TRYPSIN ACTIVITY IN CHYME AND JUXTAMURAL LAYER OF MUCUS IN EXPERIMENTAL BLOCKING OF THE BRUNNER'S GLANDS REGION OF THE RAT DUODENUM

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The secretion of the duodenal glands is a glycoprotein-rich mucus, which is incorporated into the composition of the chyme and of the supraepithelial layer of mucous deposits, readily changes into a gel during acidification of the enteral medium [6, 8]. It has been suggested [5] that this secretion may participate in the formation of the floccular structures of the chyme, which adsorb digestive enzymes and form an enteral medium with a large active surface [1]. It must be expected that reduction of the secretory activity of the duodenal glands or the complete exclusion of their secretion from processes connected with digestion may cause a change in the structure of the enteral medium and, as a result, a disturbance of its sorption properties. The aim of this investigation was to test this hypothesis by measuring activity of trypsin in different fractions of the contents of the duodenum after experimental blocking of that region of it which contains Brunner's glands.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g. In the experiments of series I, the Brunner's glands region of the duodenum was blocked by resection of its cranial part 1.5 cm long, corresponding in rats to the zone of distribution of the duodenal glands [4], with subsequent formation of an anastomosis between the stomach and intestine. Rats undergoing a mock operation and rats in which the Brunner's glands region of the duodenum was preserved, but a bypass gastroduodenostomy was formed (Fig. 1), served as the control. Trypsin activity in the animals of this series was measured 2 months after the operation. In the experiments of series II, on the anesthetized animals (0.4 ml of a 5% solution of hexobarbital intraperitoneally), after midline laparotomy, a ligature was applied to the duodenum below the zone of Brunner's glands. On animals of the control group, ligation was carried out at the level of the pyloric part of the stomach (Fig. 2). After application of the ligatures, secretion of the glands was stimulated by application of 0.2 ml of a 0.1% solution of pilocarpine to the intestine, and trypsin activity was determined in the test fractions 30-40 min later. The fractions of chyme and juxtamural mucus were obtained from standard segments of the duodenum from animals deprived of food for 12 h previously by the method in [2]. In the experiments of series I activity of the enzymes also was studied in pancreatic homogenates prepared by the usual method. Trypsin activity in all the fractions was determined by the method in [7]. The number of animals studied in each series of experiments is indicated in Table 1. Histological verification of the completeness of removal of the duodenal glands in the experiments of series I and also of the region of application of the ligature to the intestine in series II was carried out on paraffin sections through the organ, stained by the PAS reaction. The functional state of the duodenal glands in the two control groups of animals of series I (undergoing the mock operation or gastroduodenostomy) was evaluated from the concentration of PAS-positive secretion in the cytoplasm and of RNA

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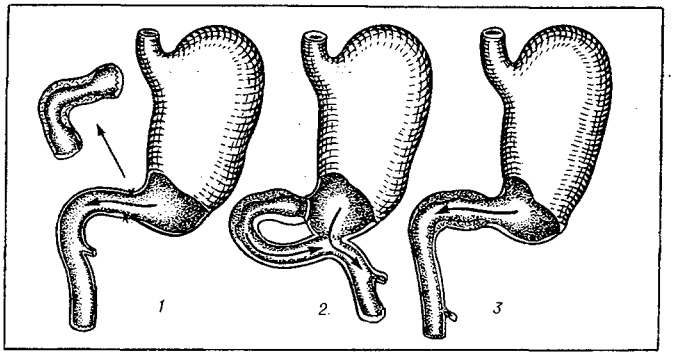

Fig. 1. Diagram showing operations performed: 1) resection of Brunner's glands region; 2) bypass gastroduodenostomy; 3) mock operation.

