# THE DIFFERENT BIOLOGICAL EFFECTS OF DUSTS APPLICATED INTRATRACHEALLY SEPARATELY OR IN MIXTURES IN RATS

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An intraperitoneal injection of pure natural anhydrite has been shown to cause a mild fibrosis, which has been attributed to the content of quartz.[8] We postulated that anhydrite enhances the effect of the quartz. Cadmium sulfide also induces pulmonary fibrosis in our rat intratracheal test.[2] It is known, that polyvinylpyridine-N-oxide (PVNO) possesses an inhibitory action on quartz. However, we were able to show that CdS behaves differently in a mixture with PbS than with PVNO.[3]

In order to investigate the behaviour of dusts in mixtures, we used the rat intratracheal test to study anhydrite, cadmium sulfide and titanium dioxide dusts separately or in mixtures with Döorentruper quartz DQ 12 or polyvinylpyridine-N-oxide. The individual dusts and the artificial dust mixtures were initially made into suspensions, which were then physically characterized.[5] The results of this characterization are presented in the poster session.

A variety of dust samples were instilled in suspension in 0.5 ml of physiological saline solution into the trachea of female SPF Wistar rats (weight approximately 200 gr). The animals were sacrificed 3 months after instillation. The half of each left lung was hydrolyzed in sealed reagent tubes with 6 N.HCl for 16 to 18 hrs at 104°C. Following cooling and filtration the corresponding hydrolysate (0.1 to 0.5 ml) was neutralized with NaCl and the total hydroxyproline content determined.[7] The other half of each left lung was weighted, homogenized and extracted with chloroform-methanol (2:1). The total lipid content was then determined.[1] The corresponding right lungs of these animals and their regional lymph nodes were fixed in 4% formalin, dehydrated in graded alcohols and embedded in paraffin. Sections were stained with HE and EvG.

## RESULTS

Those animals treated with anhydrite showed no increase in total lipid or hydroxyproline content in the lungs when compared with controls. After titanium dioxide these parameters were slightly raised. Treatment with PVNO or PVNO with CdS elicited only a mild increase in total lipid content compared with controls. Following separate instillation of quartz we found a dose-dependent increase in both total lipids and total hydroxyproline. Separate instillation of cadmium sulfide also caused significant elevation of both parameters (Table I).

Both successive and simultaneous instillation of anhydrite and quartz led to marked reduction in total lipid and hydroxyprolin content, compared with values obtained after separate quartz treatment. However, with decreasing quartz concentration or increasing anhydrite concentration an increase in lipid and hydroxyproline content was observed. Total lipids and hydroxyproline content in the lungs were also significantly elevated following a combination of titanium dioxide and quartz compared with values obtained with separate application of titanium dioxide.

The highest increase of total hydroxyproline content was established in animals which had been treated with quartz. The lowest level of hydroxyproline was achieved after simultaneous instillation of anhydrite with quartz. PVNO combined with quartz led to a clear reduction in hydroxyproline content, but significantly increased it when combined with CdS compared with that obtained after separate application of CdS.

Inflammatory changes in control lungs were not found. Following separate quartz treatment numerous disseminated histiocytic nodules with marked synthesis of collagenous connective tissue were seen both in the pulmonary tissue (Figure 1) and in the lymph nodes (Figure 2). The content of these changes was dose-dependent. In addition, in the lungs alveolar proteinosis was also observed.

After successive instillation of 35 mg anhydrite and 11 mg quartz a marked nodular histiocytic reaction without fibrosis was observed (Figure 3). In addition, simultaneous intratracheal instillation of 2 mg quartz with 35 mg anhydrite elicited in lymph nodes a minimal reaction with suggestion of nodule formation without fibrosis (Figure 4).

With increasing anhydrite dose the number of foam cells and foreign body giant cells decreased, whilst histiocytic nodules increased. In all anhydrite-quartz groups, independent of the anhydrite dose, merely numerous birefringent crystals were observed, without any increase in connective tissue component or alveolar proteinosis. In the swollen lymph nodes numerous partially confluent histiocytic nodules with a minimal collagenous reaction were found (Figure 5).

