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The Effect of Ethanol and Coal Dust on the Tracheal Mucosa — Histochemical Examinations of Animals in Experimental Conditions

Wpływ alkoholu etylowego i pyłu węglowego na błonę śluzową tchawicy — badania histochemiczne zwierząt w warunkach doświadczalnych

THE OBJECTIVE

Lack of the scientific literature on simultaneous effect of ethanol and coal dust on the respiratory tract have made the authors undertake the task of experimental study of this significant issue.

INTRODUCTION

The development of coal mining industry in the Lublin region created new environmental-existential conditions. It is thought that the main reasons of alcohol consumption are instilled in the social environment (10, 14). Chronic diseases of respiratory tract in pit-coal miners come in the first place. The observations carried out in the Department of Otolaryngology in Lublin indicated the relationship between alcoholism and the occurrence of the upper respiratory tract diseases (13). The present study was performed on the basis of observations of morphological and histochemical characteristics of the tracheal mucosa in rats exposed to environment highly contaminated with coal dust and being administered water solution of ethanol to drink.

MATERIALS AND METHODS

The white rats — 12 mature Wistar males were selected at random into two experimental groups and one control group. The experimental group animals received 25% solution of ethanol *ad libitum* to drink and were exposed to dust coal.

One of the stages of the experiment consisted in breathing in the dust coal. During this stage the rats were staying for 4 hrs daily in a specially designed chamber where electrically driven fans distributed the dust coal steadily. SiO in the dust coal was determined by application of phosphorous acid anhydride. The measurement of the total air pollution by coal dust was performed and the values of pollution of the breathed air were corresponding to the pollution with dust in the pit-coal mines.

After 4 and 20 weeks of the experiment (the experimental group I and II) the animals were decapitated and the tissue from the 1/3 of the upper part of trachea was collected. The material was fixed in Carnoy fluid, Baker fluid and some material was frozen and then was cut in a cryostat of Slee HS type. In compliance with the methodology (16) the following colouring and histochemical reactions were performed: 1) histochemical reaction acc. to Gomori on the activity of acid phosphatase; 2) acc. to Wachstein—Meisel — on the ATP-ase activity; 3) acc. to Nachlas — on the activity of succinic and lactic dehydrogenase.

MICROSCOPIC PICTURE

The mucosa of the trachea of the examined segments is covered with ciliated stratified epithelium, the thick subepithelial membrane has a strongly marked reticular lamina. The mucous membrane contains sero-mucous glands. With the PAS reaction the coloured reaction was revealed in the subepithelial membrane of the stratified epithelium, in the mucous cells cytoplasm as well as in the vascular walls. The histoenzymatic tests allowed for showing activity of regularly localized and midi-intensified hydrolases. After performing reactions on the respiratory enzymes activity, a special intensive reaction in the ciliated stratified epithelium and in the mucous membrane glands was observed.

The experimental group I (4 weeks' exposure) is presented in Figs. 1—4. The tracheal epithelium showed a regular structure in the specimens for study. Only the number of beaker cells got increased. Glands in the mucosa were greatly arborescent. After colouring with the PAS method, strong filling with secretion was shown in the glands. The reaction on acid phosphatase activity was intensified in the ciliated cells cytoplasm. A strong reaction was also revealed in the numerous macrophages of the mucosa. The vascular walls were characterized by the increased activity to ATP-ase. The activity of respiratory enzymes was intensive in the stratified ciliated epithelium and focally increased in the mucosal connective tissue.

The experimental group II (20 weeks' exposure) is presented in Figs 5—8. The cytoplasm of epithelium cells showed vacuolar degeneration, perinuclear vacuolisation was present. The cells often lacked cilia. In this area the epithelium was low. The number of beaker cells got significantly decreased as compared to

the previous group. Places lacking the epithelium could be seen. The subepithelial membrane revealed irregular thickness, the connective tissue contained many fibrous elements and inflammatory infiltrations. There were few glands in the mucosa, similarly few blood vessels were observed. The PAS reaction showed great changes in the subepithelial membrane, particularly in the places where the epithelium structure got blurred. The beaker cells contained PAS positive granules, similarly as the mucous glands. The reaction to acid phosphatase was coarse-granular, locally diffusive. ATP-ase maintained intensive activity in the less damaged areas of tracheal walls. The activity of respiratory enzymes was irregular, significantly intensified within less modified parts of the epithelium.

DISCUSSION AND CONCLUSIONS

Degenerative - inflammatory and even carcinogenic influence of ethanol on the upper parts of digestive and respiratory tracts is widely reported (2, 7, 10). Inflammatory lesions in the oral cavity (Stomatitis) may be explained as the result of lack of vitamin B, A and deficiency of iron in connection with the overconsumption of alcohol (13). It concerns particularly the chronic processes. Synergetic activity of ethanol and carcinogenic compounds is suggested. Alcohol "is making the way" to carcinogenic compounds by non-specific action and influencing the immunologic mechanisms (3, 15). Mucous secretion has anti-bacterial and anti-viral properties due to IgA and IgE particles localization in it and fixing in the secretion of numerous basophilia (4). The secretion immunoglobulins in the trachea and in the bronchi create the protective environment. The plasma cells when synthesizing IgA, are distributed mostly around the glands. The secretion part is localized in the serum cells as usual (5). In the connective tissue, besides the mast cells, there are macrophages that are significant in creating barrier which determines local immunity of the respiratory system (12).

