

MORPHOLOGY AND MORPHOMETRY OF THE LUNG IN CYNOMOLGUS MONKEYS AFTER 2 YEARS INHALATION OF QUARTZ UNDER NORMAL AND EXCESS PRESSURE (*)

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INTRODUCTION

Modern tunnelling often includes working under excess pressure to protect buildings and the environment. Tunnel workers are exposed to different kinds of dust, arising from the underground rock or from material used for mixing the concrete. The dust exposure in tunnelling depends on the working station and the total dust concentration varies about 10 mg/m^3 .⁵

In a multidisciplinary research project, the effects of "Shotcrete Tunnelling in Compressed Air" are investigated. This method is used more and more in tunnelling. Both, clinical investigations in tunnel workers and experimental studies in animals are conducted. The main inhalation experiment is carried out in monkeys with quartz dust and compressed air.⁶

This paper deals with morphological results of the experiment, especially regarding macromorphology and histopathology of the respiratory tract, preliminary morphometrical findings and some biochemical data of the lung collagen content.

MATERIAL AND METHODS

Final morphological and morphometrical evaluation as well as hydroxyproline determination was carried out on 21 cynomolgus monkeys (*M. fascicularis*) separated in 4 groups and kept for 26 months, 5 days per week and 8 hours per day under the following conditions:

- Group I (n=5) normal atmospheric pressure (1.0 bar), without dust
- Group II (n=7) quartz dust DQ-12 (5 mg/m^3) and normal pressure (1.0 bar)
- Group III (n=4) quartz dust DQ-12 (5 mg/m^3) with 2.5 bar absolute pressure and
- Group IV (n=5) 2.5 bar absolute pressure, without dust

Evaluation of the experiment was done involving different disciplines. The results of BAL-cytology and CT-radiology are presented by Krombach et al. in another paper in the proceedings of this conference.

(*) Supported by "Federal Secretary of Research and Technology," BMFT, Grant No.: 01 VD 492/7.

After 12 and 18 months, open lung biopsies were taken using standard techniques, and studied morphologically (12 mo.: n = 25; 18 mo.: n = 24).

Immediately after the last radiological examination the animals were sacrificed. The lungs were fixed by instillation under controlled pressure (2.5% glutaraldehyde, pH 7.4; 20 cm H₂O). Additionally a retrograde perfusion via the abdominal aorta was carried out.

Tissue samples from six different locations were taken after 24 hours of fixation, corresponding to the levels of computed tomography.

After washing in phosphate-buffer, the specimens were embedded in paraffin and tissue slices (6 μm) were cut. Qualitative histomorphology was done on sections stained with Haematoxylin-Eosin (H&E), Giemsa and Azan.

Morphometric evaluation was done chequered on 6 H&E stained sections of each lung, using the 'Interactive Image Analysing System' (IBAS-2, Kontron). In this equipment the histologic structures of the lung tissue are transformed into evaluable black and white pictures. In a multistep semiautomatic morphometric programme of 90 single steps four different area parameters were determined: —air space,—total lung tissue,—lung tissue without blood vessels and bronchial airways, ('respiratory lung tissue') and—areas of cellular accumulations, corresponding to granulomatous reaction tissue.

The determination of hydroxyproline content of the lungs was performed on unselected, glutaraldehyde fixed lung tissue in a modified method according to Stegemann.¹¹

For the statistical evaluation, the SAS-statistical system according to SAS—User's Guide (Statistics Version, 5. Ed., 1985) was applied.

RESULTS

Lung Biopsies

After 12 as well as after 18 months, reaction tissue can be detected in the lungs of all dust exposed animals. This granulomatous reaction tissue consists of inflammatory cells (macrophages, lymphocytes, polymorphonuclear granulocytes, mast cells), fibroblasts and collagenous fibers.

In the phagocytosing cells, quartz particles can be detected. Qualitatively, the degree of fibrosis is markedly higher in group II than in group III, especially after 12 months. But, after 18 months, the findings in group III increase comparatively.

