

The Effect of Chronical Intake of Arsenic on the Liver Tissue Respiration in Mice

In the course of our experiments on some hygienic aspects of long-termed intake of arsenic, it was noted that a chronic application of even small doses of this toxic agent in drinking water produced in mice significant histological changes of the liver¹. It was therefore of interest to study whether this effect was connected with respiratory changes of the liver tissue. This topic was studied in vitro in rat liver and kidney slices² and in mitochondria³⁻⁵; but we could not find any paper dealing with the influence of arsenic administered in vivo. The aim of the present study was to find whether, and to what degree, tissue respiration can be influenced by a chronic intake of arsenic in drinking water.

The experiments were done with hairless mice used throughout all our studies of arsenic action. Arsenic was applied as trioxide in the drinking water in concentrations of 5 and 50 mg/l respectively. These solutions were given ad libitum. After 256 days the mice were decapitated, the liver was rapidly removed and homogenized in icecold sucrose solution (0.25 M). Oxygen consumption in the homogenate was measured by the conventional Warburg technique on air and related to the dry weight of the homogenate.

In another set of experiments, arsenic trioxide was added to the assay system in the final concentration of 10^{-3} M. The scope of these experiments was to find whether a chronic administration of arsenic contributes to activation of adaptative mechanisms.

The results are summarized in the Table. It can be seen that arsenic markedly suppresses the oxygen consumption of the liver tissue. However, the difference between the

groups is not proportional to the differences in concentrations and it is also not statistically significant.

The presence of arsenic in the incubation medium caused a significant decrease of the respiration in normal mice. On the other hand, in animals exposed to the lower concentration of arsenic, only slight and insignificant decrease of oxygen consumption could be observed. Even in the group drinking 50 mg arsenic/l the decrease was much lower than in the experiment without in vitro addition of arsenic. The lower sensitivity to arsenic may be due probably to an adaptation of the organism to a prolonged exposure to low doses of the toxic agent, or to a decreased level of oxidizable substrates. The first presumption seems to be supported by our finding of a decrease in acute toxicity of arsenic during its chronic intake⁶.

In addition, the present study shows that the method used can be a valuable and sensitive indicator of arsenic intoxication which registers even small changes not detectable with current methods.

Zusammenfassung. Es wird gezeigt, dass eine langfristige Zufuhr von Arsen in Trinkwasser bei Mäusen eine wesentliche Abnahme des Sauerstoffverbrauches des Leberhomogenates hervorruft. Die chronische Verabreichung von niedrigen Arsengaben blockiert die respirationshemmende Wirkung von Arsen in vitro, was wahrscheinlich auf eine Adaptation des Organismus zurückzuführen ist.

V. BENCKO and Z. ŠIMÁNEŠ

Institute of Hygiene and Department of Pharmacology, Charles University Medical School of Hygiene, Praha (Czechoslovakia), 29 January 1968.

The oxygen consumption in liver homogenates of mice exposed to arsenic

Concentration of arsenic in drinking water	No arsenic added $\mu\text{l O}_2/\text{h}/\text{mg}$ dry weight	Arsenic added $\mu\text{l O}_2/\text{h}/\text{mg}$ dry weight
None	4.88 ± 0.52^a (6)	2.89 ± 0.18 (5)
5 mg/l	2.99 ± 0.51^b (4)	2.46 ± 0.30 (6)
50 mg/l	2.59 ± 0.12^b (4)	1.88 ± 0.16^b (5)

^a Mean \pm standard error of the mean. ^b Value significantly different from control. No. of animals in brackets.

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Adrenergic Fibres in the Mucous Membrane of Guinea-Pig Alimentary Tract

A rich nerve supply to the mucous membrane of the small intestine was recognized by DOGIEL¹ and by CAJAL² ('plexus periglandulaire' and 'plexus des villosités'); according to the latter author, these fibres are motor nerve fibres, being intimately related to smooth muscle cells and to secretory cells. By electron microscopy, HONJIN et al.³ have observed bundles of nerve fibres in the muscularis mucosae of tunica propria of human small intestine: these fibres contain agranular vesicles and are situated in close contact with CAJAL's interstitial cells. SILVA⁴ described nerve fibres around mouse colon glands, running in

bundles and containing both granular and agranular vesicles. By fluorescent microscopy, in cat and monkey small intestine, JACOBOWITZ⁵ has followed adrenergic fibres as far as the tunica propria, where they branch over the basal aspect of the glandular epithelium.

By employing the FALCK and HILLARP method⁶ for the histochemical detection of biogenic amines, we have investigated the distribution of adrenergic nerve fibres in the mucosa of different segments of the digestive tract. The stomach, the ileum and the rectum of 6- to 8-month-old guinea-pigs have been the object of the present

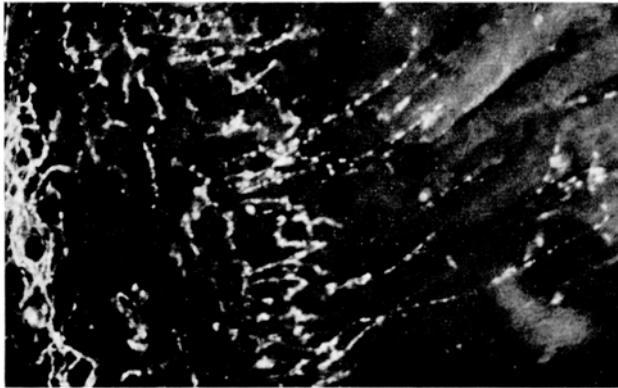


Fig. 1. Rectum. Oblique section through mucosa and submucosa. To the left fluorescent fibres of the submucosa and the perivascular net; in the middle fluorescent fibres of tunica propria and muscularis mucosae; to the right fluorescent fibres running parallel to the axis of the crypts. $\times 210$.