In the rat lung the separate intratracheal instillation of titanium dioxide failed to elicit a fibrous reaction. The foam

Table I

Means (x) and Standard Deviations (s) of Total Lipids and Total Hydroxyproline in Rat Lung 3 Months After Intratracheal Instillation

| group (dust sample) | Total lipids/ lung mg | | hydroxiproline/lung mg | |
|---|---|---|---|---|
| | x | s | x | s |
| 35 mg anhydrite | 43,47 | 5,06 | 3,40 | 0,52 |
| 2 mg DQ 12 | 122,80 | 49,19 | 4,94 | 1,12 |
| 5 mg DQ 12 | 191,95 | 49,93 | 5,85 | 0,67 |
| 7 mg DQ 12 | 202,94 | 45,48 | 8,20 | 1,33 |
| 11 mg DQ 12 | 207,13 | 58,46 | 8,22 | 1,29 |
| 20 mg PVNO | 48,11 | 4,55 | 3,66 | 0,31 |
| 20 mg CdS | 66,25 | 3,46 | 5,18 | 2,24 |
| 5 mg anhydrite + 11 mg DQ 12 | 156,96 | 13,37 | 3,81 | 0,70 |
| 20 mg anhydrite + 11 mg DQ 12 | 78,76 | 25,60 | 4,12 | 1,20 |
| 50 mg anhydrite + 11 mg DQ 12 | 78,83 | 13,04 | 3,73 | 0,92 |
| 35 mg anhydrite + 2 mg DQ 12 | 37,99 | 2,52 | 3,10 | 0,26 |
| 35 mg anhydrite + 5 mg DQ 12 | 40,34 | 3,42 | 3,22 | 0,14 |
| 35 mg anhydrite + 11 mg DQ 12 | 66,34 | 9,39 | 3,15 | 0,17 |
| 30 mg $TiO_2$ | 53,14 | 12,65 | 3,30 | 0,37 |
| 30 mg $TiO_2$ + 5 mg DQ 12 | 101,78 | 20,80 | 4,81 | 0,65 |
| 2 mg PVNO+11 mg DQ | 55,53 | 5,97 | 3,47 | 1,21 |
| 11 mg PVNO+11 mg DQ | 55,52 | 6,09 | 4,14 | 0,51 |
| 2 mg PVNO+20 mg CdS | 58,55 | 3,87 | 5,72 | 0,70 |
| 11 mg PVNO+20 mg CdS | 58,46 | 4,65 | 6,24 | 0,81 |
| 20 mg PVNO+20 mg CdS | 59,76 | 5,22 | 6,50 | 0,84 |
| control | 44,17 | 5,00 | 3,26 | 1,03 |

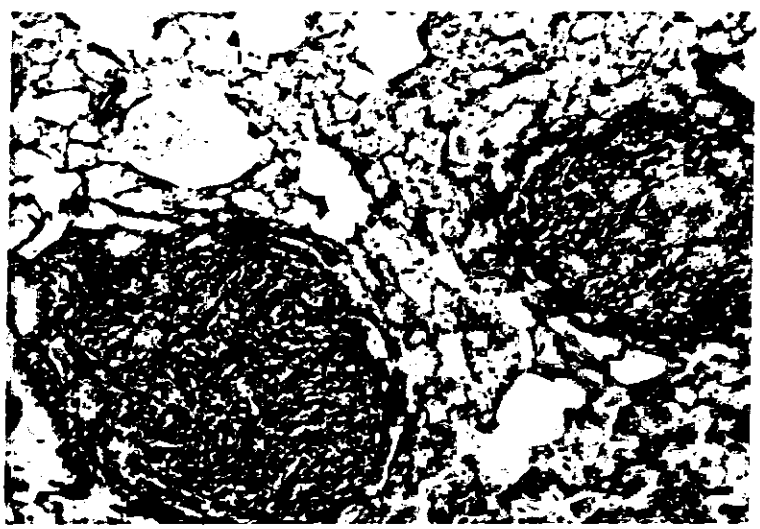

Figure 1. Rat lung with histiocytic nodules, a fresh formed collagen tissue and alveolar proteinosis 3 months after intratracheal instillation of 5 mg Dörentruper quartz.

