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Experimental Investigations of the Effect of Electroenergetic Ashes on Duodenal Mucous Membrane

Badania eksperymentalne nad wpływem popiołów elektroenergetycznych na błonę śluzową dwunastnicy

Экспериментальные исследования влияния электроэнергетической золы на слизистую оболочку двенадцатиперстной кишки

In the face of rapid development of industry intoxication of atmosphere with by-products increases; this involves the exposure of human organism to the noxious activity of ecotoxins. Dusts produced by big heat and power generating plants belong here. Inhaled through the respiratory system they constitute the greatest menace to the organism. The influence of electroenergetic ashes on the respiratory system has already been studied by numerous authors (7, 9, 13).

In view of the easiness of absorption of the dusts containing heavy metals and their readily soluble salts through the respiratory system, their transition through vesicular barrier into blood as well as the possibility of permeation to various organs (1, 6, 11), we shall seek to analyse if, and in what way electroenergetic ashes introduced through inhalation influence the morphology and activity of the duodenal mucous membrane.

MATERIAL AND METHODOLOGY

White rats of Wistar strain were used for investigations. Their body mass was ca 200 g, their number was 36. The animals were divided into three experimental groups and one control group. In experimental groups I, II, III the animals were given once, intratracheally, 0.2 ml electroenergetic ashes suspension in 0.9% NaCl solution. The ashes solution contained 50 mg of the examined ashes sample in 0.6 cm^3 of 0.9% NaCl. The animals from the control group were once administered 0.2 ml physiological salt into trachea.

The experimental animals from the group I were given ashes collected from Wrocław heat and power plant, the animals from the group II — from Lęg (Cracow) heat and power plant, and those from the group III — from Rybnik power station. Respirable fractions (10) were used for making suspensions. They contained 4.6% free silica on the average as well as trace elements, whose contents for the group I and II were presented in the paper by Królikowska-Prasał and co-authors (7).

After 3 months since administering electroenergetic ashes the animals were decapitated and the duodenal segments were collected for histochemical studies. PAS reaction to polysaccharides was performed after McManus method (15), using control reactions with diastase and dimedone. The diaze reaction with the use of Fast Red B (Gurm firm, London) (14) was also carried out in order to reveal serotonin (5-hydroxytriptamine) in enterochromaffin cells of intestine. The basic staining with hematoxylin and eosin was performed.

OWN INVESTIGATIONS

Animals of control group

Staining with hematoxylin and eosin showed correct structure and staining faculty of duodenal mucous membrane. In the epithelium enterocytes and mucocytes became visible in proper proportion. The enterocytes cytoplasm stained pink. Dark-violet nuclei were placed at the base of the cells (Fig. 1). Among enterocytes were also non-stained mucocytes. The structure and staining faculty of intestinal glands (Lieberkühn gland) did not deviate from the norm. PAS reaction displayed red granules in mucocytes found in the epithelium covering duodenal villi and in the intestinal glands. The intensity of their staining was different (Fig. 2). Enterochromaffin cells became visible after carrying out Fast Red B reaction. They were observed in the epithelium of duodenal villi and in the intestinal glands (Fig. 3). Specific granules got intensively stained dark brown, they densely filled the cells's cytoplasm at its base, round the nucleus and in the apical parts. In single cells a weaker reaction was observed (Fig. 4).

Animals of experimental group I

The structure and staining faculty of all layers of duodenal mucous membrane were similar to those of the control group (Fig. 5). No morphological changes were found in the particular types of epithelium cells. The enterocytes cytoplasm stained normally. Their nuclei were placed at the base of the cells. The number of mucocyted giving intensive PAS reaction increased both in the epithelium covering villi and in the intestinal glands. Mucocytes were barrel-like expanded and they contained a great amount of granules assuming an intensively red colour (Fig. 6). The number and distribution of enterochromaffin cells in the epithelium covering villi and in the glands was similar as in the control group. Enterochromaffin cells were largely filled with granules (Fig. 7). In single cells much weaker reaction, alike as in the control group, was observed.

