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EXPERIMENTAL INVESTIGATION OF THE EFFECT OF PERORALLY APPLIED ALUMINIUM ON THE STRUCTURE AND FUNCTION OF SOME INTERNAL ORGANS. LUNG ALTERATIONS IN RATS

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The authors examined the lung lesions caused by peroral aluminium application as a 0,1 % solution of $AlCl_3$ in a dose of 3 mg Al^{3+}/kg b. w. for 40 days in a total of 126 white male rats (70 experimental and 56 control). Visible structural alterations were established on the15th day of the trial consisting in desquamation of bronchial epithelium, enhanced amounts of acid glucosaminoglycans in the cell cytoplasm and irregular disposition of cells. Later on, the activity of SDH, alkaline phosphatase, and ATP-ase decreased but acid phosphatase activity increased. Connective-tissue fibres grew up in the wall of some bronchi. These morphological findings suggest that although perorally accepted, AlCl3 induced structural lesions in the lungs as well as metabolic disturbances in the bronchial mucosa which depended on the duration of treatment.

Key-words: Aluminium, peroral application, lungs, histopathology, histoenzymology, rats

INTRODUCTION

Aluminium is a widespread element. It is used in the chemical industry where one contacts with its compounds. In the human body aluminium (Al) causes different organic alterations. Al effect on the tissues depends on the way of introduction into the organism and the subsequent metabolic processes. Perorally applied Al induces structural lesions in the brain, liver, heart, kidneys and bones (3, 7, 8, 11 - 13, 15, 19). It exerts also an embryotoxic effect (5, 11, 18) while its inhalation by the bronchial tree attacks mainly the lungs (1, 10, 14, 16, 20).

We could not find any publications dealing with lung lesions as a result from peroral application of Al or its compounds in the literature available.

The purpose of the present study was to reveal the structural alterations in the lungs caused by Al administered in

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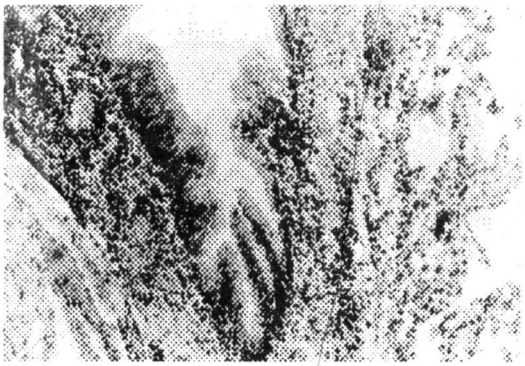

Fig. 1. Rat lung 15 days after peroral $AlCl_3$ application. Multilayered bronchial epithelium. Cell nuclei are located in a different distance from the basement membrane. Staining HE. Magn. 10 x 10

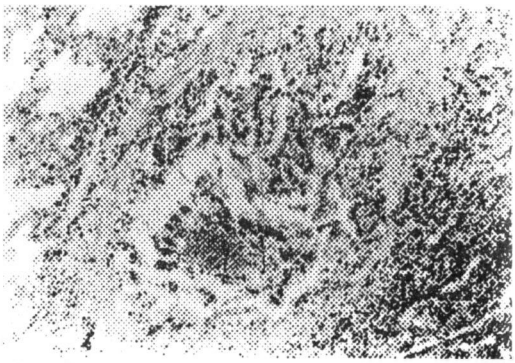

***Fig. 2**. Rat lung 40 days after peroral $AlCl_3$ application. Desquamated bronchial cells and uncovered basement membrane. Staining HE. Magn. 10 x 10*

minimal doses and the mechanism of the development of these changes.

MATERIAL AND METHODS

The examination covered 126 white male rats with body weight of 120 ± 10 g. Some 70 experimental animals were perorally given 3 mg /kg of Al^{3+} as a 0.1 % solution of $AlCl_3$ for 40 days while the control group of 56 rats received distilled water during the same period. Ten experimental and eight control rats each were killed by decapitation on the 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 30^{th}, and 40^{th} day of the trial. Lung pieces were fixed in 10 % formalin solution and in Carnua solution as well. Paraffin sections (5 μm tick) were stained with HE, after Gomori (for reticular fibres), after van Gieson (for collagen ftissue), with PAS reaction after McManus (for glycoproteins), with toluidine blue at pH 2 and pH 4 (for acid glucosaminoglycans), with Brachet reaction (for RNA) and Feulgen reaction (for DNA). The succinate dehydrogenase (SDH) activity was estimated on cryostat sections after the method of Nachlas, the alkaline phosphatase and acid phosphatase activity - after that of Gomori, and the adenosine triphosphatase (ATP-ase) activity - after that of Padycula and Herman.