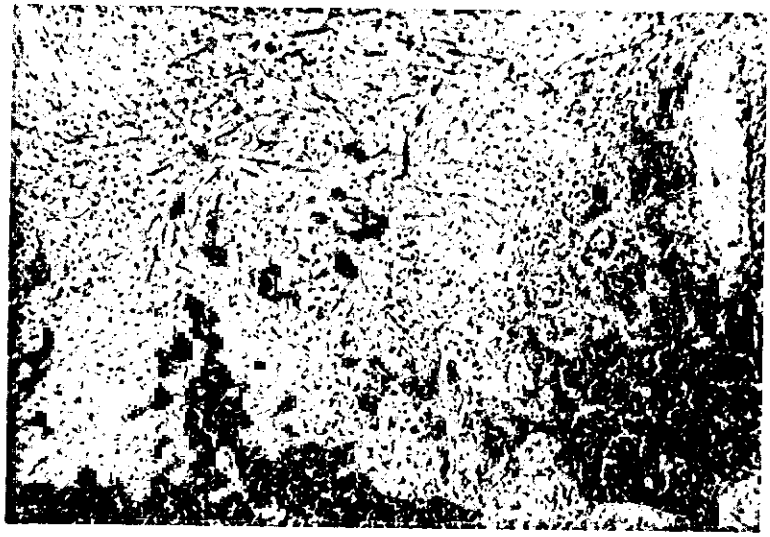

Figure 2. Partial compact fibrosis in the lymph nodes after intratracheal instillation of 5 mg Dörentruper quartz. EvG stain, magnification 50 fold.

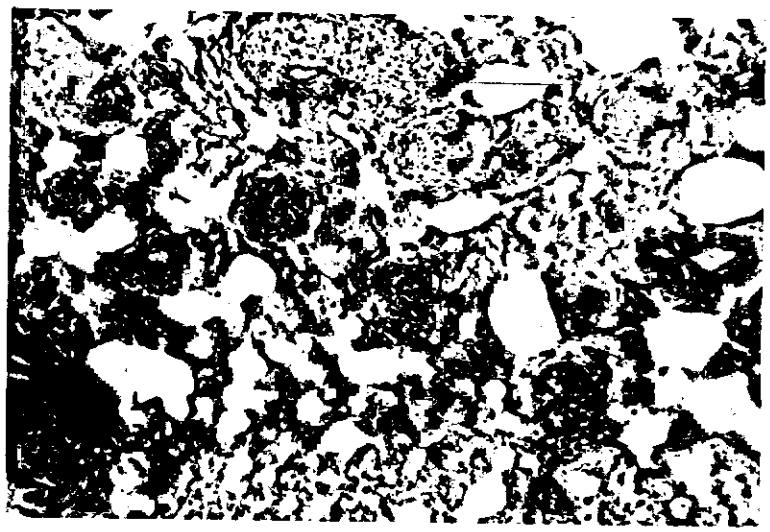

Figure 3. Rat lung with small histiocytic nodules without fibrosis after successive intratracheal instillation of 35 mg anhydrite and 11 mg Dörentruper quartz DQ 12. EvG stain, magnification 50 fold.

cell reaction in the lung after separate titanium dioxide instillation (Figure 6) was markedly enhanced following a combination of titanium dioxide and quartz (Figure 7). The alveolar septae beiing markedly thin and the alveolar epithelia much flattened. After combined treatment with titanium dioxide and quartz, the lymph nodes showed dense deposits of brown pigment and a focal histiocytosis.

After instillation of 20 mg PVNO there was no evidence of inflammation. Nodules of fibrosis did not occur.

After intratracheal instillation of 20 mg CdS, in the lungs, inflammation, moderate fibrosis within the nodular granulomata and single groups of foam cells were seen (Figure 8). Lymph nodes contained dense deposits of finally granular pigment but did not give evidence of fibrosis.

Figure 4. Rat lymph node with focuslike histiocytic reaction without fibrosis after simultaneous intratracheal instillation of 2 mg Dörentruper quartz with 35 mg anhydrite. EvG stain, magnification 50 fold.