Organ Weights

Weights of lung, mediastinal lymph nodes, heart, spleen, liver, and kidney were determined at necropsy. The weights of these organs, except the kidney, are increased in dust exposed groups (Figure 1). Due to intratracheal instillation, lung weights shown in Figure 1 do not give an exact information.

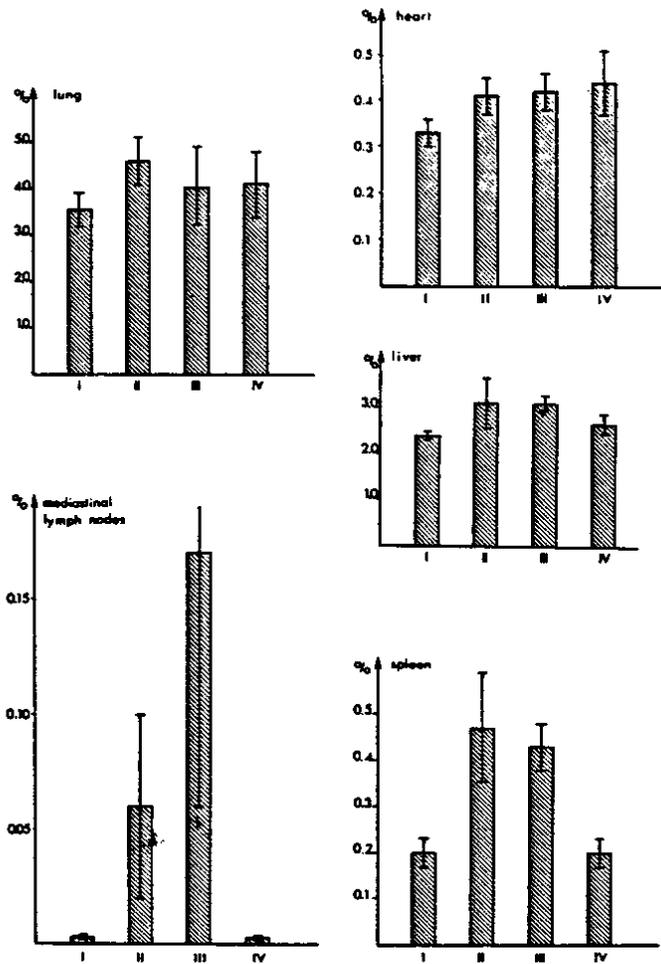


Figure 1. Relative organ weights of heart, lung, liver, spleen, and mediastinal lymph nodes, determined at necropsy, after intratracheal instillation and perfusion.

Macroscopy

Macroscopically, in control animals, the fixed lungs reveal a smooth surface, pale, red coloured and a soft elastic texture. Quartz exposed animals have more voluminous lungs with slightly rough surface, many whitish coloured nodules and a tight tissue texture. The mediastinal lymph nodes are extremely enlarged. In many cases, they are narrowing the

lumen of the trachea and the main bronchi. In this way, they are hindering the respiration of the animals.

Histopathology

The qualitative morphologic evaluation of control lungs shows normal lung structure with thin alveolar septa (Figure 2a). Some macrophages and very few cellular accumulations are detectable. In dust exposed animals, the structure of lung tissue is totally altered. Extended areas of fibrosis (Figure 2b) with mast cells incorporated between fibroblasts, macrophages and collagenous fibers are visible in the animals of groups II and III. Fibrotic nodules often occur perivas-

