 days. Under the same conditions regional changes in tone of the resistive vessels did not undergo qualitative changes. Predominant constriction of the arteries of the descending aorta during fixation of intact rats [5] under conditions of obturation of the biliary tract was supplemented by a relative decrease in the lumen of the resistive vessels in some other parts of the skin-muscle region and in some abdominal organs.

LITERATURE CITED

1. O. A. Kovalev, Patol. Fiziol., No. 3, 84 (1977).
2. O. A. Kovalev, Cor Vasa, 20, 230 (1978).
3. O. A. Kovalev and O. N. Nepochatov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 3, 425 (1980).
4. O. A. Kovalev and S. K. Sheremetevskaya, Fiziol. Zh. SSSR, No. 7, 1021 (1977).
5. O. A. Kovalev and S. K. Sheremetevskaya, Fiziol. Zh. SSSR, No. 7, 95 (1980).
6. R. I. Novikova, A. F. Gredzhev, K. Z. Minina, et al., Vestn. Khir., No. 5, 113 (1979).
7. S. A. Seleznev, S. M. Vashetina, and G. S. Mazurkevich, Combined Evaluation of the Circulation of the Blood in Experimental Pathology [in Russian], Leningrad (1976).
8. B. I. Tkachenko, The Venous Circulation [in Russian], Leningrad (1979).
9. S. K. Sheremetevskaya and O. N. Nepochatov, in: Systemic Reactions of the Organism under Pathological Conditions [in Russian], Leningrad (1979), pp. 24-25.
10. O. S. Better, G. A. Aisenbergs, T. Berl, et al., Clin. Sci., 58, 493 (1980).
11. D. S. Bloom, L. Bomzon, C. Rozendorff, et al., Clin. Sci. Mol. Med., 37, 1P (1974).
12. D. S. Bloom, B. H. Eidelman, and T. A. McCalden, Gut, 16, 732 (1975).
13. L. Bomzon, P. B. Wilson, and T. A. McCalden, Clin. Sci. Mol. Med., 55, 109 (1978).
14. D. R. Hunt, Aust. N. Z. J. Surg., 49, 733 (1979).
15. A. Latzina, H. Brown and M. E. Brown, Surg. Forum, 19, 340 (1968).
16. A. M. Rudolf and M. A. Heymann, Circ. Res., 21, 163 (1967).
17. K. Sacoda and M. Atic, Am. Surg., 36, 731 (1970).
18. L. A. Sapirstein, Gastroenterology, 52, No. 2, 365 (1967).

CHANGES IN THE GASTRIC MUCOSA OF RATS AFTER INTRAGASTRIC INJECTION OF GASTRIC JUICE FROM HEALTHY SUBJECTS AND PATIENTS WITH DUODENAL ULCER

N. Sh. Amirov and N. I. Belostotskii

UDC 616.33-018.73-02:[616.33-008.8-02:616.342-002.44]-092.9

KEY WORDS: ulcer formation; proteolytic enzymes; glycosaminoglycans; lysosomal enzymes.

An important role in the development of lesions of the gastric and duodenal mucosa during ulcer formation is played by the gastric juice, which exhibits increased aggressiveness toward the gastric mucosa in patients with peptic ulcer [1, 4, 5]. Ulceration of the gastric mucosa may be facilitated by the lowering of its resistance possibly on account of diminished production of components forming a protective covering for the stomach [10, 11] and also with a disturbance of the structure of the protective gel [10].

Laboratory of Pathophysiology, Central Research Institute of Gastroenterology, Moscow.

[Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh (Deceased).] Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 17-19, November, 1982. Original article submitted June 14, 1982.

0007-4888/82/9411-1483$07.50

TABLE 1. Number of Animals With Changes in Their Gastric Mucosa (in % of total number of animals in group)

Changes in mucosa	Control	Experiment	P
Hyperemia	44	68	$<0,05$
Hemmorrhages	40	68	$<0,05$
Erosions	24 (0,48)	56 (1,24)	$<0,02$
Ulcers	0	16 (0,16)	$<0,001$

Legend. Erosion and ulcer indices given in parentheses (ratio of number of erosions and ulcers to number of animals in group).

The object of this investigation was to study changes in degradation of substrate protein by gastric mucosal enzymes at different pH values and to compare these changes with lesions in the mucosa and with the electrophoretic picture of proteins, glycoproteins, and glycosaminoglycans in mucosal extracts of rats after intragastric injection of gastric juice from healthy human subjects (control) and patients with duodenal ulcer (experiment). The proteolytic activity of gastric mucosal extracts of the experimental animals also was compared with the autolytic activity of these extracts against autologous mucosal proteins on acidification of the extracts to different pH values.

