It is well known that TEP, for example, of the intestinal epithelium is the difference between membrane potentials of the basolateral and apical surfaces of the epitheliocytes, and that it averages 4-8 mV [6]. It is possible that the TEP recorded in the liver is also the difference of membrane potentials of hepatocytes facing the biliary and sinusoidal capillaries. The fall of TEP of the liver during ischemia and its rapid recovery on resumption of the blood flow evidently reflect depolarization of the hepatocyte membranes in response to ischemia.

An advantage of the use of TEP to assess the state of the liver function is that it can be recorded continuously.

Thus as a result of these investigations it was shown that a TEP exists in the liver between the sinusoidal and biliary capillaries, with a mean value of 10 mV, and highly sensitive to changes in the hepatic blood flow; it also correlates in the character of its responses to changes in blood flow with the responses of the membrane potential of the hepatocytes.

The method of continuous recording of TEP may be promising as a means of assessing the functional state of the liver and its blood flow in vivo.

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LYSOSOMAL ENZYME ACTIVITY IN THE GASTRIC MUCOSA OF RATS WITH EXPERIMENTAL GASTRIC ULCER

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The formation of experimental gastric ulcers is linked with disturbances of the stability of lysosomal membranes. It has been shown [8] that during stress-induced ulcer formation their stability is reduced. The ability of prostaglandin E_1 to prevent ulcer development may be due to its stabilizing effect on lysosomal membranes [9]. Disturbances of the integrity of lysosomal membranes have been found as a result of exposure of the gastric mucosa (GM) to a combination of bile and hydrochloric acid, which was accompanied by the development of damage to GM [15]. Increased acid phosphatase (AP) activity in GM was observed in [2] during the development of experimental ulcer. There is much evidence to show that ulcerogenic factors acting on GM have a labilizing effect on lysosomal membranes [6, 14]. Information on labilization of lysosomal membranes and activation of lysosomal enzymes in the presence of injury to GM provided a basis for formulation of the general hypothesis that lysosomes can participate in the development of gastric ulcer [8, 9, 14, 15]. Some workers, however, deny the pathogenetic role of lysosomes in the development of gastric ulcer and, in particular, of ulcer due to histamine [11].

The aim of this investigation was to study the resistance of lysosomal membranes to mechanical injury during isolation of lysosomes from GM, and the intravital release of lysosomal enzymes from mucosal cells into the lumen of the stomach during the formation of an

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TABLE 1. Number of Animals with Lesions of GM (in %) and Index of Damage to DM in Rats 2 h after Intraperitoneal Injection of Histamine (10 mg/kg) and Serotonin (25 mg/kg)

Parameter	Control	Histamine	Serotonin
Number of animals with lesions of GM, % of total number of animals in group	8	100	100
Index of damage: Area of lesions / Number of animals with lesions	0,54	9,36	6,78

TABLE 2. Changes in Unsedimented Activity, Activity of Lysosomal Fraction, and Activity of Gastric Washings after Intraperitoneal Injection of Histamine (10 mg/kg) and Serotonin (25 mg/kg)

Enzyme	Control			Histamine			Serotonin		
	UA	LS	AW	UA	LS	AW	UA	LS	AW
AP	0,2	0,08	0	0,29	0,06	0,05	0,26	0,05	0,08
p	—	—	—	0,05	0,05	—	0,05	0,05	—
Elastase	0,012	0,022	0,06	0,072	0,002	0,041	0,027	0	0,036
p	—	—	—	<0,05	<0,05	<0,05	<0,05	—	<0,05
Cathepsin:									
G	0,042	0,037	0,017	0,082	0,037	0,082	0,042	0,007	0,032
p	—	—	—	<0,05	—	<0,05	—	<0,05	<0,05
B	0,36	0,07	0,14	0,52	0,04	0,32	0,46	0,01	0,14
p	—	—	—	<0,05	0,05	<0,05	0,05	<0,05	—
D	0,79	0,26	0,35	1,09	0,16	0,82	0,98	0,01	0,4
p	—	—	—	<0,05	<0,05	<0,05	<0,05	0,05	<0,05
E	0,98	0,36	0,37	1,31	0,48	0,82	1,1	0,19	0,36
p	—	—	—	<0,05	0,05	<0,05	>0,05	0,05	>0,05

Legend. Here and in Table 3: UA) unsedimented activity, LS) activity of lysosomal fraction, AW) activity of washings.

acetate ulcer, formed 30 min after application of acetic acid, and in erosive lesions of GM after intraperitoneal injection of histamine and serotonin.