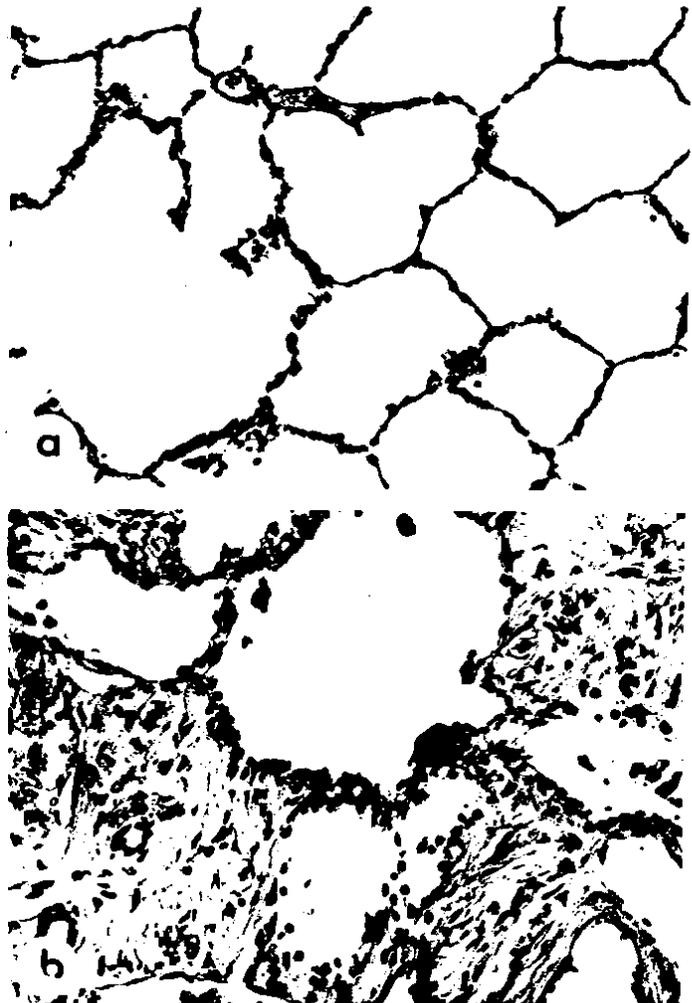


Figure 2. Lung histopathology after 26 months of inhalation: a. Normal lung structure in a control animal (group I); b. extended fibrosis in dust exposed animal (group II); H&E staining, obj. 10x.

cularly. Due to intratracheal instillation, almost all intra-alveolar cells are washed out. Many transformed alveolar pneumocytes II can be seen at the border of fibroses (Figure 3a). Using polarized light, birefringent phagocytosed quartz particles and collagenous fibers can be observed (Figure 3b). Furthermore, the quartz induced connective tissue is infiltrating those areas which do not reveal severe changes.

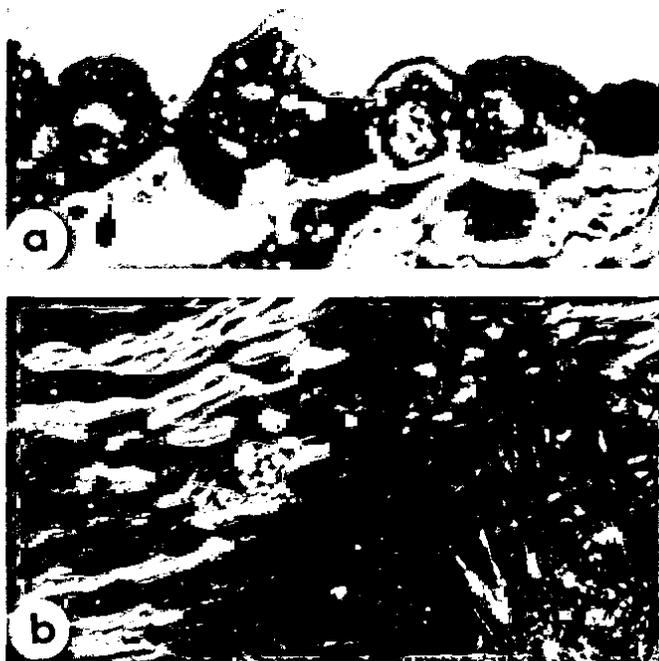


Figure 3. Detailed morphology of quartz induced lung alterations:

- a. Pneumocytes typ II, with lamellar bodies in the cytoplasm, are lining alveolar surface; semithin section, methylene-bue staining, obj. 63x;
- b. in polarized light, birefringent quartz particles as well as collagenous fibers are detectable; mast cells, as dark spots, included in the fibrosis; Giemsa staining, obj. 25x.

