

EFFECT OF OPERATIVE TRAUMA, CIRCULATORY
HYPOXIA, AND IMMUNIZATION ON THE HISTOCHEMICAL
CHARACTERISTICS OF SECRETORY CELLS OF THE
APPENDIX

V. I. Platash

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Sialomucin, dermatan sulfate, keratan sulfate, and neutral mucopolysaccharides (MPS) were determined by histochemical methods in the goblet cells of the rabbit appendix. Sialomucin and neutral MPS were found in the glands. After immunization with human γ -globulin, increased secretory activity of the mucous membrane of the appendix was found in the productive phase of immunogenesis. Increased formation of mucus in the appendix was observed also after laparotomy and in the initial period of experimental appendicitis. However, against the background of increased functional activity of the secretory cells in the immunized animals, operative trauma gave the opposite effect, and in the course of experimental appendicitis no period of activation of mucus formation was found. The effect of these factors on secretory activity of the mucous membrane is accompanied by qualitative changes in the mucus secreted.

KEY WORDS: appendix; operative trauma; circulatory hypoxia; immunization with γ -globulin.

Carbohydrate-containing biopolymers present in the composition of mucin are among the most important components of the protective mechanisms of the mucous membranes. The object of this investigation was to make a histochemical study of the mucopolysaccharides of secretory cells of the rabbit appendix under the influence of immunization, operative trauma, and experimental inflammation. Data on these problems in the literature are incomplete [1-3, 10].

EXPERIMENTAL METHOD

Experiments were carried out on 92 rabbits weighing 2-2.5 kg. In series I laparotomy was performed on the animals and the peritoneal cavity inspected. In series II laparotomy was performed on the animals after immunization with human γ -globulin. In series III experimental appendicitis was produced by tying the appendicular vessels. In series IV the appendicular vessels of immunized animals were ligated. The animals were killed 3, 6, 12, 24, 48, 72, and 96 h after the operations and material was taken for examination. The appendices from seven intact and three immunized, but otherwise intact animals were used as the control. Material for investigation was fixed in Carnoy's fluid and embedded in paraffin wax. Serial sections were stained by the PAS-reaction [4], by a combined method with alcian blue and the PAS-reaction [7], with solutions of alcian blue at pH 2.6 [6] and 1.0 [5], and with a solution of alcian blue with magnesium chloride in concentrations of 0.05, 0.3, 0.6, 0.8, and 1.0 M [9]. Diastase, testicular hyaluronidase, chondroitin mucinase, hydrolytic removal of sialic acids [8], acetylation, acetylation followed by saponification, and treatment with phenylhydrazine solution during the PAS reaction were used as enzymic and chemical controls.

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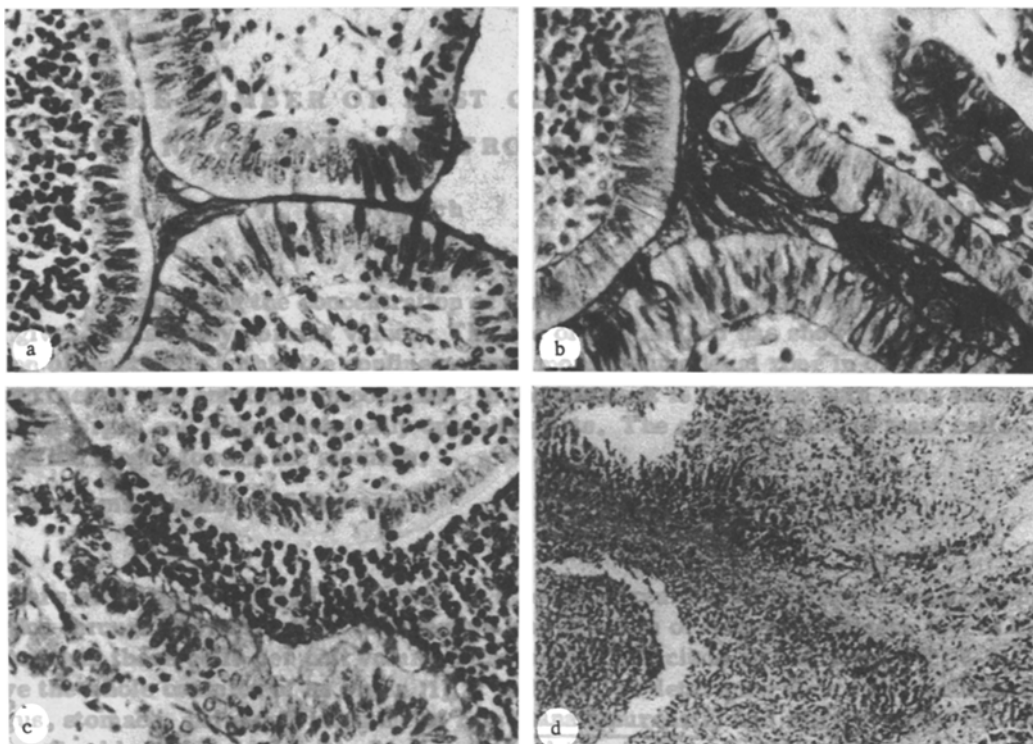


Fig. 1. Dynamics of changes in mucus formation in appendix after ligation of appendicular vessels. PAS reaction: nuclei counterstained with Ehrlich's hemalum. a) Crypt of appendix of intact rabbit (objective 40, ocular 6.3); b) increased amount of mucus in lumen of crypt of appendix 3 h after ligation of appendicular vessels (objective 40, ocular 6.3); c) reduced amount of PAS-positive mucus and accumulation of lymphocytes in lumen of crypt 12 h after operation (objective 40, ocular 6.3); d) destructive changes in appendix 72 h after ligation of appendicular vessels (objective 10, ocular 6.3).