Animals of experimental group II

Staining with hematoxylin and eosin did not reveal any significant differences in staining faculty and structure of epithelium and intestinal glands. The proportions between the number of absorbing, secreting and endocrine cells in)!'('the epithelium covering villi were preserved. PAS reaction disclosed differentiation in mucocytes activity. They were in various stages of secretion. Their different size and shape proved this (Fig. 8). A considerable number of mucocytes filled with intensively saturated granules were observed in some intestinal glands (Fig. 9). No changes were found in intensification and distribution of diaze Fast Red reaction in enterochromaffin cells of epithelium and of intestinal glands (Fig. 10).

Animals of experimental group III

The structure of epithelium and of proper mucous membrane during staining with hematoxylin and eosin did not differ from the image obtained in control group. The intensity of PAS reaction in mucocytes of intestinal glands increased. A greater number of mucocytes filled densely with intensively stained granules were observed at the bottom of these glands (Fig. 11). In mucocytes of epithelium covering duodenal villi the reaction was similar as in the intestines of control animals. The intensification of Fast-Red diaze reaction as well as distribution and number of enterochromaffin cells in this group did not differ from the results obtained in the group of control animals (Fig. 12).

DISCUSSION OF RESULTS

Dust molecules intratracheally administered may be partially expectorated and swallowed and they may get into alimentary duct in this way. In our experiment respirable fractions were used for preparation of suspensions given to animals. These fractions reach more easily the lower parts of respiratory system. The respirable fractions are the most important toxicologically, because they easily get through air-blood barrier of air-sacs into blood circulation (10). They can be also phagocyted by vesicular macrophages and with them get through the lympha into venous circulation and then, through the liver, into the duodenum, together with bile. In the intestines they may undergo revolving resorption, which adds to longer retention of toxic substance in the organism. Direct excretion of some elements through intestinal mucous membrane was also described. Among others, this pertains to lead. Other elements parenterally introduced, e.g. through inhalation, may be permanently bound by melatothioneine playing the part of detoxifying system for heavy metals (3).

Ashes introduced intratracheally in our experiment contained trace elements of Mn, Zn, Pb, V, Cr, B, Cu, Ni, Ca, Co, Ge, Tl, Sn, Sb, Be and free silica. Chemical analysis of ashes used for obtaining respirable fraction given to experimental group carried out in the paper by Królikowska-Prasał and co-workers (7) showed similar content of most of the trace elements in the experimental groups I and II. Small differences concerned Pb, B, Ca, Co, Tl, which were more abundant in the Ist experimental group and Mn, Zn, Sn, whose greater content was found in the group II. The average content of silica in all ash samples was 4.6%.

In our investigations the influence of ashes on duodenal mucous membrane and specially on its epithelium was observed. The cells of the epithelium show great functional differentiation. Enterocytes take part in absorbing processes, mucocytes are secretory cells, whereas enterochromaffin cells are endocrine cells of alimentary duct. No morphological changes were observed in the intestinal epithelium. Histochemical investigations displayed an increased PAS reaction in mucocytes of the epithelium covering duodenal villi and in mucocytes of intestinal glands of the animals from the Ist experimental group. They were given ashes containing, among others, more lead. In the experimental groups II and III an increase in the intensity of PAS reaction was observed in mucocytes localized only in intestinal glands. The animals of the group III were administered the ashes from Rybnik power station. In this region the concentration of respirable dust largely exceeding the norm (2) was described. Some authors described, among others, the greatest cumulation of absorbed lead in glandular cells of alimentary duct (12).

In our investigations no changes were observed in morphology and localization of enterochromaffin cells in duodenum of any of the examined experimental groups. The duodenum is the main reservoir of EC cells and organic serotonin and may be a sensitive indicator of changes in metabolism of this amine in the whole organism (4, 5, 8). Lack of significant changes in the intensity of diaze reaction in these cells manifests lack of mobilization of the main serotonin store in the organism with changed conditions.

The carried out investigations proved that the ashes intratracheally administered did not cause morphological changes in any layer of mucous membrane. The described changes in the intensity of PAS reaction in mucocytes of epithelium covering villi and in mucocytes localized in the intestinal glands indicate only the intensification of the cells activity, connected with processes of secretion in the groups of experimental animals. Though no close interdepenence was found between this process and the content of trace elements in the particular groups, this may prove the share of these cells in eliminating some of the components of electroenergetic ashes.