RESULTS

We establish visible structural lesions in the lungs on the 15^{th} day of the experiment. Some single and grouped bronchial mucosa cells in the large and middle bronchi are desquamated. Remnants of the cells cover the basement membrane with one or more layers. Nuclei are circled or thinned situated in a different distance from the basement membrane (Fig. 1). In the cytoplasm there is an enhancement of mucinous substances such as glycoproteins and acid glucosaminoglycans.

The activity of SDH, ATP-ase and alkaline phosphatase remains unchanged. Acid phosphatase activity is slightly expressed. Reticular and collagen fibres in bronchial walls are irregularly thick but

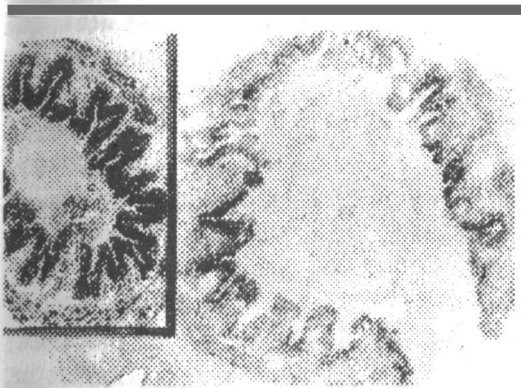

Fig. 3. Weak SDH-ase actibity in bronchial cells. Left - controls. Magn. 10 x 10

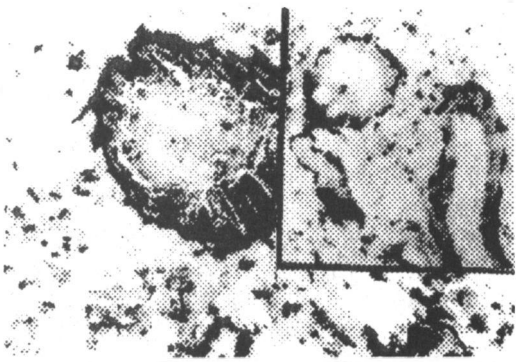

Fig. 4. Reduced alkaline phosphatase activity (in the square) as compared to that of controls. Magn. 10 x 10

some of them are rather rough. Interalveolar septi are focally thickened. There is infiltration of lymphocytes, plasmocytes and single eosinophils. High activity of both alkaline phosphatase and ATP-ase is proved in endothelial cells and adventitia of blood vessels. Desquamated pneumocytes mixed with macrophages with high cytoplasmic activity of both alkaline and acid phosphatases are observed in the lumen of alveolar groups.

These changes aggravate until the 20th day after treatment. In intact cells there is a higher concentration mainly of acid glucosaminoglycans while pyroninophilic granules are irregularly scattered in the cytoplasm being reduced in number in most cells. The staining of the nuclei of bronchial epithelial cells is of different intensity. The activity of SDH, ATP-ase and alkaline phosphatase is diminished in some cells but enhanced in others. Acid phosphatase activity is elevated.

During the interval between the 30th and 40th day, desquamation affects a large number of epithelial cells of bronchi of different caliber and the basal membrane is completely uncovered (Fig. 2). The amounts of glycoproteins, acid glucosaminoglycans, nuclear DNA and cytoplasmic RNA are significantly reduced on the 40th day after treatment. SDH activity is also strongly decreased (Fig. 3) being detectable in single bronchial epithelial cells and alveolar macrophages only. The activity of ATP-ase and acid phosphatase varies. Alkaline phosphatase activity remains high in vascular walls but reduces and even disappears in most bronchial epithelial cells and pneumocytes as well (Fig. 4).

In some bronchial walls certain inflammatory alterations along with growing up of fine peribronchially located connective-tissue fibres are observed. Alveolar septi are thickened and contain lymphocytes, plasmocytes and other cells. Desquamated pneumocytes and macrophages can be detected in alveolar lumens. Focal atelectasis is also present (Fig. 5).

DISCUSSION

Our morphological findings demonstrate that peroral Al administration

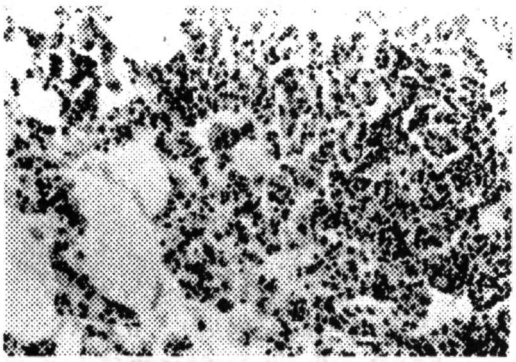

Fig. 5. *Desquamated pneumocytes and macrophages in alveolar lumen. Focal atelectasis. Staining HE. Magn. 10 x 10*