EXPERIMENTAL METHOD

The experimental material (gastric juice) was obtained from 25 healthy subjects and 25 patients with duodenal ulcer. Histamine-stimulated gastric juice was tested because it was shown previously that in response to histamine stimulation the aggressiveness of the gastric juice is manifested more clearly. Gastric juice, pH 4.5-5.0, was injected in a dose of 2 ml by the intragastric route [6]. The animals were killed 24 h after injection of the gastric juice. The mucosa was examined under a binocular loupe and the presence of hemorrhages (multiple and single), hyperemia, erosions, and ulcers and the number of the latter were recorded. After the changes had been recorded the mucosa was stripped off and homogenized in a glass homogenizer with the addition of distilled water (1 ml to 100 mg tissue) and centrifuged at 8000 rpm for 30 min. Proteolytic activity was then determined in the supernatant fraction at different pH values, using a 1% solution of dry bovine serum as the substrate, and autolytic activity of proteins of the homogenates was determined at the same pH values and after the same period of incubation (10 min). Proteolytic activity was determined by Kunitz' method [9] and the amount of protein hydrolyzed was determined from a calibration curve and expressed in micrograms of protein hydrolyzed during the experiments. Electrophoretic investigation of samples of mucosal extracts was carried out on 7.5% polyacrylamide gel [8]. Protein, glycoprotein, and glycosaminoglycan fractions were determined by a specific staining method [8]. Zones of proteolysis were found by the method described previously [3]. The numerical results were subjected to statistical analysis by the use of nonparametric criteria for tied samples [7]. Altogether 25 control experiments and 25 experiments with injection of gastric juice from duodenal ulcer patients were carried out.

EXPERIMENTAL RESULTS

In animals receiving gastric juice from patients with duodenal ulcer the vascular reaction was more marked: their severe hyperemia, more cases of hemorrhage and more frequent hemorrhages per animal, and an increase in the number of erosions. In the experimental group ulcers were found in 16% of cases, compared with none in the control (Table 1).

Investigation of the proteolytic activity of the gastric mucosal extracts showed that after injection of the patients' gastric juice into rats autolytic processes in the mucosal extracts were distinctly increased compared with the control: autolytic destruction of proteins under the influence of autologous enzymes on acidification of the extracts was intensified. This increase in autolysis was observed at all pH values but was more marked in the weakly acid range. The increase in autolytic destruction of proteins in the gastric mucosal extracts thus took place under experimental conditions both at strongly (1.0-3.0) and at weakly (4.0-6.0) acid pH values. Simultaneously with the changes described above in autolytic

TABLE 2. Changes in Proteolytic Activity and Autolysis of Proteins of Rat Gastric Mucosal Extracts after Intragastric Injection of Gastric Juice from Patients with Duodenal Ulcer and Healthy Subjects

Parameter tested	Group of animals	pH						
		1,0	1,5	2,0	3,0	4,0	5,0	6,0
		Proteolysis						
Protein hydrolysis, μg	Control	3900	3895	3925	1160	260	200	320
	Experiment	3764	3380	2900	1150	630	500	400
Changes, % of control	Control	100	100	100	100	100	100	100
	Experiment	96	87	88	99	242	250	125
P		>0,05	0,05	0,05	>0,05	<0,05	<0,05	<0,05
		Autolysis						
Protein hydrolysis, μg	Control	1250	1196	876	610	120	40	40
	Experiment	1436	1328	996	784	130	140	120
Changes, % of control	Control	100	100	100	100	100	100	100
	Experiment	115	111	114	128	108	350	300
P		0,05	0,05	<0,05	0,05	>0,05	<0,05	<0,05

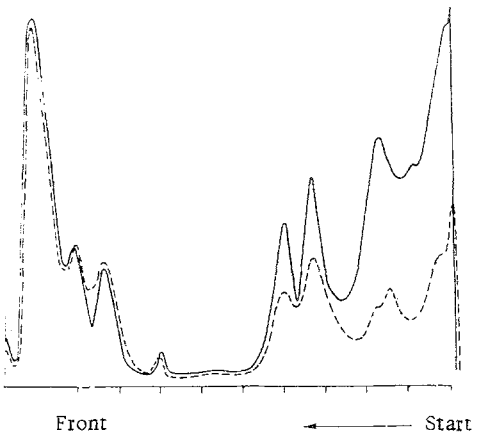

Fig. 1. Electrophoresis of glycosaminoglycan fractions of samples of gastric mucosal homogenates from rats after intragastric injection of gastric juice from healthy subjects (continuous line) and from patients with duodenal ulcer (broken line). Arrow shows direction of movement of fractions.