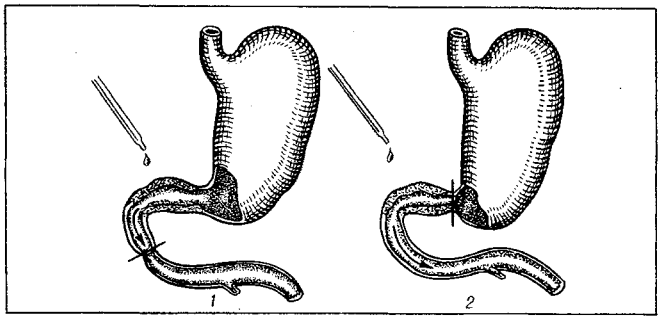

Fig. 2. Diagram of acute experiment: 1) ligation of duodenum distally to Brunner's glands region + pilocarpine; 2) ligation of duodenum on boundary with pylorus + pilocarpine.

in the nucleoli of the granulocytes, stained with gallocyanin and chrome alum. Concentrations of these substances were judged from the results of cytophotometric measurement of transmittance in the corresponding structures by the method in [3]. The results were subjected to statistical analysis by the Student–Fisher test.

EXPERIMENTAL RESULTS

On histological study of the zone of anastomosis 2 months after resection of the Brunner's glands region of the duodenum, no duodenal glands were found in the submucosa of the duodenum. Consequently, in the experimental group the passage of secretion of the duodenal glands into the enteral medium and its possible influence on trypsin activity in the corresponding fractions of the duodenal contents were totally ruled out. In rats with a bypass gastroduodenostomy, compared with animals undergoing the mock operation, there was only a very small and not significant increase in the fraction of cells characterized by lower optical density of their nucleoli (Fig. 3). However, the mean values of the RNA concentration in the nucleoli did not differ significantly. No difference likewise was found in the concentration of PAS-positive secretion in the glandular cells of animals of the two control groups. Consequently, blocking of the Brunner's glands region of the duodenum from the passage of chyme by the formation of a bypass gastroduodenostomy caused no significant changes in functional activity of the duodenal glands.

Trypsin activity in fractions of the chyme and juxtamural mucus was significantly lower in rats with a resected Brunner's glands region of the duodenum than in the control groups (Table 1). Activity of the enzyme in the chyme was 53.1% of its level in rats undergoing the mock operation and 52.4% of the value obtained in animals undergoing gastroduodenostomy. In the fraction of the juxtamural layer of mucus this value was 59.1 and 64.4% respectively of the values in the two control groups. The changes discovered are evidently unconnected with a disturbance of pancreatic function in response to the operation, for no significant differences in trypsin activity were found in homogenates of the pancreas obtained from animals of the experimental and control groups.

TABLE 1. Trypsin Activity (in nmoles/mg protein/min) in Chyme, Juxtamural Layer of Mucus, and Pancreas in Control and Experimental Groups (M ± m)

Series of experiments and groups of animals	Number of animals	Trypsin activity in fractions		
		chyme	layer of mucus	pancreas
Series I				
Experiment: resection of Brunner's glands	13	28,2±3,6	10,3±1,3	61,7±5,1
Control				
rats undergoing mock operation	13	53,1±6,5**	17,2±2,2*	71,1±9,0
gastroduodenostomy	13	53,8±9,5*	16,0—2,3*	61,9—12,3
Series II				
Experiment: ligation of duodenum distally	9	27,2±9,3	8,6±2,3	—
Control: ligation of duodenum on boundary	9	69,2±8,8**	27,0±5,4**	—

Legend. Asterisks indicate differences between parameters in experiment and control: *$p < 0.05$, **$p < 0.01$.

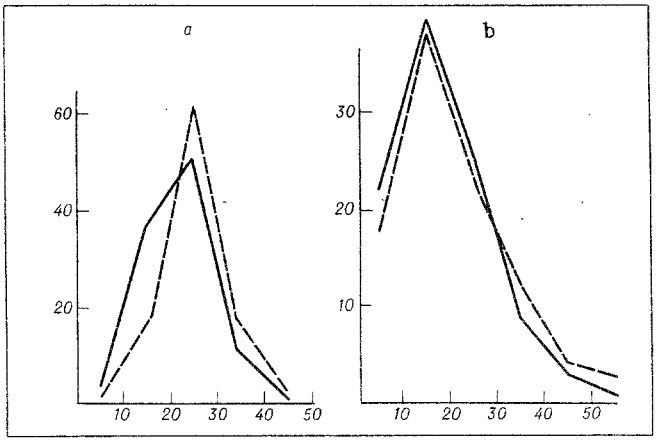

Fig. 3. Distribution of duodenal gland cells by optical density of nucleoli (a) and cytoplasm (b) in rats undergoing the mock operation (continuous line) and in animals undergoing gastroduodenostomy (broken line). Abscissa, transmittance, in conventional units; ordinate, fraction of cells.