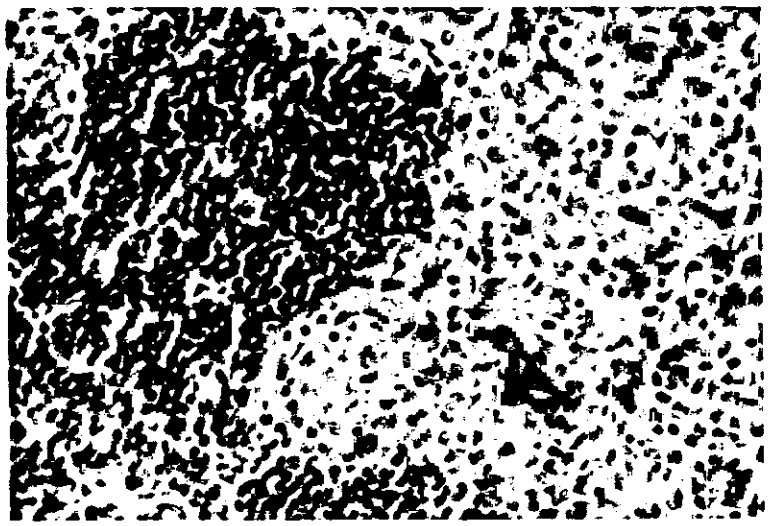

Figure 5. Rat lymph node with a slight fibrosis after simultaneous intratracheal instillation of 11 mg Dörentruper quartz with 35 mg anhydrite. EvG stain, magnification 50 fold.

With increasing PVNO dose at a constant level of CdS, the lungs revealed abundant granular material, which was not birefringent. An inflammatory reaction without nodule formation or foam cells were present. The inflammatory component in the lungs and the dust deposits in both lungs and lymph nodes increased with the total dose.

After the simultaneous instillation of 20 mg PVNO and 20 mg CdS a focal increase in connective tissue in the lung in the form of fibrosis (Figure 9) was observed. In the lymph nodes a focal fibrosis, occasionally, in the form of nodules was seen.

The simultaneous instillation of PVNO and quartz, neither in the lungs nor in the lymph nodes was there any fibrous reaction. Dust deposits could not be found in the lymph nodes.

Numerous nodular granulomata, pricipally histiocytosis with

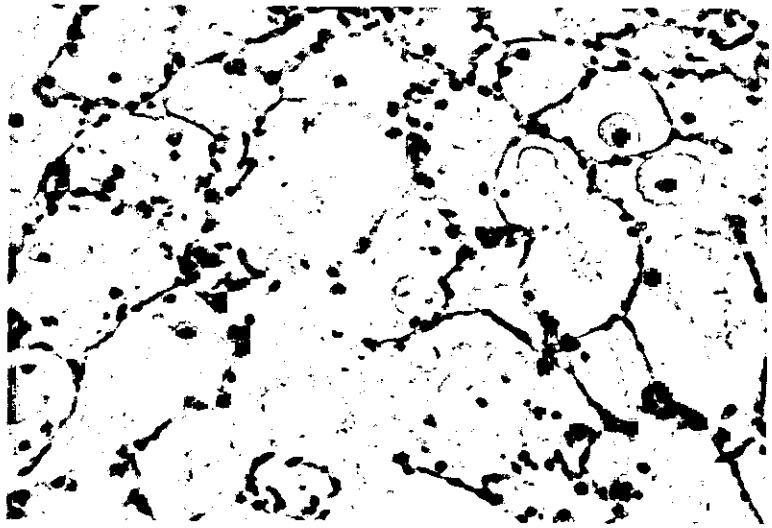

Figure 6. Rat lung, a slight foam cell reaction after intratracheal instillation of 30 mg titanium dioxide. EvG stain, mgnification 125 fold.

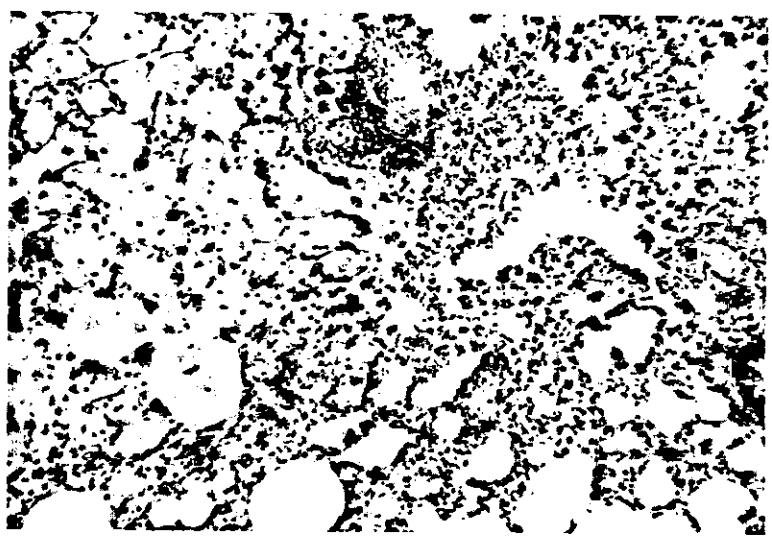

Figure 7. Rat lung, a intensifier foam cell reaction after intratracheal instillation of Dörentruper quartz with titanium dioxide. EvG stain, magnification 63 fold.

a tendency toward confluence, were not in evidence in the lung until 6 months following combined instillation of 2 mg PVNO and 11 mg Dörentruper quartz.

