

MORPHOLOGY AND PATHOMORPHOLOGY

CYTOCHEMICAL ANALYSIS OF MUCOPOLYSACCHARIDES IN THE STOMACH OF NORMAL RATS AND OF RATS WITH EXPERIMENTAL HYPO- AND HYPERTHYROIDISM

A. A. Turevskii and S. N. Fedchenko UDC 612.321.1.1:547.963.1+[616.441-008.61+
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An excess of thyroid hormones depresses the synthesis of sulfomucins in the gastric gland cells of albino rats, appreciably inhibits the synthesis of sialomucins, but has little effect on the content of neutral mucopolysaccharides. Thyroidectomy or administration of 6-methylthiouracil leads to an increase in the content of all these compounds.

The role of thyroid hormones in the secretory activity of the stomach and, in particular, their influence on glycoprotein synthesis are still unexplained, and conflicting results have been obtained by different workers. Depression of thyroid function by methylthiouracil has been shown [3] to give good clinical results in patients with chronic gastritis and gastric ulcer, by increasing the content of mucopolysaccharides in the stomach, for these substances are an important protective factor of the organ [1, 6, 11, 12]. Similar results have been obtained in experiments on rats [4], in which a decrease in the number of ulcers produced in the gastric mucosa by Shay's method was observed after administration of methylthiouracil or after thyroidectomy. On the other hand, Lipovskii [5] asserts that thyroidectomy in rats is followed by manifestations of destruction of the glandular apparatus of the stomach and by a sharp decrease in the content of mucoid secretion.

It was accordingly decided to examine the changes in the content of mucopolysaccharides in the gastric mucosa of albino rats with experimental hypo- and hyperthyroidism. Attention was concentrated on analysis of the individual fractions of epithelial mucins: sialomucins, sulfomucins, and neutral mucopolysaccharides. Another feature selected for study was the types of cells which are responsible for the secretion of these compounds, because there is no general agreement in the literature on this question [2].

EXPERIMENTAL METHOD

Hypothyroidism was produced in noninbred albino rats weighing 120-150 g by total thyroidectomy (30 animals) or by daily oral administration of 6-methylthiouracil (6-MTU) for 10 days through a special tube in a dose of 50 mg/100 g body weight (40 animals); hyperthyroidism was produced by administration of thyroid under the same conditions in a dose of 50 mg/100 g body weight (40 animals). For each of the experimental groups 30 control animals of the corresponding sex and weight, kept under the same conditions and receiving the same food, were taken. Near the end of the experiment the rats were kept for 36 h on a minimal diet (water ad lib.), and under ether anesthesia small pieces were excised from the wall of the three different parts of the stomach and immersed in Shabadash's neutral fixative [8]. Paraffin sections were treated by histochemical methods: differential staining of neutral mucopolysaccharides by Shabadash's method, staining for acid mucopolysaccharides with colloidal iron by Hale's method, with toluidine blue at different pH values, and with alcian blue at pH 1.0 and 2.5 by Spicer's method [13]. Control sections were treated with testicular hyaluronidase, with amylase, by vigorous methylation, and by methylation followed by saponification [14]. The state of hypo- and hyperthyroidism was verified by the change in basal metabolism,

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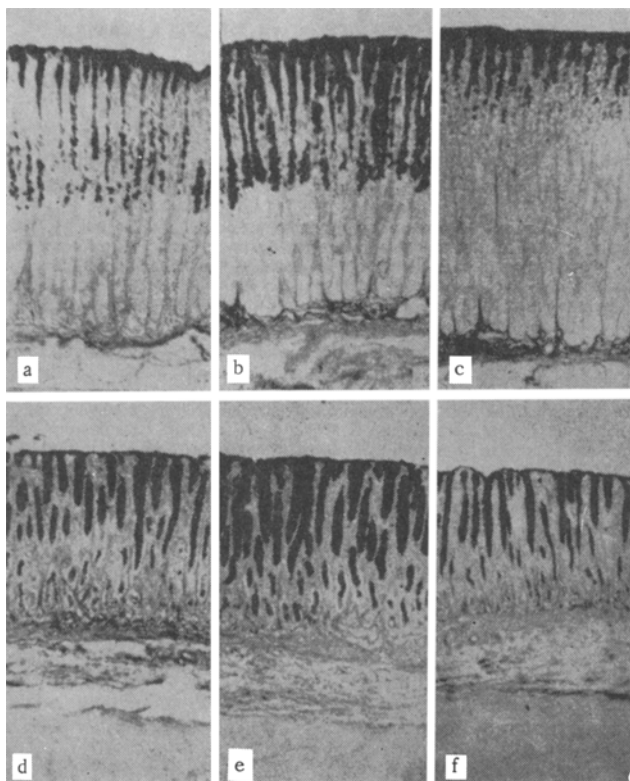