destruction of gastric mucosal extract proteins a tendency was observed for hydrolysis of substrate protein to be reduced at acid pH values (Table 2). In other words the tendency was for proteolysis of substrate protein and autolysis of mucosal proteins to move in opposite directions at pH 1.0-3.0, i.e., at pH values when pepsins exhibit their activity: an increase in autolytic destruction of mucosal proteins was observed at a time when activity of acid proteases against substrate protein was depressed. Hydrolysis of proteins at weakly acid pH values was increased, as also was autolysis at these same pH values (Table 2).

The protein content in protease fractions of experimental and control animals did not differ significantly. The number of fractions of glycosaminoglycans in mucosal extracts from animals of the experimental group was less than in the control: for instance, on average in the control nine fractions of glycosaminoglycans were found, compared with six in the experiment. The content of glycosaminoglycans also was reduced in the fractions, more especially in those near the starting line compared with enzyme-bound fractions located in the frontal half of the gel (Fig. 1). The content of glycoproteins in the frontal fractions was unchanged, whereas in fractions located in the central part of the gel it was very slightly increased.

Gastric juice from patients with duodenal ulcer thus is significantly more aggressive toward the gastric mucosa of rats after intragastric injection than healthy human gastric

juice. These results agree with those obtained previously when gastric juice from patients with duodenal and gastric ulcer was injected into rats [1, 5, 6]. An important role in this effect is undoubtedly played by proteolytic enzymes [2, 6]. At the same time it must be borne in mind that the gastric mucosal tissue defects in the experimental animals could be due not only to increased aggressiveness of the patients' gastric juice as a result of pathological changes in proteolytic activity of changes in the enzyme spectrum [2], but also to a reorganization of the proteolytic activity of the autologous enzymes of the mucosa under the influence of the injected patient's gastric juice. Activation of proteolysis and autolysis in the weakly acid pH zone observed under these circumstances may be due to lysosomal enzymes [12]. The increase in mucosal extract protein degradation on acidification to pH 1.0-3.0 with a simultaneous lowering of activity of the mucosal pepsins toward substrate protein is evidence of the lowered resistance of the mucosal proteins, and lowering of the resistance of the proteins of the mucosa leads to disturbance of its integrity even when activity of proteolytic enzymes is normal or depressed, as was the case in the presented experiments. An important role in the protection of the mucosa against the harmful effects of proteolytic enzymes and acid is ascribed to the mucosal barrier of the stomach [10]. The reduction in the number of fractions of glycosaminoglycans and of their content in the fractions is evidence of disturbance of the structures protecting the mucosa, and this may lead to the development of mucosal lesions in experimental rats.

LITERATURE CITED

1. N. Sh. Amirov, Byull. Éksp. Biol. Med., No. 12, 101 (1971).
2. N. Sh. Amirov, D. V. Antonov, and I. E. Trubitsyna, in: Current Problems in Gastroenterology [in Russian], Part 8, Moscow (1975), pp. 114-117.
3. N. Sh. Amirov and N. I. Belostotskii, Fiziol. Zh. SSSR, 67, 600 (1981).
4. A. A. Akhlakova and Kh. É. Gadzhiev, in: Proceedings of the 3rd Republican Conference of Daghestan Internists [in Russian], Makhachkala (1976), pp. 88-89.
5. A. A. Akhlakova, "The aggressive properties of the gastric juice in peptic ulcer," Candidate's Dissertation, Makhachkala (1978).
6. N. Sh. Amirov and D. V. Antonov, Byull. Éksp. Biol. Med., No. 3, 300 (1981).
7. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medico-Biological Research [in Russian], Leningrad (1973).
8. H. Maurer, Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis, DeGruyten (1971).
9. A. A. Shelagurov, Diseases of the Pancreas [in Russian], Moscow (1970).
10. A. Allen and A. Garner, Gut, 27, 249 (1980).
11. J. Schrager and M. D. Oates, Br. Med. Bull. 34, 79 (1978).
12. J. C. Delhier and J. C. Soule, Gastroent. Clin. Biol., 5, No. 1, 6A (1981).