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MORPHOLOGICAL ANALYSIS OF ACTIVITY OF MUCUS-PRODUCING STRUCTURES OF THE DUODENUM OF RATS FED WHEAT BRAN OF DIFFERENT PARTICLE SIZE

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It was shown previously that an increase in the cellulose content, in the form of addition of wheat bran, in the diet of laboratory rats for a sufficiently long period leads to an increase in the degree of development of the duodenal glands [3]. These experimental conditons also were shown to stimulate secretory activity of the duodenal glands and also of the goblet cells of the intestinal mucosa [4]. These changes correlated with a change in pH of the chyme toward the acid side. It was suggested that this state of affairs could be one cause of morphological and functional changes discovered. Meanwhile the role of the mechanical factor in the realization of the secretory response of the mucus-producing cells due to the passage of chyme containing fairly coarse bran particles along the intestine, cannot be ruled out. The duodenal glands are known to secrete not only in response to the introduction of hydrochloric acid solutions into the intestinal lumen, but also in response to direct mechanical stimulation of its inner surface [6]. The aim of this investigation was to assess the possible effect of the mechanical stimulation of the duodenal mucosa factor on the morphological and functional state of mucus-producing structures contained in it.

EXPERIMENTAL METHOD

Male Wistar rats weighing initially 62.7 g were used. For 3 months the animals, divided into three groups, with five in each group, received a diet identical in nitrogen content (10% protein by calorific value), identical in calorific value (468 kcal/100 g diet), and balanced with regard to mineral and vitamin composition, in unrestricted amounts; the main

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TABLE 1. Optical Density (T) of PAS-Positive Secretion (conventional units) in Mucus-Forming Cells of Duodenum of Rats on a Diet with Addition of Bran of Different Particle Size (M±m)

Group of animals	Goblet cells		Cells of duodenal glands	
	villi	crypts	superficial parts	deep parts
1	12,83±0,36	13,96±0,40	34,69±0,53	24,53±0,43
2	13,82±0,24*	16,44±0,29**	39,55±0,64**	30,85±0,68**
3	15,19±0,26**	15,15±0,30*	37,11±1,21	34,98±1,25**

Legend. *$p<0.05$, **$p<0.001$ Compared with values in group 1.

component of the diet was wheat bran, which differed in weighted mean particle size for each group. The particle size of the diet of the animals of group 1 was under 200 μ, group 2 about 300 μ, and group 3 — 800 μor more. Since in rats bran does not undergo enzymatic hydrolysis in the stomach and subsequently passes along the small intestine [7], it was assumed that the intensity of mechanical stimulation of the intestinal mucosa depends on the degree of dispersion of the bran particles. At the end of the experiment the animals were killed with chloroform, the duodenum removed, and pieces were excised from its proximal end for subsequent histological examination. The rest of the duodenum was used to measure the glanduloduodenal index [4]. The material was fixed in 10% buffered neutral formalin for 48 h. Paraffin sections were cut and stained by the PAS reaction after preliminary treatment or not with phenylhydrazine, and also by a combination of the PAS reaction and alcian blue at pH 2.5. The average number of goblet cells to a villus and in a crypt of the duodenum was estimated, for which purpose no fewer than 30 villi and crypts of each animal were analyzed. The concentration of secretory material in the goblet cells and duodenal glands was estimated as the transmittance (T), measured on a "Reichert" cytophotometer at a wavelength of 550 μ. No fewer than 50 cells in the deep and superficial terminal divisions of the duodenal glands and the same number of goblet cells in the crypts and on the villi were analyzed in each animal. Mean values were compared, allowing for dispersions connected with internal and individual variation [2].

EXPERIMENTAL RESULTS

The glanduloduodenal index, reflecting the extent of the duodenal glands from the pyloric sphincter in the caudal direction relative to the total length of the duodenum, was the same in the animals of all three groups, namely: 9.1 in group 1, 9.1 in group 2, and 9.0 in group 3. Consumption of increased amounts of cellulose by the rats caused an increase in this parameter [3]. Meanwhile, as the experimental results show, a change in particle size of the bran, which was the source of the excess of cellulose in the diet, did not affect the degree of development of the duodenal glands. It had no effect either on the chemical composition of the secretion of the mucus-producing cells. In animals of all groups the cells of the duodenal glands contained neutral, whereas the goblet cells contained neutral and acid glycoproteins.

The mean number of goblet cells in the duodenal crypts was 23.1 ± 0.6 in group 1, 22.4 ± 0.5 in group 2, and 25.1 ± 0.6 in group 3. In the villi the value of this parameter was 33.2 ± 1.4, 46.9 ± 1.8, and 34.5 ± 1.9 respectively. A statistically significant ($p<0.05$) increase in the number of goblet cells on the duodenal villi was observed in the animals of group 2, receiving bran with a particle size of about 300 μ. This was probably due to slowing of migration of cells in the epithelial layer covering the villi rather than to an increase in their proliferative activity, for the number of goblet cells in the crypts was virtually unchanged. This conclusion, at first glance, contradicts the observed increase in the rate of migration of cells in the crypt—villus system in response to the addition of cellulose to the rats' diet [5]. However, it may be that the character of the cellular response of the duodenal mucosa to the stimulating action of bran particles depends on their size. It can be postulated on the basis of these results that finely dispersed (under 200 μ) and coarse (800 μ or more) bran particles contained in the chyme induce more rapid renewal of the goblet cells and, probably, of the whole epithelial layer of the mucosa compared with bran particles of average size.

On measurement of the optical density of the cytoplasm of the goblet cells and cells of the duodenal glands an increase in transmittance was found in those animals whose diet contained the larger bran particles (Table 1). This is evidence of a decrease in concentration of the secretory material in the mucus-producing cells of the duodenum as a result of activation of its elimination. It can be tentatively suggested that mechanical stimulation of the duodenal wall by chyme containing larger bran particles was the cause of the more active secretion of mucus from the goblet cells and duodenal glands. This mucus is necessary for the formation of a more powerful protective layer on the surface of the duodenal mucosa. The response of cells located in the superficial and deep parts of the duodenal glands differed. Cells of the deep terminal parts of the glands responded most intensively to stimulation of the mucosa, as shown by the more active release of secretion from them. It was shown at one time that the content of secretory material is always higher in these terminal parts of the duodenal glands of normal rats than in the superficial parts [1]. Clearly cells in the stage of accumulation of secretion respond first to stimulation, so that there was a greater decrease in the optical density of their cytoplasm. In the animals of groups 2 and 3, compared with those of group 1, it amounted to 25.8 and 42.6% respectively for cells of the deep terminal parts compared with 14 and 7% for the superficial parts. These differences are evidence that the secretory process in the duodenal glands is not synchronized.

The results thus suggest that mechanical stimulation of the duodenal mucosa by bran particles of different size contained in the chyme affects the level of secretion of the mucus-producing cells of the duodenum. More intensive stimulation by large particles potentiates this process. In the duodenal glands cells in the deep terminal parts react more strongly to this stimulation. Changes in particle size of the bran added to the animals' diet did not affect the extent of the glandular area of the duodenum. The composition of the glycoproteins in the mucus secretion of the duodenal glands and goblet cells also was unchanged under these conditions. The rate of renewal of the goblet cells may perhaps depend on the size of the bran particles in the chyme, but this requires further verification.

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