induces structural and functional pulmonary disorders. Already on the 15th day after Al introduction, some signs of damage of the epithelial cells of the bronchial mucosa can be established. Later on the activities of SDH and ATP-ase which participate in the oxidative phosphorization and cellular preservation (9) decrease along with acid phosphatase activity enhancement. These changes argue convincingly of the development of dystrophic processes in the cells and of disturbances of cell metabolism. On the other hand, there is an intensive mucous secretion in the bronchial epithelial cells on the account of glycoproteins and particularly of acid glucosaminoglycans. This is considered an expression of a defense reaction against the action of toxic substances and their derivatives (2). The reduction of mucous substances and, especially, that of acid glucosaminoglycans established by us at the end of experiment is assumed by some authors (6) to influence unfavourably upon the mechanisms of compensation and adaptation of the bronchial mucosa.

The diminution of alkaline phosphatase activity in bronchiolar epithelial cells considered like pneumocytes of type 2 the most sensitive cells towards toxic influences (4) 30 days after the treatment is of essential interest. It could be presumed that due to aluminium-induced disorders in phosphate metabolism (17) the synthesis of phospholipids in the pneumocytes of type 2 is destroyed resulting in altered metabolic processes in these cells.

In contrast to lung alterations after Al inhalation characterized by granulomas, diffuse pulmonary fibrosis, etc. (1, 10, 14), the changes after peroral Al application remain more discrete. It seems possible that, however, the alterations in reticular fibres such as roughness, fragmentation, etc. along with inflammatory changes in the wall and peribronchial growing up of thin connective-tissue fibres could determine the development of bronchial and peribronchial fibrosis after Al treatment of a longer duration.

CONCLUSION

The analysis of our results indicates that the development of both structural and metabolic disturbances in the lungs depends on the duration of Al treatment.

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Experimentelle Untersuchung der Aluminiumseinwirkung über die Struktur und Funktion der Organen der Ratte bei der peroralen Applikation. Veränderungen in den Lungen

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Zusammenfassung: Es wurde ein Versuch über 126 weißen männlichen (70 experimentelle und 56 Kontrolltiere) Ratten angestellt. Das Aluminium wurde peroral durch eine Sonde als 0,1 %-ige Lösung von $AlCl_3$ in einer Dose von 3 mg Al^{3+}/kg innerhalb von 40 Tage lang verabreicht. Man studierte die Lungenveränderungen der Ratten. Schon am 15. Tag nach dem Beginn des Experiments wurden einige strukturelle Beeinträchtigungen in den Lungen festgestellt: eine Desquamation des Bronchialepithels, eine Steigerung der Menge der Glukoseaminoglykans im Zytoplasma und eine unregelmäßige Verteilung der Zellen. Die Aktivität der SDH-ase, der alkalischen Phosphatase und der ATP-ase nahm ab, während sich diese der sauren Phosphatase erhöhte. In der Wand einiger Brochi stellte man außerdem Kollagenfaserwachstum fest. Diese morphologischen Veränderungen zeugen davon, daß, obwohl das $AlCl_3$ peroral

eingenommen wurde, seine Wirkung über die Lungen- und Bronchialstruktur ausgeübt wurde. Die Schwere dieser Läsionen hing von der Dauer der Einwirkung ab.

Etude expérimentale sur la structure et la fonction des organes sous l'effet de l'aluminium accepté oralement dans l'organisme. Changements dans les poumons des rats

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Résumé: Dans les conditions expérimentales on a étudié en dynamique l'action de l'aluminium sur 126 rats blancs mâles (70 - d'expériment et 56 - de contrôle). L'aluminium a été admis comme une solution de 0.1 % d'$AlCl_3$ en dose de 3 mg Al^{3+}/kg au cours de 40 jours. On a étudié les changements de la structure des poumons. Au 15^e jour du début du traitement on a éxaminé des changements dans les bronches - écaillage des cellules épithéliales de la muqueuse bronchiale, un contenu muqueux augmenté dans la cytoplasme, un arrangement irrégulier des cellules, etc. Le caractère de ces changements s'est approfondit au cours de l'expériment. On a constaté une diminuation de l'activité de la succindéhydrogénase, de la phosphatase alcaline, de l'adénosine-triphosphatase; une augmentation de l'activité de la phosphatase acide; la quantité des substances muqueuses diminue, et dans quelques des bronches se développe une fibrose. Les changements nous montrent que $AlCl_3$ admis oralement provoque des modifications dans la structure des poumons et de changements métaboliques dans la muqueuse bronchiale. Ces changements dépendent de la continuité du traitement