## DISCUSSION

In the various mixtures PVNO showed variable behaviour, i.e. with quartz inhibitory, with CdS stimulatory. A similar behaviour was found with anhydrite: It acted inhibitory on quartz, whereas with the bounding catalyser, a mixture of iron and potassium sulfide, no inhibition could be found.[8]

Furthermore, the physical characteristics of the dust mixtures employed[6] indicates that the act of mixing imparts to the dusts properties which cannot be calculated on an additive basis. The mixture will thus react totally differently from the individual dusts applied separately. The observations lead us to the suggest that we may be dealing with a general principle, namely that each dust can demonstrate different behaviour in different dust mixtures.

Qualitative differences in histological appearance were estab-

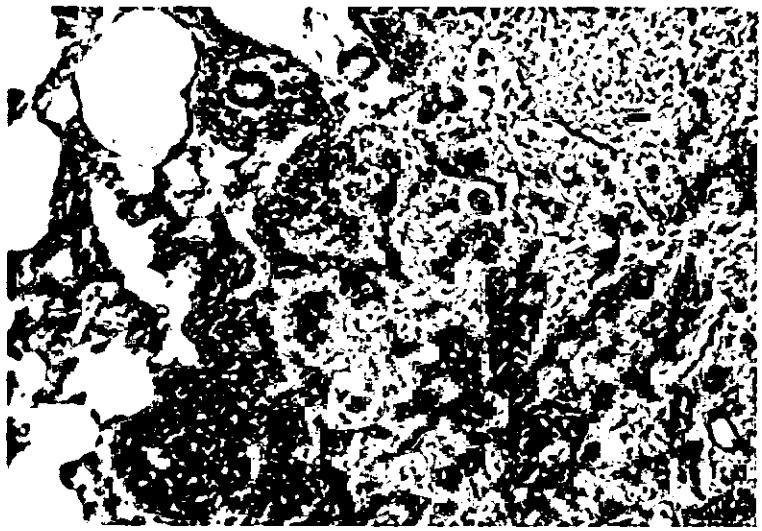

Figure 8. Rat lung, occasional foci of inflammation, moderate fibrosis within nodular granulomata after intratracheal instillation of 20 mg CdS. EvG stain.

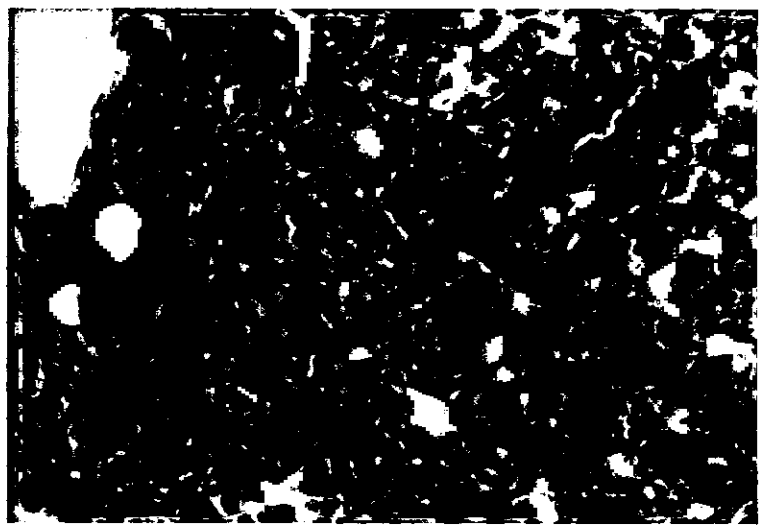

Figure 9. Rat lung, focal fibrosis after simultaneous intratracheal instillation of 20 mg Cds and 20 mg PVNO. EvG stain.

lished between the various experimental groups. The intratracheal instillation of CdS elicited a marked inflammatory reaction in the lungs. However, in contrast with the quartz effect, no sinus histiocytosis could be found in the lymph nodes. The addition of PVNO enhanced the effect of CdS by increasing the degree of penetration of the dust, resulting in fibrosis in the lymph nodes, too.