The lung structure of animals just exposed to compressed air (group IV) is not apparently altered compared with group I animals. In some areas alveolar septa seem to be slightly thickened. In all investigated locations of the lungs no morphological signs of any other pathological process or cancerogenic effect can be seen.

The mediastinal lymph nodes reveal severe fibrosis. They consist at almost 80% of collagenous fibers. Additionally, in the liver of all quartz exposed animals, a severe granulomatous reaction with quartz particles and marked fibrosis, so called "quartz induced nodules," is detectable. Quartz particles are also obvious in the spleen, mesenterial lymph nodes, but not in the kidney.

Morphometry

In lung biopsies, at both times the amount of reaction tissue is more extended in animals which inhaled quartz under normal pressure than under excess pressure. The number of reaction nodules decreases from 12 to 18 months, due to an enlargement of the nodules. The percentage amount of cellular accumulations is significantly increased in group II ($p < 0.01$) and only very slightly in group III (Figure 4).

The final morphometrical evaluation, however, shows a relative alignment between the two dust exposed groups

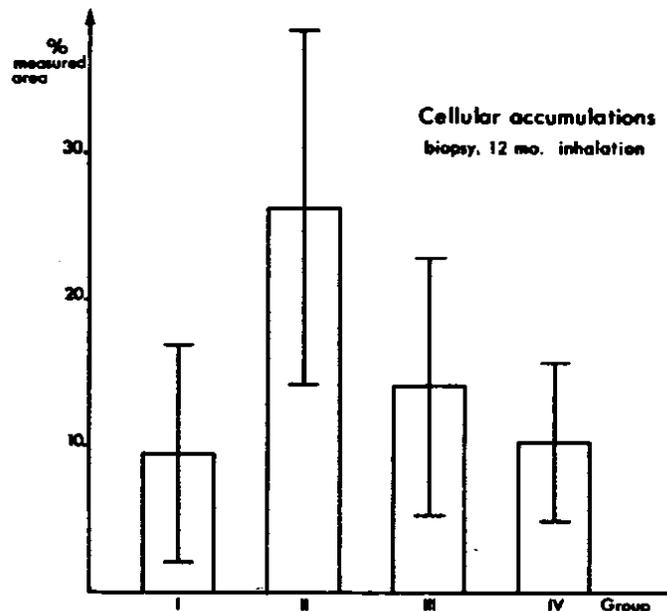


Figure 4. Percentage of cellular accumulations, corresponding to granulomas, in lung biopsies, taken after 12 months of inhalation; arithmetic mean and SEM.

(Figure 5). The significance values are group I/group II $p < 0.001$ and group I/group III $p < 0.05$. Marked differences between right and left lung, as well as between upper, middle and lower level could not be observed.

OH-proline

The hydroxyproline content of the lung, determined as 'mg per gram lung tissue,' shows a severe and significant increase in both dust exposed groups (I/II $p < 0.001$; I/III $p < 0.01$). In animals under excess pressure, however, this increase is somewhat lower than under normal baric conditions (Figure 6).

DISCUSSION

The comparison of findings in lung biopsies with final histomorphology indicates that a delay seems to exist in dust and excess pressure exposed animals, regarding the development of silica induced granulomas. The deposition of inhaled particles also depends on the animals breathing pattern.³ As this breathing pattern may be changed under excess pressure, the delay may be due to another deposition of particles. But, the differences between groups II and III are almost equalized after 26 months. Therefore, group III animals eventually might have developed more extended granulomas, if the exposure time would have been longer.

And, further morphological and morphometrical studies have to be done on bronchial epithelia. A complete morphological characterization of the lung requires informations on the gas exchange tissue as well as on the airway tree.¹² Due to an eventually changed particle deposition, alterations possibly occur in the airway tree, too.