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EXPLANATION OF FIGURES

Fig. 1. White rat duodenum. Control group. Staining with hematoxylin and eosin. Magn. ca 200 ×.

Fig. 2. White rat duodenum. Control group. PAS reaction. Magn. ca 200 ×.

Fig. 3. White rat duodenum. Control group. Fast Red B diaze reaction. Magn. ca 200 ×.

Fig. 4. Enterochromaffin cell in intestinal gland. Control group. Fast Red B reaction. Magn. ca 400 ×.

Fig. 5. White rat duodenum. Experimental group I. Staining with hematoxylin and eosin. Magn. ca 200 ×.

Fig. 6. White rat duodenum. Experimental group I. PAS reaction in mucocytes. Magn. ca 200 ×.

Fig. 7. White rat duodenum. Experimental group I. Fast Red B reaction. Magn. ca 400 ×.

Fig. 8. Mucocytes in duodenal epithelium. Experimental group II. PAS reaction. Magn. ca 400 ×.

Fig. 9. PAS reaction in rat's duodenum. Experimental group II. Magn. ca 200 ×.

Fig. 10. Fast Red B diaze reaction in enterochromaffin cells of rat's duodenum. Experimental group II. Magn. ca 400×.

Fig. 11. PAS reaction in mucocytes of rat's duodenum. Experimental group III. Magn. ca 200×.

Fig. 12. Fast Red B reaction in rat's duodenum. Experimental group III. Magn. ca 400×.

STRESZCZENIE

Przebadano wpływ popiołów elektroenergetycznych na błonę śluzową dwunastnicy szczura białego. Zwierzętom doświadczalnym (3 grupy) podawano dotchawiczo zawiesinę popiołów pobranych z trzech różnych elektrociepłowni. Na pobranym materiale wykonywano reakcję PAS na mukopolisacharydy, reakcję dwuazową Fast Red B na serotoninę oraz barwienie hematoksyliną i eozyną.

Zmian morfologicznych w błonie śluzowej dwunastnicy nie stwierdzono. Badania histochemiczne wykazywały wzmożoną intensywność reakcji PAS w mukocytach nabłonka pokrywającego kosmki i wyścielającego gruczoły jelitowe w grupie I doświadczalnej oraz w mukocytach gruczołów jelitowych w grupie II i III doświadczalnej. Intensywność reakcji dwuazowej była podobna we wszystkich badanych grupach.

РЕЗЮМЕ

Исследовано влияние электроэнергетической золы на слизистую оболочку двенадцатиперстной кишки белой крысы. Опытные животные (3 группы) получали в трахею суспензию золы взятой из трех разных теплоэлектоцентралей. Проведено реакцию PAS на мукополисахариды, диазовую Fast RedB на серотонин и окраску гематоксилином и эозином.

Морфологических изменений в слизистой оболочке двенадцатиперстной кишки не обнаружено. Гистохимические исследования показали повышенную интенсивность реакции PAS в мукоцитах эпителия покрывающего ворсинки и выстилающие кишечные железы в I опытной группе и в мукоцитах кишечных желез в II и III опытной группе животных. Интенсивность реакции диазовой была сходна во всех исследованных группах.