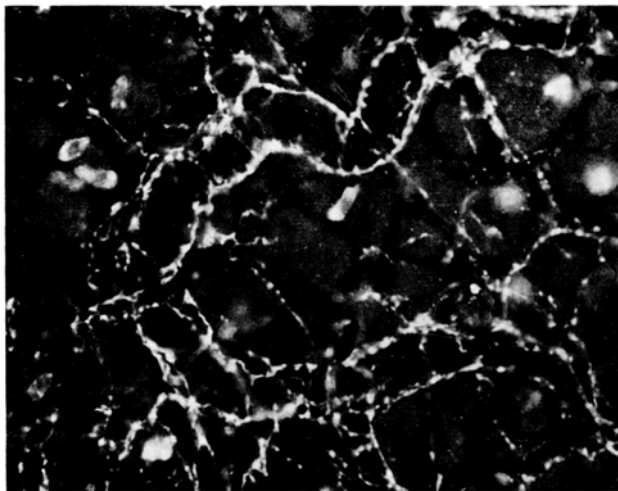


Fig. 2. Stomach. Tangential section through tunica mucosa. Among the innermost part of gastric glands (transversally sectioned) perivascular fluorescent fibres and thin isolated fluorescent fibres are apparent. $\times 390$.

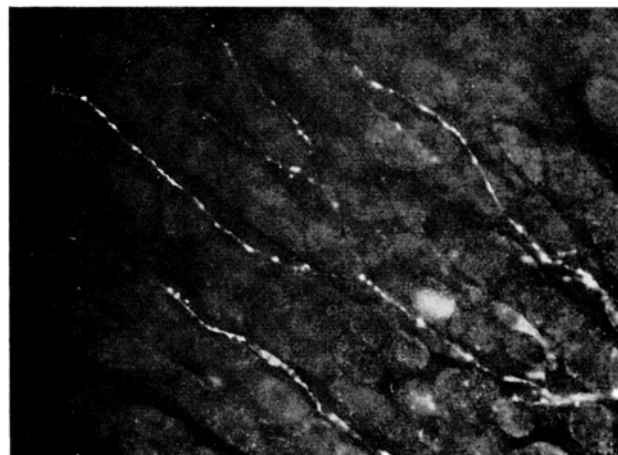


Fig. 3. Stomach. Transverse section through tunica mucosa. Thin fluorescent fibres with the typical varicosities are observed running parallel to the axis of gastric glands (longitudinally sectioned). $\times 390$.

investigation. According to the indications given in a former paper⁷, transverse, oblique and tangential sections were prepared together with flat whole mounts of the mucous membrane. The specificity of the reaction has been controlled according to the indications given by CORRODI and JONSSON⁸.

The distribution of fluorescent nerve fibres is very similar in all the segments examined. From the fluorescent fibres net of the submucosa several branches were seen crossing the muscularis mucosae and penetrating the tunica propria. Within the muscularis mucosae, bundles of fluorescent varicose fibres follow the length of smooth muscle cells. The fibres which have branched within tunica propria form a thick net around the bottom of the crypts (Figure 1). In sections tangential to the free surface of the wall fluorescent varicose fibres apparently surround the glands (transversally sectioned) running not far from the epithelial cells (Figure 2). In addition, around the glands, a dense vascular net with its typical perivascular nervous supply is apparent. In stomach and rectum walls, thin fluorescent fibres run along the glands and crypts and can be followed to beneath the free surface of the wall (Figure 3). In small intestine, similar fibres are apparent within the stroma of the villi, up to the tip.

Unfortunately, with the present method, neither in smooth muscle cells of muscularis mucosae and of blood vessels nor in epithelial cells of gland bottoms, could we define how intimate the relationship is between adrenergic fibres and muscular and glandular effectors. In order to elucidate the latter aspect of the problem, ultrastructural investigations are in progress in our laboratory.

In conclusion, in guinea-pig stomach ileum and rectum adrenergic fibres with varicose aspect have been demonstrated within the tunica propria of the mucosa, in intimate relationship with the secretory cells. Adrenergic fibres were detected also in the texture of muscularis mucosae, parallel to bundles of smooth muscle cells⁹.

Riassunto. Con il metodo in microscopia a fluorescenza di FALCK e HILLARP è stata studiata l'innervazione adrenergica della tonaca mucosa del canale alimentare della cavia adulta. Sono state messe in evidenza fibre adrenergiche nella lamina propria della tonaca mucosa, in intimo rapporto con le cellule epiteliali secernenti. Fibre adrenergiche sono presenti anche nello spessore della muscularis mucosae parallele ai fasci di cellule muscolari lisce.

G. GABELLA and M. COSTA

*Department of Histology and General Embryology of the University of Torino, 10126 Torino (Italy),
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