Recently, the dust content of the bronchial mucosa was discussed with regard to lung cancer.² Also because of this,

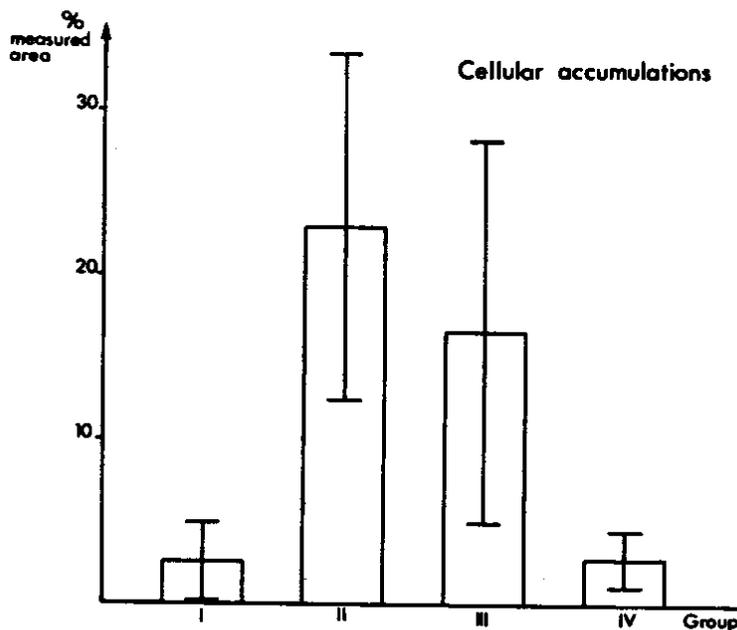


Figure 5. Percentage of cellular accumulations, corresponding to granulomas in the lungs, determined on 6 different locations after 26 months of inhalation; arithmetic mean and SEM.

studies on the morphology of the bronchi are necessary. But of course, because of the relatively short experimental period, our experiment cannot serve as a study on cancerogenicity of quartz dust.

The differences between the percentage amount in biopsy and necropsy cellular accumulations in control animals are caused by different kinds of tissue fixation, immersion and instillation, respectively.

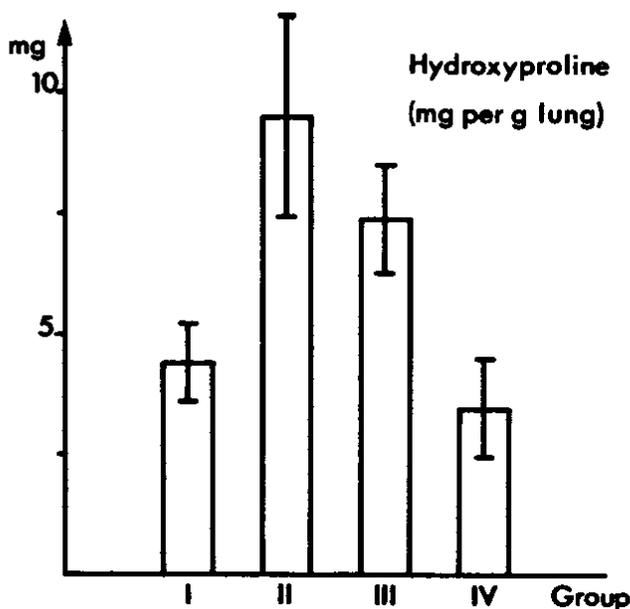


Figure 6. Hydroxyproline content of lung tissue.

A significantly increased lung hydroxyproline content could also be detected in monkeys after repeated paraquat treatment.⁸ However, the absolute amount of hydroxyproline in those animals was lower than in our animals. This is probably due to different lung tissue treatment before the determination of hydroxyproline content. We used glutaraldehyde fixed samples, and in the other case⁸ frozen lung tissue was taken.

Pathogenetically, the findings described develop in the following manner: the clearance of inhaled particles from the lung firstly takes place via bronchi and trachea and secondly via regional lymph nodes. The lymph nodes are overflowing and in this way macrophages with phagocytosed particles reach the blood and are deposited in other tissues, such as liver, spleen and mesenteric lymph nodes (Figure 7).