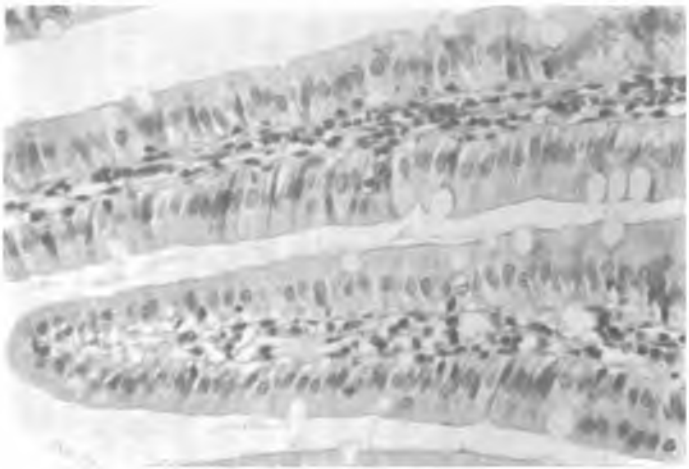


Fig. 1

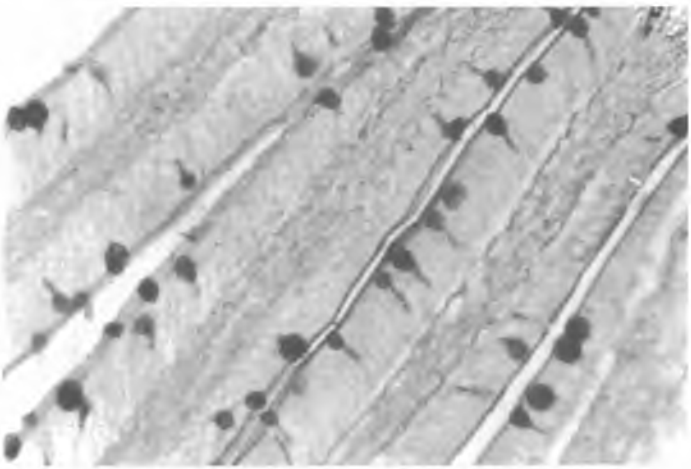
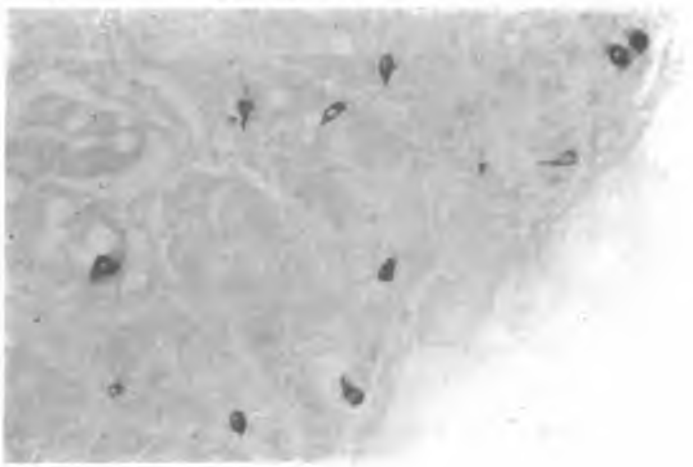


Fig. 2



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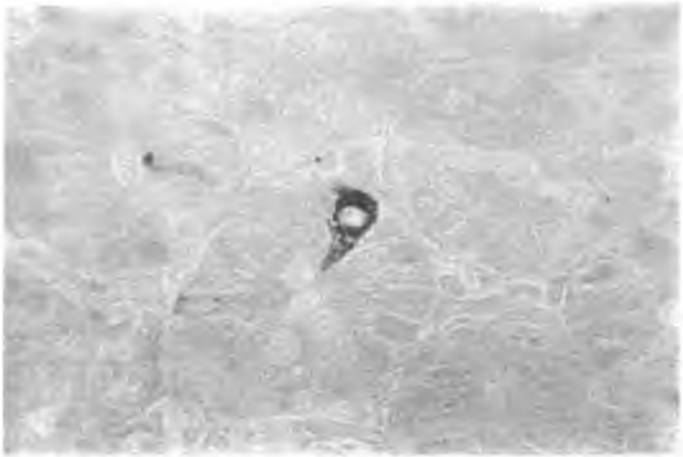