Fig. 1. Neutral mucopolysaccharides in the gastric mucosa of rats. Shabadash's staining method (PAS reaction), 112.5 \times , photomicrograph: a) fundal portion in controls; b) in hypothyroid animals; c) in hyperthyroid animals; d) pyloric portion in controls; e) in hypothyroid animals; f) in hyperthyroid animals.

pressure at the moment of testing [9]. A further histological examination of the thyroid glands was made at the end of the experiment.

EXPERIMENTAL RESULTS

Feeding 6-MTU to the rats as indicated above led to a decrease of 56.8% in the oxygen consumption by the end of the experiment, while the oxygen consumption of the animals receiving thyroid was increased on the average by 29.6% over the control. The content of mucoid compounds was represented by the following notation: very strong reaction +++++, strong +++, moderately strong ++, weak +, and indefinite reaction \pm .

The investigation showed that, except for the chief cells, all other types of glandular cells gave a reaction for neutral mucopolysaccharides (PAS reaction), but the intensity of their staining varied in different types of cells. The strongest reaction was obtained in the apical parts of the cytoplasm of the surface epithelium, the epithelium covering the gastric pits, and in the mucus lying on their surface (++++). In the zone of the fundal glands the content of PAS-positive material was greatest in the surface epithelium of the mucous membrane, while in the pyloric part it was greatest in the gastric pits. The accessory cells gave a strong reaction (+++), while the parietal cells gave a weakly positive (+) or indefinite (\pm) reaction when certain technical precautions were taken. The terminal portions of the pyloric glands synthesize a comparatively small amount of neutral mucopolysaccharides (++; Fig. 1a, d). In the hypothyroid and thyroidectomized rats a regular and sharp increase in the content of these compounds was found in all the structures enumerated (Fig. 1b, e). In the hyperthyroid animals, on the other hand, a very slight decrease was observed in the content of neutral polysaccharides in the cells of the surface epithelium and the epithelium of the pits, it was practically unchanged in the terminal portions of the pyloric glands, and in the accessory cells of the fundal portion the decrease in their content was quite definite (Fig. 1c, f). The accessory cells,

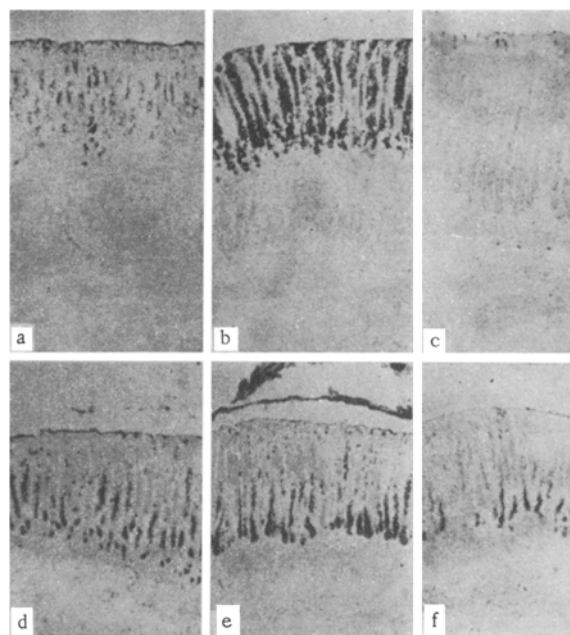


Fig. 2. Sialomucins in the gastric mucosa of rats. Alcian blue, pH 2.5, 112.5 \times , photomicrograph: a) fundal portion, control; b) in hypothyroid rat; c) in hyperthyroid rat; d) pyloric portion, control; e) in hypothyroid rat; f) in hyperthyroid rat.

which was determined in the experimental (except the thyroidectomized) animals and the controls in a Krogh's apparatus and expressed in milliliters of oxygen absorbed per hour, with allowance for the temperature, the air humidity, and the barometric