Increasing anhydrite dose in the dust mixture with quartz led to a decrease in form cell content but an increase in the number of histiocytotic nodules. By contrast, the combination of titanium dioxide with quartz not only enhanced the foam cell reaction but also caused flattering of the alveolar epithelium with the formation of thin alveolar septae. After the combined application of titanium dioxide and quartz the lymph nodes exhibited sinus histiocytosis with no evidence of fibrosis. Combining 35 mg anhydrite with 11 mg quartz elicited a minimal connective tissue reaction in the lymph nodes, but not in the lung.[4] The morphological differences correlated well with the alterations in the hydrogen concentration in suspensions of saturated solutions.[6]

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# THE PROPORTION OF LONG FIBRES IN ATTAPULGITE AND SEPIOLITE CONTAINING ADSORPTION GRANULATES

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## INTRODUCTION

In the Federal Republic of Germany every year more than one million tons of adsorption granulates are used by consumers as animal bedding, adsorption material for oil, or as additive in colours and glues. The majority of these granulates consists of the fibrous minerals attapulgite resp. palygorskite and sepiolite. Depending on the location of the mine, rather different lengths and compositions of the fibres can be found. It is suggestive that the content of fine fibres (D $<0.1$ µm) may have carcinogenic properties.[4] Intrapleurally and intraperitoneally injected attapulgite fibres of the long type caused cancer in rats whereas the short type was not effective.[3,4,5,6,7] In addition, Spanish sepiolite proved to be uneffective as revealed by intrapleural injection experiments.[6,7] Nevertheless, a Finnish sample of a remarkably long fibrous sepiolite, supposedly a rarity, was found to cause cancer in intraperitoneal injection experiments. (Pott et al. unpublished) Thus, the durability of the fibres in the lung has at least to be considered.

Morbidity and mortality studies on employees of American attapulgite mines and mills as well as X-ray examinations of workers in decomposition and processing factories of sepiolite in Turkey have been published.[1,8] However, even mortality studies, conducted during the processing of short fibrous attapulgite deposits in Georgia, distinctively neither excluded nor supported any tumour risk.[8]

Thus, carcinogenic properties of the minerals attapulgite and sepiolite can only be excluded by the use of injection experiments. Especially the content of long fibres in commercially available products has to be kept at least as small as in the tested samples. This communication describes the mineralogical composition and the content of fibres with a length of L $\geq$ 5 µm of adsorption granulates, used as animal bedding.

## MATERIALS AND METHODS

### Mineralogical Investigations

The mineralogical composition of 75 commercially available samples of adsorption granulates used as animal bedding was studied by means of:

- X-ray diffraction
- polarization—and phase contrast microscopy
- differential thermoanalysis.

Samples supposedly composed of fibrous components were subsequently classified according to number and length of fibres.

### Scanning Electron Microscopy (SEM) Classification of Fibre Quality

Of each sample, by the use of sonication (30'), 5 mg were suspended in 50 ml distilled water. Aliquots were filtered with nucleporefilters (poresize 0.2 µm, previously sputtered with gold) to obtain a substrate density of 5 µg/cm$^2$. These specimens were analysed by SEM (13000x). In addition to the mineralogical study, the specimens were also investigated by energy dispersive X-ray analysis to obtain the elemental composition of each sample.

### Qualification of Fibres by Scanning Transmission Electron Microscopy (STEM)

The concentration of fibres contained in 5 samples was revealed by the use of STEM. As described above, an aqueous suspension of the sample was prepared and filtered on an untreated nucleporefilter (poresize 0,2 µm). Subsequently, the specimen was sputtered with carbon, the filter material dissolved in a Jaffe washer, and the remaining transmission sample was analyzed at a magnification of 29000 times (STEM) for fibres of any length and at a magnification of 10000 times (TEM) for fibres with a length of L $\geq$ 5 µm.[4]

## RESULTS

Attapulgite resp. palygorskite were characterized as main components in 7 samples and sepiolite in 19 samples, (Figure 1). In 9 other samples sepiolite was also found in minor quantities. These 35 products as well as 14 other samples containing various calciumsilicahydrates were analyzed for their fibrous components by the use of scanning electron microscopy. Twenty six products which were mainly composed of attapulgite and sepiolite proved to be aggregated of fine fibres. Although the overwhelming majority of these fibres did not exceed the length of L $\geq$ 5 µm, a smaller portion of the fibres was longer than 5 µm in all cases.