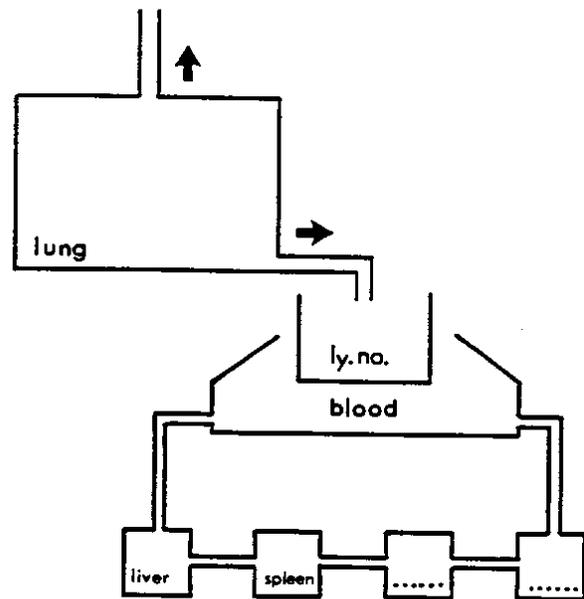


Figure 7. Pathogenesis of the lesions in various organs after inhalation of quartz dust.

Liver granulomas have been reported previously in rats, intravenously treated with silica⁴ and as single case reports in humans due to inhalation of various dusts.^{1,10} Because of this distinct cellular reaction the increase of the organ weight of the liver can be explained.

Interestingly the heart weights of group IV animals, just exposed to excess pressure, are increased compared with group I animals. This is an indication that just excess pressure causes effects on the respiratory and circulatory system. But, a direct effect of hyperbaric oxygen on the vascular wall has already been presumed elsewhere.⁹

Regarding morphometry, additional to the area measurements the evaluation of two 'mean chord lengths' will be carried out. These data are also known as 'mean linear intercept'.⁷ Firstly, the chord lengths of the respiratory lung tissue will

provide informations concerning an interstitial fibrosis, and secondly, chord lengths of the alveolar diameter, to get an answer about any possible tendency of emphysema in the dust and/or excess pressure exposed animals. A more sophisticated discussion will be possible when these morphometric data are available.

Additional investigations shall be carried out in the same animals, using electron microscopy and enzyme histochemistry. This will provide more detailed morphological informations. And, furthermore some steps still have to be done:

1. The comparison of the results obtained with different morphological methods, such as cytology, radiology and pathomorphology, and
2. Morphological data have to be compared with lung function tests.

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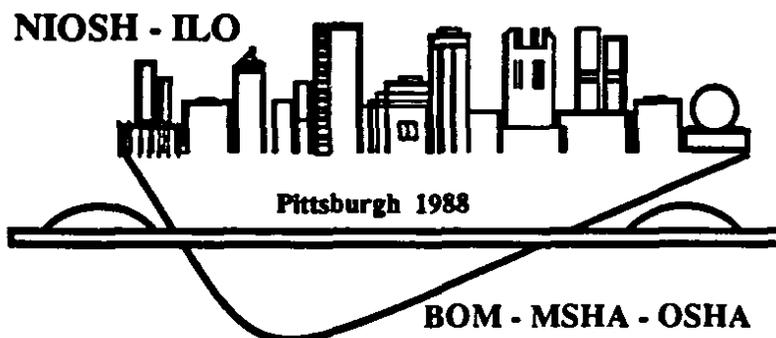
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ACKNOWLEDGEMENT: The technical assistance of Mrs. R. Grover, Mrs. G. Lauer mann, Mrs. H. Metes, Mrs. I. Spiekermann, and Mrs. Y. Steinfartz is gratefully acknowledged.

Proceedings of the VIIth International Pneumoconioses Conference
Transactions de la VIIe Conférence Internationale sur les Pneumoconioses
Transacciones de la VIIa Conferencia Internacional sobre las Neumoconiosis

Part **I**
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Pittsburgh, Pennsylvania, USA—August 23–26, 1988
Pittsburgh, Pennsylvanie, Etats-Unis—23–26 août 1988
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September 1990

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DHHS (NIOSH) Publication No. 90-108 Part I