Respiratory tract diseases, chronic and non-specific diseases of unknown ethiology in particular, occupy the first place among coal miners (8, 9). It is thought that when establishing the highest permissible concentration of toxic substances in the air at the work place, the results of tests carried out on animals should be considered (6). In this paper it has been stated that the lesions of tracheal mucosa in rats after 4 weeks' exposure to ethanol and coal dust are of irreversible character and show characteristics of mid-intensified non-specific inflammation. The secretion processes are increased and are seen both on study specimens and after the PAS reaction performance. Prolonged action of toxic substances causes gradual atrophy of glandular elements and lesions in the tracheal epithelium. There was a considerable degree of lesion as well as its irreversible character. However, squamous cell metaplasia and total glandular atrophy were not reported. It is obvious that the lesions observed in animals

cannot be fully applied to human beings. However, the initial growth of glandular cells observed in this study seems to be an important phenomenon. It can be attributed to the immuno-reaction to the toxic substances.

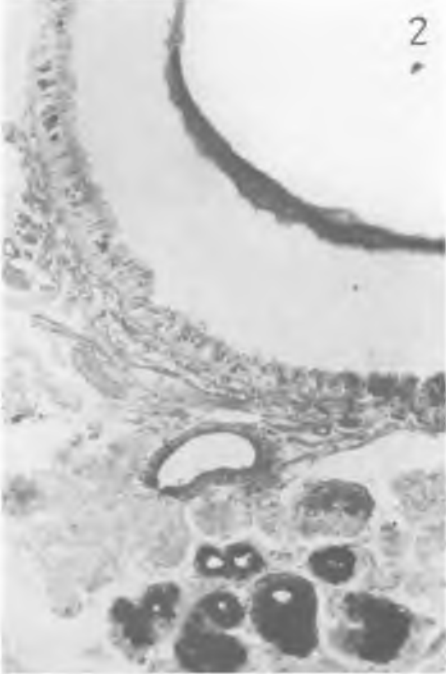
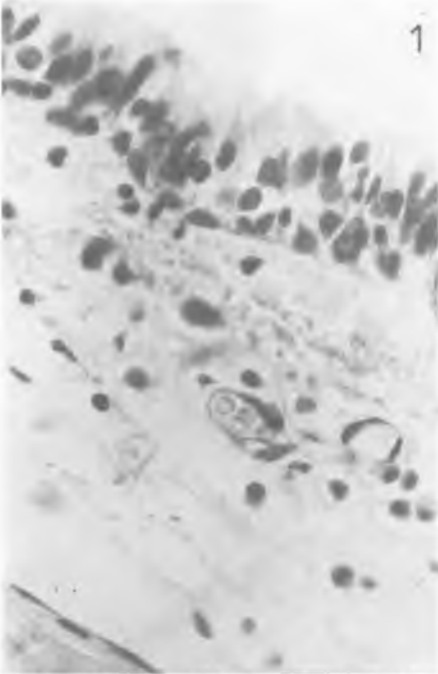
On the other hand, the increase of mucocides number negatively influences the effectiveness of ciliary apparatus activity as the mucous cells replace ciliated elements, and besides, the excess of mucus causes agglutination of cilia (1). It makes removal of coal dust difficult. The resulting transformation of mucosa towards the decrease of secretion process may be the phenomenon beneficial for the organism.

Conclusions

1. In experimental animals after 4 weeks' exposure to coal dust and ethanol, the lesions in trachea have reversible character.
2. Prolonged effect (20 weeks) of coal dust and ethanol causes irreversible lesions of tracheal mucosa as well as the atrophy of glandular elements.
3. The experimental tasks with application of animal model and histochemical reactions, may be the reference for the clinical tests of similar syndromes but with considering the experiment peculiarity.

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EXPLANATION TO FIGURES

Fig. 1. Experimental group I. Colouring with hematoxylin and eosin. Regular structure of stratified epithelium. Magn. 400 × .

Fig. 2. Experimental group I. PAS colouring. Intensive reaction in glandular cells. Magn. 100 × .

Fig. 3. Experimental group I. Reaction to acid phosphatase activity. Numerous cells seen in mucosa that are filled with reaction products. Magn. 100 × .

Fig. 4. Experimental group I. Reaction to ATP-ase activity. Intensive reaction in vascular walls. Magn. 100 × .

Fig. 5. Experimental group II. Colouring with hematoxylin and eosin. Significant lesions of the structure, inflammatory infiltrations. Magn. 100 × .

Fig. 6. Experimental group II. PAS colouring. Single PAS-positive granules in beaker cells. Magn. 200 × .

Fig. 7. Experimental group II. Reaction to acid phosphatase activity, coarse-grained, focally diffusive. Magn. 100 × .

Fig. 8. Experimental group II. Reaction to lactic dehydrogenase. Intensified reaction in the segments of maintained epithelium. Magn. 200 × .

STRESZCZENIE

Zwierzęta doświadczalne (szczury białe szczepu Wistar) pocono 25% wodnym roztworem etanolu i poddano działaniu pyłu węglowego. Po upływie 4 i 20 tygodni trwania doświadczenia na preparatach tchawicy wykonano badania histochemiczne (odczyny na obecność mukopolisacharydów, na aktywność enzymów hydrolitycznych i enzymów oddechowych) oraz barwienie hematoksyliną i eozyną. Po upływie 4 tygodni wystąpiły objawy zapalenia nieswoistego, po 20 tygodniach stwierdzono nieodwracalne uszkodzenie błony śluzowej tchawicy.

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