

ON SOME HISTOCHEMICAL CHANGES IN THE MUCOUS MEMBRANE OF THE ESOPHAGUS IN RATS UNDER EXPERIMENTAL CONDITIONS.

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Abstract: The article studies the features of some histochemical reactions in the esophagus in rats under normal conditions and when exposed to a certain irritant. Histological sections were stained with hematoxylin and eosin and van Gieson. Neutral polysaccharides were detected using the PASS reaction (saliva amylase control), and acidic mucopolysaccharides were detected with toluidine blue (medium pH 3.2 and 7.4). Histological examination revealed that the thickening of the organ mucosa occurred mainly due to hyperplasia of cells of all layers of the stratified squamous epithelium.

Keywords: rats, esophagus, hematoxylin and eosin, van Gieson, mucopolysaccharides, basal layer, nucleoproteins.

О НЕКОТОРЫХ ГИСТОХИМИЧЕСКИХ ИЗМЕНЕНИЯХ СЛИЗИСТОЙ ОБОЛОЧКИ ПИЩЕВОДА У КРЫС В УСЛОВИЯХ ЭКСПЕРИМЕНТА.

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Резюме: В статье изучены особенности некоторых гистохимических реакций в пищеводе у крыс в норме и при воздействии на него определенным раздражителем. Гистологические срезы окрашивались гематоксилин-эозином и по ван-Гизон. Нейтральные полисахариды выявлялись при посредстве ПАРС-реакции (контроль амилазой слюны), а кислые мукополисахариды - толуидиновым - синим (РН среды 3,2 и 7,4). При гистологическом исследовании выяснилось, что утолщение слизистой оболочки органа происходило, в основном, за счет гиперплазии клеток всех слоев многослойного плоского эпителия.

Ключевые слова: крысы, пищевод, гематоксилин-эозином, по ван-Гизон, мукополисахариды, базальный слой, нуклеопротейдов.

Introduction. Usually, two diseases of the esophagus lead to the formation of a group of patients requiring esophagoplasty (esophageal reconstruction): congenital developmental anomaly — atresia, and cicatricial stricture following a burn caused by ingestion of a corrosively acting chemical substance [1,2]. Reconstructive surgery of the esophagus currently remains one of the most complex areas of clinical surgery. This is due to the technical difficulties of surgical interventions, the high postoperative mortality rate, the large number of complications, and the low quality of life of operated patients [3]. In this regard, studying the functional state of the pre-epithelial mucous layer (supraepithelial mucus layer) of the gastrointestinal tract after various types of esophagoplasty may be promising for understanding the physiological role, conditions of formation and functioning of the mucous layer, for correcting emerging disorders, and for choosing the optimal method of esophageal replacement [4,5].

Aim of the study. We investigated the features of certain histochemical reactions in the esophagus of rats under normal conditions and under the influence of a specific irritant on it.

Materials and Methods. Twenty-five esophagi from rats that received 3,4-benzpyrene orally at a dose of 0.2 ml of 0.1% solution for a prolonged period were studied (experimental series). Another 25 rats served as the second, control group. These animals were not subjected to any interventions. The material under study was fixed in Carnoy's fluid, Shabadash's fluid, and 12% neutral formalin. Histological sections were stained with hematoxylin-eosin and by the van Gieson method. Neutral polysaccharides were detected using the PAS reaction (control with salivary amylase), while acid mucopolysaccharides were revealed with toluidine blue (at pH 3.2 and 7.4 of the medium). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were studied by staining the sections with gallocyanin solution (control with 0.1% ribonuclease solution at 37°C for 1–3 hours). DNA was also detected using the Feulgen reaction. The content of DNA and RNA was determined by the cytophotometric method using the MUF-5 instrument. The amount of nucleic acids was calculated as the product of optical density and nuclear volume. Optical density was computed from curves obtained with the instrument's recording device. Nuclear volume was determined as the product of its two radii ($r_1 \times r_2$)². Nuclear radii were measured with an ocular micrometer.

Results. The conducted studies showed that after prolonged exposure to the carcinogen (after 150 days), the experimental series of rats exhibited focal dense thickenings of the esophageal mucosa of a whitish color, round or irregular in shape. Histological examination revealed that the thickening of the organ's mucous membrane occurred mainly due to hyperplasia of cells in all layers of the stratified squamous epithelium. Predominantly, the basal layer proliferated. Cells of the latter had irregular or polygonal shapes with large hyperchromatic nuclei. Elongated cells of the middle epithelial layers contained pale nuclei. Flat cells of

the superficial epithelial layers lacked nuclei. Basal layer epithelial cells proliferated and formed cords and nests invading the underlying tissues of the mucous membrane. The latter were infiltrated by lymphocytes, histiocytes, and plasmocytes. Examination of neutral polysaccharides showed that PAS-positive material in the rat esophageal epithelium was distributed unevenly. It was present in large amounts in the superficial layers of hyperplastic epithelial areas, where cell cytoplasm stained violet-cherry with periodic acid-Schiff reagent. This specific staining disappeared after treatment of sections with amylase, indicating that the polysaccharide contained in the cells was glycogen. Glycogen granules were evenly distributed throughout the cell cytoplasm. In contrast, neutral polysaccharides were not detected in cells of other layers of the hyperplastic esophageal epithelium, nor in areas of the esophagus without epithelial hyperplasia. The noted features of neutral polysaccharide distribution in the esophageal epithelium are significant in light of the generally accepted view that glycogen serves as the primary energy reserve substance in cells. An increase in RNA content was observed in the cytoplasm of intensively dividing cells of the basal layer of hyperplastic esophageal epithelium and in its detached nests invading the underlying mucous membrane tissues, resulting in diffuse gray-blue staining of the cell cytoplasm. The nuclei of these cells also showed elevated DNA content. It is known that nucleoproteins, particularly RNA, accumulate and play a major role during intensive cell growth. A decrease in mitotic activity of cells is accompanied by a reduction in nucleoprotein content. In our experiments on rats exposed to the carcinogen, these characteristic patterns of nucleoprotein distribution were clearly evident, as RNA and DNA were present in large amounts only in areas of enhanced cell growth. Thus, in normal epithelium of the esophagus, the amount of DNA was 3.81 ± 0.23 , while in foci of its hyperplasia it was 4.18 ± 0.21 (an increase of 9.7%). For RNA, the corresponding values were 3.84 ± 0.32 and 4.72 ± 0.31 (an increase of 22.9%). The metachromasia reaction was not pronounced in either intact or hyperplastic epithelium, nor in the

underlying connective tissue of the esophageal mucous membrane, indicating the absence of acid mucopolysaccharides in these structures.

Conclusion. Based on the conducted studies, it can be concluded that in foci of hyperplasia of the stratified squamous epithelium of the rat esophagus induced by carcinogen exposure, there was a significant increase in the content of RNA and DNA, with RNA increasing at a higher rate than DNA. At the same time, a significant increase in glycogen content was observed in the superficial layers of cells of the hyperplastic epithelium. No changes were detected in the content of acid mucopolysaccharides in the hyperplastic epithelium or in the underlying tissues of the rat esophageal mucous membrane.

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