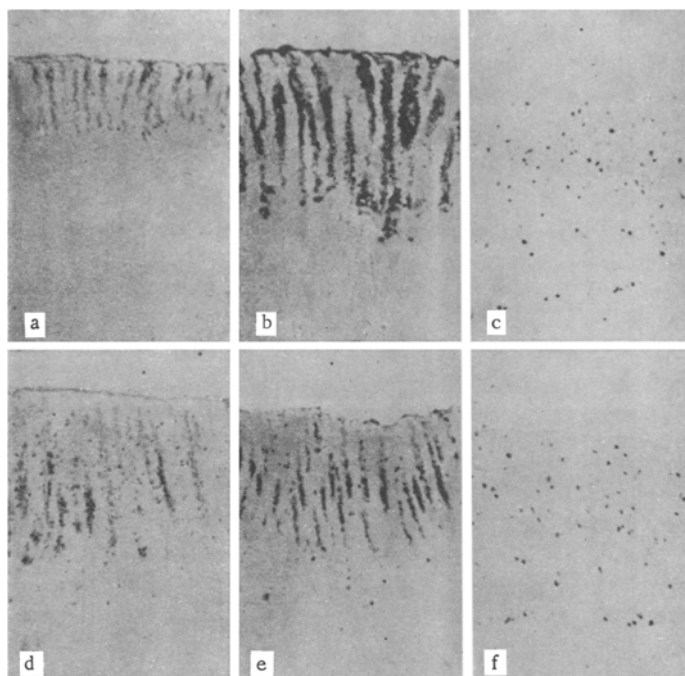


Fig. 3. Sulfomucins in the gastric mucosa of rats. Alcian blue, pH 1.0, 112.5 \times , photomicrograph: a) fundal portion in control rats; b) in hypothyroid; c) in hyperthyroid animals; d) pyloric portion in control rats; e) in hypothyroid; f) in hyperthyroid rats.

which responded by a definite increase or decrease in synthesis of neutral mucopolysaccharides to the hypothyroid or hyperthyroid state, were the most sensitive cells to the action of thyroid hormones. The secretory cells of the rat stomach were much more reactive with respect to synthesis of sialomucins and sulphomucins.

Under normal conditions the highest content of sialomucins is found in the terminal portions of the pyloric glands and the mucus lining the interior of the stomach (+++). Their content in the accessory glands is smaller (++), and in the surface epithelium it is low (+). The chief and parietal cells do not react for sialomucins. By the action of 6-MTU or thyroidectomy the content of sialomucins rises sharply both in the fundal and in the pyloric glands. Their content in the accessory glands and in the epithelium of the gastric pits of the fundal portion was visibly higher than in the control (Fig. 2b). The greatest loss of sialomucins, or even their total disappearance, occurred in this same portion of the stomach of the hyperthyroid animals (Fig. 2c); their content was also reduced in the pyloric glands.

The content of sulphomucins in the stomach of intact rats is low. They are found in the epithelium of the gastric pits (++) and in the accessory cells (+) of the fundal part of the stomach and the cells of the neck of the pyloric glands (+) (Fig. 3a, d), and also in the wandering cells of the connective tissue (++) . The surface epithelium does not give a well marked reaction (\pm). The reports of the presence of sulfomucins in the chief cells of the rat stomach [2] could not be confirmed. None were likewise found in the parietal cells.

The sulfomucin content in the stomach of the animals receiving 6-MTU was greatly increased. The increase in intensity of the reaction was particularly marked in the accessory cells of the fundal glands (Fig. 3b). An excess of thyroid hormones inhibited the synthesis of sulfomucins to such an extent that it was practically impossible to detect them (Fig. 3c, f), and only the connective-tissue cells, mainly the mast cells infiltrating the gastric mucosa of the hyperthyroid animals, gave a stronger reaction for sulfomucins than normally. Compounds of the hyaluronic acid and chondroitin sulfate C type could not be found in the rats' stomach.

The results of these experiments thus show that thyroid hormones play an active part in mucopolysaccharide synthesis. Their effect is exhibited mainly on the accessory cells of the fundal glands. An

excess of thyroid hormones sharply inhibits the synthesis of sulphomucins by all the cells; the synthesis of sialomucins is inhibited to a lesser degree, and that of neutral mucopolysaccharides less still. This is in agreement with the modern view that an excess of thyroid hormones acts by disturbing the normal course of oxidation-reduction processes in the cells by interfering with phosphorylating mechanisms [7, 10].

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