

HISTOCHEMICAL CHANGES IN THE MUCOUS MEMBRANE OF THE GASTRO-INTESTINAL TRACT OF ALBINO RATS DEPRIVED OF WATER

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The morphological and histochemical properties of the mucous membrane of the stomach and small intestine were compared in rats receiving moist food and water and rats kept on a dry diet without water. During water deprivation there is a marked increase in mucin production, and in the stomach this takes place not merely by cells of the glandular layer but also by cells in the neck of the fundal glands. The number of goblet cells in the small intestine is increased.

Physiological investigations have revealed the part played by the gastro-intestinal tract in maintenance of the water balance of the body during water deprivation [1, 3, 4, 6]. However, very few morphological studies of this problem have been undertaken. Only the distribution of zones of glandular secretion in various rodents has been at all carefully studied [2, 5].

In this investigation the histochemical properties of the mucous membrane of the gastro-intestinal tract were studied in animals deprived of water.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing about 200 g.

For 6 days the animals were kept on the same diet: they received oatmeal porridge (moisture content about 9.7%), carrot (moisture content about 87.4%), bread (moisture content about 37.1%), and water. The animals were then divided into two groups: group 1 (control) remained on the same diet, while the animals of group 2 were deprived of water and were given dried food—oatmeal (moisture content about 9.7%), carrot (moisture content about 12%), and bread (moisture content about 12%).

The animals were sacrificed with ether after 6-12 days. A strip along the whole length of the greater curvature of the stomach and part of the small intestine were excised for histological and histochemical investigation. Fixation was carried out in 15% formalin, and the material was embedded in paraffin wax. Sections were stained with hematoxylin-eosin, azure-eosin, by Hale's method, with alcian blue and toluidine blue, by the PAS reaction, and by a reaction for "latent" metachromasia.

EXPERIMENTAL RESULTS

The intermediate portion of the stomach in albino rats is lined by stratified keratinizing epithelium and the secretory portion of the mucous membrane by high prismatic cells, forming a single glandular zone producing a mucous secretion. The product of the secretion of these cells, in its histochemical properties, belongs to the class of neutral mucins. In the region of the gland pits a definite "latent" metachromasia is found, admittedly weak in intensity, yet indicating the presence of bound sialic acid in the composition of the secreted mucoprotein. Evidently, the mucin produced by the cells of the glandular zone may belong to the sialomucin category. Its neutral character is presumably due to subsequent splitting of the sialic acid from the mucoprotein under the influences of the highly acid gastric juices.

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The contents of the cells of the cardiac gland stained weakly with the PAS reaction. The tonal distribution of PAS-positive staining of these glands differed from that of the cells of the glandular zone (more lilac tones were present). These cells stained clearly by Hale's method; no staining whatever was found after treatment of the preparation with hyaluronidase. Since the phenomenon of "latent" metachromasia could not be detected in the cells of the cardiac glands, it may be concluded that the mucoproteins of their acid mucin did not contain sialic acid.

The chief cells of the fundal glands were almost free from Hale-positive material. The intensive basophilia of the chief cells was due to their high content of ribonucleoproteins. Numerous protein granules (containing pepsinogen) were seen in the apical portions of the cells.

The cytoplasm of the parietal cells was oxyphilic and granular in structure. Granules giving a weak reaction for mucoid substances were present in the apical part of the intermediate cells.

The secretory divisions of the pyloric glands are formed mainly on cells resembling the accessory cells of the fundal glands in their morphology. The cells of the pyloric glands stained moderately intensively by Hale's method and with the PAS reaction, indicating the presence of weakly acid mucoproteins present in the secretion of the cardiac glands. However, these or similar mucoproteins were evidently present in the secretion of the pyloric gland cells in lower concentration than in the mucin of the cardiac gland cells.

The mucous membrane of the stomach in the animals receiving a dry diet showed changes which were most marked in the fundal part of the stomach. With the PAS reaction not only the cells of the glandular zone itself stained intensively, but also a large proportion of cells from the neck and adjacent parts of the body of the glands. On the 6th day of water deprivation, intensive "latent" metachromasia was found in the region of the gland pits. By the 12th day it was also observed at the neck of the fundal glands and had become very persistent. The increase in number of cells giving an intensive PAS-positive staining at the expense of cells of the neck and part of the body of the fundal glands may be regarded as a morphological expression of increased mucin production, with involvement of new zones of cells in this process not taking part in mucin production under normal conditions. The intensive "latent" metachromasia of the mucin from these new secretory zones confirmed that the "neutral" mucin of the glandular zone was sialomucin containing bound sialic acid. At the same time the intensive "latent" metachromasia could also serve to some extent as an index of the fall in active acidity of the secretion of the fundal glands.

In other portions of the mucous membrane (in the zones of the cardiac and pyloric glands and in the interstitial tissue) no clearly defined changes could be found.

In the mucous membrane of the small intestine attention was concentrated on the study of goblet cells. With the PAS reaction the cells appeared loosely packed, they stained weakly, and as the goblet cells matured and moved toward the surface of the villi, accumulation of PAS-positive substance took place. Droplets of mucin in such cells appeared dense and compact. The opposite picture was seen after staining by Hale's method: the crypt cells stained more intensively than the goblet cells of the villi, which were very palely stained. However, in the lumen of the intestine above the goblet cells of the villi, intensively stained "caps" of mucin (PAS- and Hale-positive) could be seen. In the course of formation of the goblet cells, mainly substances of acid nature (Hale-positive) were perhaps formed initially, and later a neutral material accumulated, leading to the intensive PAS-positive reaction of the newly formed complex and to a sharply decrease in intensity of staining by Hale's method. In the lumen of the intestine this complex breaks up into the initial PAS- and Hale-positive components. The latter component of mucin is a compound containing hexuronic acids. As formation of the goblet cells continues, sialic acid and ester-bound sulfate groups accumulate in them.

In the intestine of the rats deprived of water the number of goblet cells was sharply increased, and this was especially noticeable in the region of the crypts. Cells containing intensively staining Hale-positive material were present only in the crypts.

In the goblet cells lying at the base of the villi ability to bind colloidal ferric hydroxide was considerably reduced. These cells appeared loosely packed. However, when stained with Schiff's reagent their mucin gave a clear PAS-reaction unlike the control, where the PAS-reaction was clearly defined only in the cells of the villi. At the apex of the villi absolutely no cells with Hale-positive mucin were found. Liberation of mucin from the newly formed goblet cells took place sooner than normally.

The observed changes demonstrate increased mucin production by the goblet cells, shown both by an increase in their total number and in their rate of "maturation."

It may be postulated that the increased mucin production by the gastro-intestinal tract during water deprivation is aimed at compensating for the deficiency of water taken with the food, and it may be regarded as a morpho-histochemical expression of the adaptive reaction.

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