EXPERIMENTAL RESULTS

Investigation of the secretory cells of the appendix of intact rabbits showed that the mucus they synthesized contained a complex of carbohydrate-containing biopolymers. The secretion of the goblet cells in the crypts of the appendix contained polysaccharides identified histochemically as sialomucin, dermatan sulfate, keratan sulfate, and neutral mucopolysaccharides (MPS). Sialomucin and neutral MPS were discovered in the glands of the appendix.

The effect of immunization on secretory activity of the mucous membrane of the appendix was studied at the height of the productive phase of immunogenesis (7 days after the last antigenic stimulation). In this period, a marked increase in functional activity of the secretory cells of the appendix was observed in the immunized animals. Many goblet cells distended with mucin were found in the epithelium of the crypts and the amount of secretion was increased in the spaces of the crypts and glands. The histochemical analysis found no qualitative changes in the mucus. Increased secretion of mucin by the goblet cells of the crypts was accompanied by a proportional increase in the synthesis of neutral MPS, sialomucin, dermatan sulfate, and keratansulfate. A proportional increase in the intensity of synthesis of sialomucin and neutral MPS was found in the glands.

After laparotomy (experiments of series I) increased mucin production was found in the glands of the appendix, but after 12-24 h this was replaced by a slight decrease; later the control level was restored.

These changes in the secretory activity of the glands were accompanied by synthesis of neutral MPS and sialomucins in them. At the same time, when mucin production had fallen to its lowest level (after 24 h) the secretion of the glands stained with alcian blue at pH 1.0 or if contained in a solution 0.3 M magnesium chloride. The color remained unchanged after treatment of the preparations with testicular hyaluronidase or chondroitin mucinase. Evidently at this period sulfated MPS of the dermatan sulfate type were synthesized by the glands. No marked changes in mucin production by the goblet cells were observed in the crypts

after laparotomy. The histochemical characteristics of the mucin synthesized by them corresponded to those of intact animals.

After laparotomy on the immunized animals (experiments of series II) a diminution of mucus formation was observed in the appendix in the early stages, and it was more marked in the glands. Histochemical analysis showed a decrease in the production of both sialomucins and neutral MPS in the glands. Synthesis of neutral MPS and dermatan sulfate was reduced in the goblet cells of the crypts but the content of highly sulfated MPS of the keratan sulfate type showed little change. The decrease in mucus production in the appendix after laparotomy lasted 12 h, and the secretory activity and histochemical characteristics of the secreted mucus returned to normal after 24-48 h.

Ligation of the appendicular vessels (experiments of series III) caused a temporary but considerable increase in mucus formation in the appendix. The secretion of mucus in the glands was increased chiefly on account of the synthesis of neutral MPS. The content of sialomucins rose a little 3 h after ligation of the vessels, but no sialomucins could be detected in the secretion of the glands after 6 h. An increase in secretion by the goblet cells of the crypts took place at the same time as the increase in synthesis of sulfated MPS. This period was followed by diminution of secretory activity of the mucous membrane (after 6-12 h) and destructive changes subsequently developed in the appendix (Fig. 1).

After ligation of the appendicular vessels of the immunized animals (experiments of series IV) mucus formation was reduced in the early stages, and this progressed to the development of destructive changes in the mucus membrane. In the goblet cells and lumen of the crypts the content of sulfated MPS fell, and after 3-6 h none could be detected. The decrease in the synthesis of neutral MPS took place more slowly. Control reactions for determining the content of sialomucins became rather stronger during the first 3 h after the operation, but later they weakened again. The decrease in mucus formation in the glands of the appendix was accompanied by a decrease in the synthesis of sialomucins and neutral MPS. With the development of destruction of the mucus membrane no functioning secretory cells could be found in the appendix, but a certain amount of mucin containing neutral and acid MPS still remained in the lumen of the crypts.

In the productive phase of immunogenesis the secretory activity of the mucous membrane of the appendix is thus increased. An increase in mucus formation in the appendix also is observed after laparotomy and in the initial period of experimental appendicitis. However, operative trauma gives the opposite effect when applied against the background of increased functional activity of the secretory cells in immunized animals, and no period of activation of mucus formation can be found in the course of experimental appendicitis. The effects of these factors on the secretory activity of the mucous membrane are accompanied by qualitative changes in the mucus synthesized.

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