Fig. 4

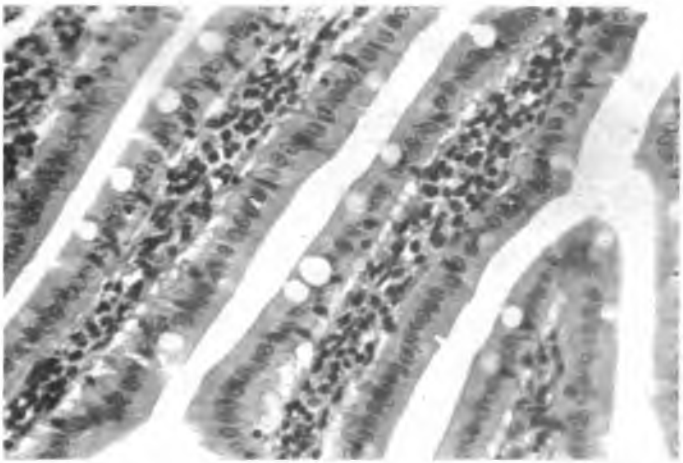
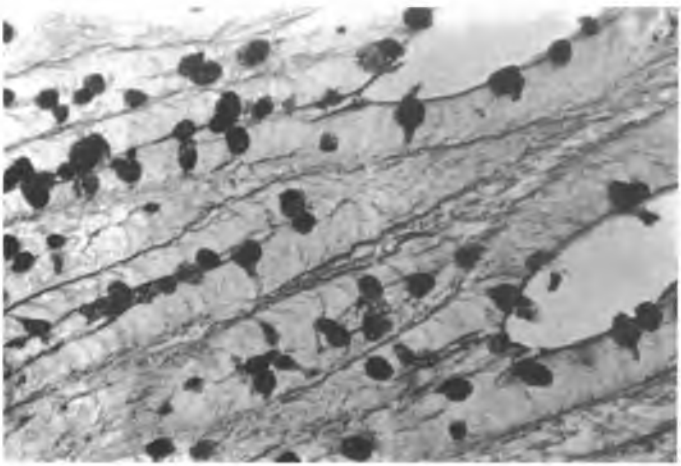


Fig. 5



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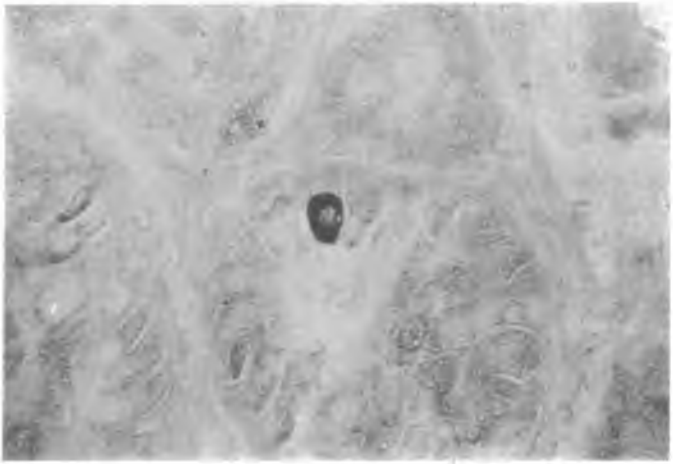

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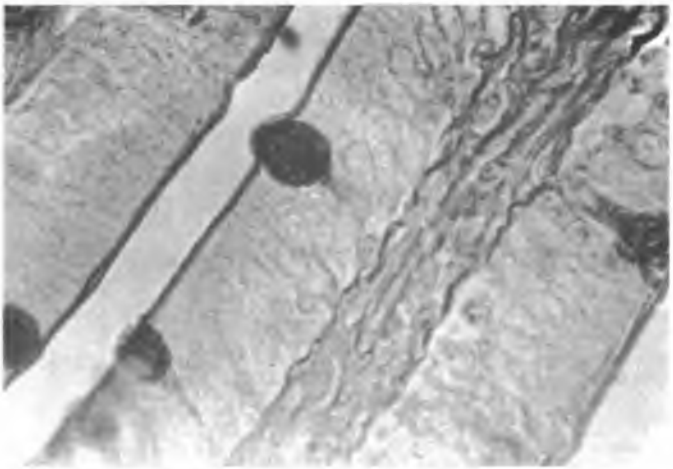