EXPERIMENTAL METHOD

Experiments were carried out on 60 albino rats weighing 200 g. Before the experiments the rats were deprived of food for 24 h but allowed free access to water. There were three series of experiments: I) the formation of an acetate ulcer by Okabe's method [13], II) intraperitoneal injection of histamine in a dose of 10 mg/kg, III) intraperitoneal injection of serotonin in a dose of 25 mg/kg combined with ligation of the pylorus. The animals were killed 30 min after application of acetic acid and 3 h after injection of serotonin or histamine. Before sacrifice, under ether anesthesia, laparotomy was performed on the rats and 2 ml of physiological saline was injected into the stomach; the solution was later withdrawn into a test tube. After injection of histamine or serotonin the number of rats with lesions of the mucosa was counted and expressed as a percentage of the total number of animals. The index of involvement of the mucosa also was calculated: total area of damage to GM divided by the number of animals with lesions of the mucosa. After sacrifice of the animals the stomach was washed out with ice-cold physiological saline, and dried with filter paper. GM was dissected with a scalpel and a homogenate of it prepared in 0.25 M sucrose solution with 0.001 M EDTA. Homogenization was carried out in a glass homogenizer with teflon pestle (gap 0.16 mm), by hand with 10 excursions of the pestle at 4°C. Centrifugation was carried out by the scheme described previously [5]. Activity of the lysosomal enzymes was determined in gastric washings, in the supernatant after centrifugation containing unsedimented enzymes, and in the lysosomal fraction. The lysosomal membranes were disintegrated by freezing and thawing of the lysosomal fraction 10 times. AP activity was determined at pH 5.0 and activity of proteolytic enzymes at various pH values. To determine AP activity, 4-nitrophenyl phosphate was used as

TABLE 3. Changes in Activity of Lysosomal Enzymes in Different Fractions in GM Homogenates from Rats with Acetate-Induced Ulcer

Enzyme	Control			Acetate-induced ulcer				
				zone of ulcer		intact GM		AW
	UA	LS	AW	UA	LS	UA	LS	
AP	0,13	0,03	0	0,16	0,01	0,3	0,09	0,05
p	—	—	—	0,05	0,05	<0,05	<0,05	—
Elastase	0,01	0,01	0	0,01	0,03	0,05	0	0,06
p	—	—	—	—	0,05	<0,05	<0,05	—
Cathepsin G	0,01	0,01	0	0,06	0	0	0,28	0,06
p	—	—	—	<0,05	<0,05	—	<0,05	—
B	0,12	0,02	0,02	0,17	0,03	0,23	0,04	0,08
p	—	—	—	>0,05	0,05	0,05	0,05	<0,05
D	0,64	0,2	0,18	0,77	0,22	0,95	0,34	0,42
p	—	—	—	0,05	>0,05	<0,05	<0,05	<0,05
E	0,75	0,32	0,12	0,75	0,35	0,76	0,44	0,32
p	—	—	—	—	>0,05	>0,05	<0,05	<0,05

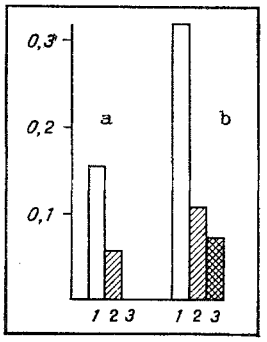

Fig. 1. Changes in AP activity of the stomach 30 min after application of acetic acid to the serous membrane. 1) Activity of unsedimented enzymes, 2) activity of lysosomal fraction, 3) activity of washings; a) control, b) experiment.

the substrate. The results were expressed in micromoles of 4-nitrophenol liberated during the reaction (30 min). Proteolytic activity was determined with the use of a 1% solution of hemoglobin as the substrate; elastase activity at pH 8.5, neutral proteinase (cathepsin G) at pH 7.0, and cathepsins B, E, and D at pH 4.3, 3.5, and 2.0, respectively. The results were expressed in extinction units with measurement of optical density of the solution after precipitation of proteins with 10% TCA solution (λ = 280 nm). For isolation of lysosomes material was taken from five animals. The damage to GM was assessed in each animal. The significance of changes was determined by nonparametric tests [4].

EXPERIMENTAL RESULTS

Injection of histamine into rats with a ligated pylorus was accompanied by an increase in the index of damage to GM (Table 1). After injection of serotonin the number of animals with mucosal lesions increased, as also did the index of damage to GM. Injection of histamine was followed by increased activity of the unsedimented enzymes and release of lysosomal enzymes into the lumen of the stomach (Table 2). After injection of serotonin, increased activity of the unsedimented lysosomal enzymes (AP, elastase, cathepsins B and D) was observed, accompanied by a decrease in enzyme activity in the lysosomal fraction, which does not contradict previous data [8, 9] and is evidence of increased fragility of the lysosomal membranes [5]. Comparison of the effects of histamine and serotonin showed a stronger effect of histamine on enzyme release from the lysosomes, evidently due to the indirect action of serotonin [1]. The ulcerogenic effect of histamine on GM is probably a combination of its labilizing action on the lysosomal membranes and its effect on secretion and on the microcirculation [6, 7, 10]. It must be noted that, by contrast with serotonin, histamine did not reduce enzyme activity in the lysosomal fraction significantly. In the animals with acetate-induced ulcer (30th minute) the resistance of the lysosomal membranes isolated from the mucosa not only in the zone of the newly formed ulcer, but also in the surrounding intact GM (Table 3) was reduced,

and as a result, increased release of enzymes took place from the lysosomes and cytosol into the lumen of the stomach (Fig. 1). These data confirm the previous hypothesis that the lysosomal apparatus of the entire mucosa is involved in ulceration of the stomach [3].

The development of gastric ulcers, whether induced by application of acetic acid to the serous membrane or by injection of pharmacological doses of histamine and serotonin, is thus accompanied by increased release of lysosomal enzymes from the lysosomes into the cell cytosol, and then into the lumen of the stomach. This process is responsible for the initial formation of the ulcer defect of GM, and subsequent involvement of the system of secretory proteases [3] ensures the final formation of the ulcer defect in the mucosa.

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