In the experiments of series II, on rats deprived of food before the experiment began, accumulation of a large quantity of secretory material was observed in the cells of the duodenal glands. After application of pilocarpine solution to the duodenum the quantity of PAS-positive secretion in the cytoplasm of the cells was reduced, evidence of its release into the lumen of the intestine (Fig. 4). On histological investigation of samples of duodenum from animals of the experimental group, in all cases the ligature was found to be distally to the zone of the duodenal glands, and it consequently prevented access of their secretion into the distal part of the duodenum, from which the corresponding fractions were obtained. By comparison with the control group, in the experimental animals a statistically significant decrease in trypsin activity was observed, both in the chyme fraction and in the juxtamural layer of mucus (Table 1). The changes were more marked than the results obtained in the experiments of series I. Trypsin activity amounted to 39.3% of the level in the control animals in the chyme and 31.9% in the fraction of juxtamural mucus.

It can be tentatively suggested that changes in trypsin activity discovered in the two series of experiments are the result of weakening of the sorption properties of the structures of the chyme and of the supraepithelial layer of mucus, arising as a result of the absence of secretion of the duodenal glands in their composition. Another possibility is that the secretion of the glands contains factors promoting activation of the enzyme as it arrives from the pancreas in the intestine in the form of trypsinogen. It has been shown [9] that the intestinal juice collected through a fistula leading to the Brunner's glands region of the duodenum contains large quantities of enterokinase. Direct proof of the formation of this enzyme by cells of the duodenal glands is not yet available, and this hypothesis accordingly requires special verification.

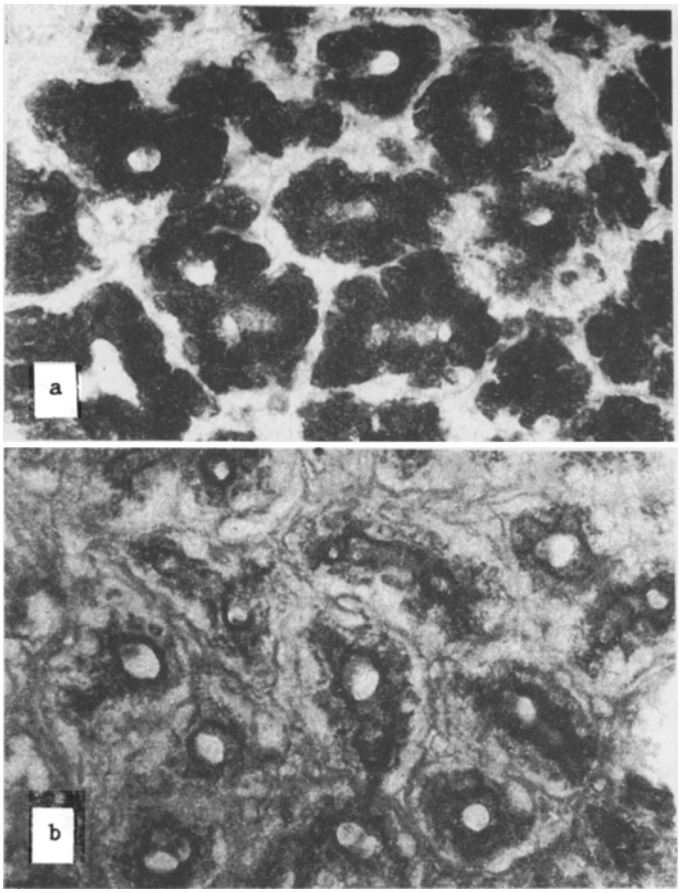

Fig. 4. Content of secretion in duodenal glands of intact rats (a) and 1 h after application of pilocarpine (b). Staining: PAS reaction. Magnification: ×400.

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