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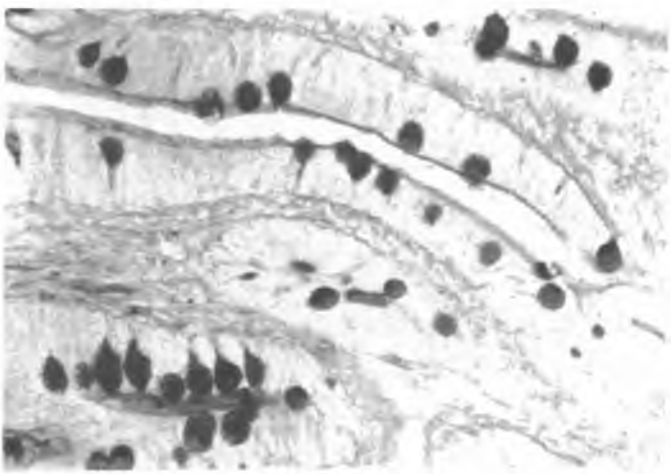

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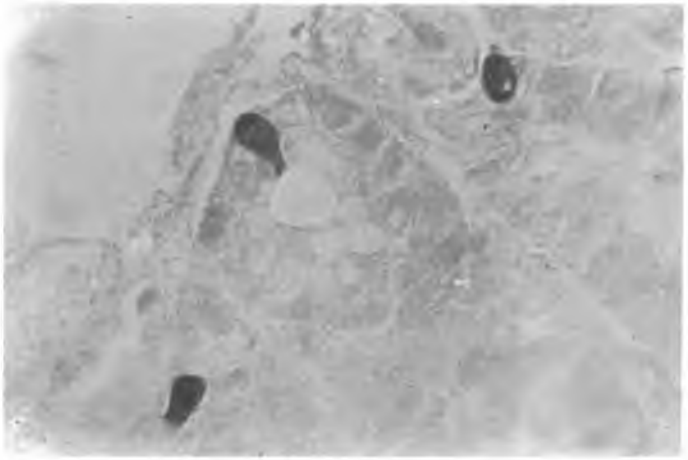

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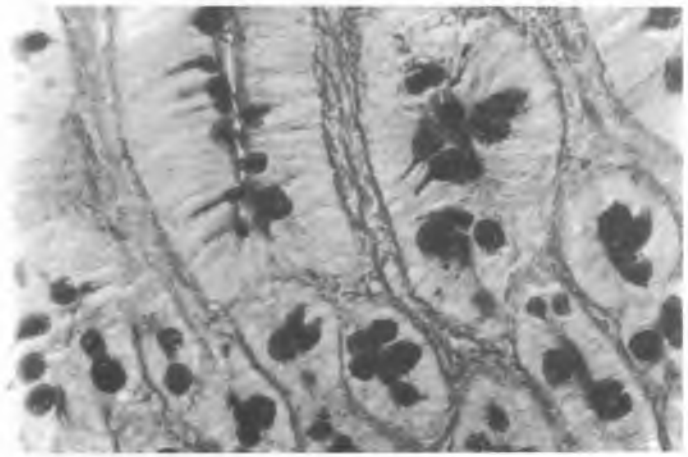

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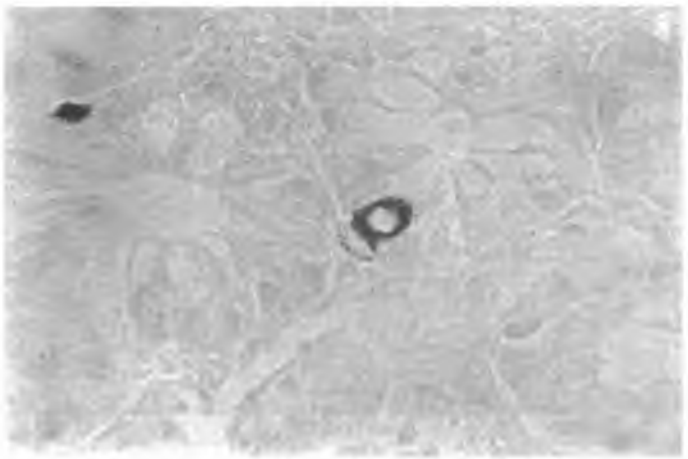

Fig. 12