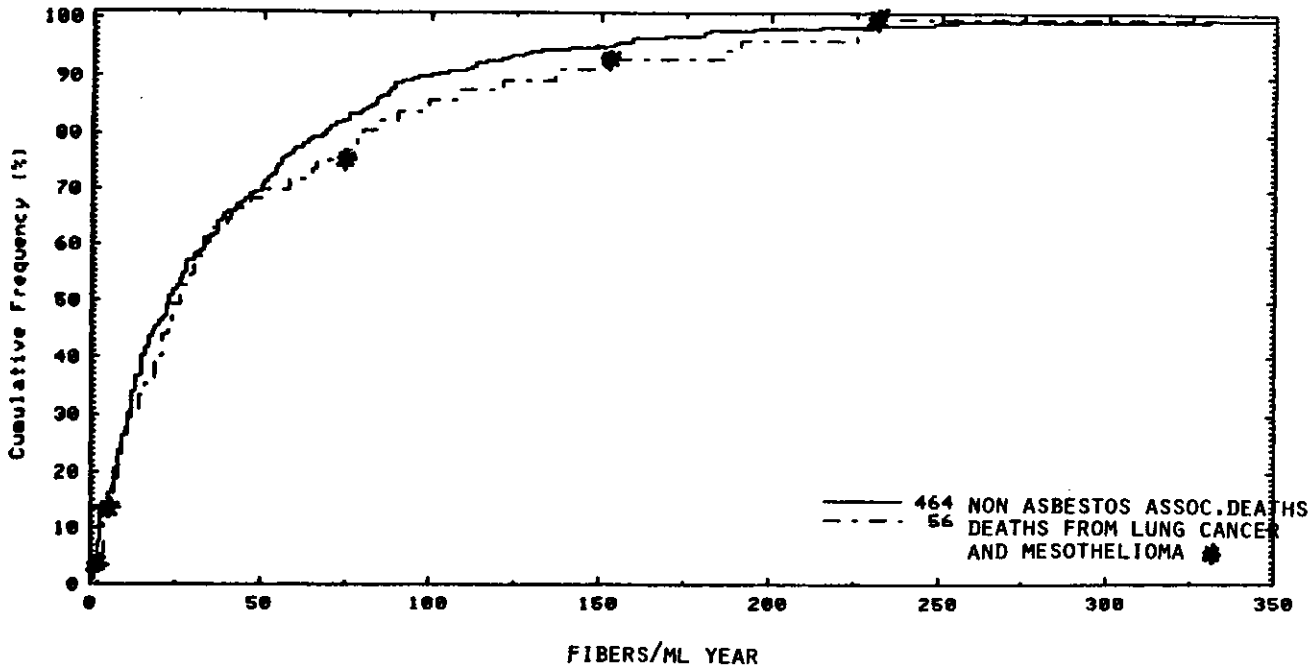


Figure 1. Cumulative doses of cases of lung cancer, mesothelioma and non-asbestos-associated deaths.

The results of X-ray diffraction and differential thermoanalysis studies were confirmed by the elemental analysis which showed a higher Al, Fe portion in attapulgite. However, the distinction between attapulgite and sepiolite with elemental analysis was not completely feasible.

Of the 26 products, 4 samples with relatively long and 1 sample with short fibres as revealed by qualitative fibre characterization were selected for a quantitative analysis by scanning transmission electron microscopy (STEM). The results are summarized in Table I. The median length of the fibres was measured as L = 0.7 to 1.3 µm, the median diameter as D = 0.03 to 0.05 µm and the ratio was calculated as L/D = 20 to 29. The remarkably constant number of all fibres was counted as 71 to 135 • $10^9$ F/mg. Despite the generally short fibres, longer fibres (L ≥ 5 µm) were found in all samples. The concentration of fibres with a length of L ≥ 5 µm was found to be 1.8 and 26.4 • $10^6$ F/mg for attapulgite and 12.2, 12.7, and 1240 • $10^6$ F/mg, respectively, for sepiolite. The latter one was already found to consist of more long fibres than all other products by the use of SEM-analysis.

## DISCUSSION AND CONCLUSION

The concentration of fibres longer than 5 µm in 5 adsorption granulates achieve a crucial importance if compared with results obtained from experimentally injected attapulgite and sepiolite in animals.[4] Figure 2 summarizes a comparison between the findings of both investigations. In 4 of the adsorption granulate samples the concentration of fibres longer than 5 µm was lower than the concentration yielding carcinogenic effects in the injection experiment. However, it

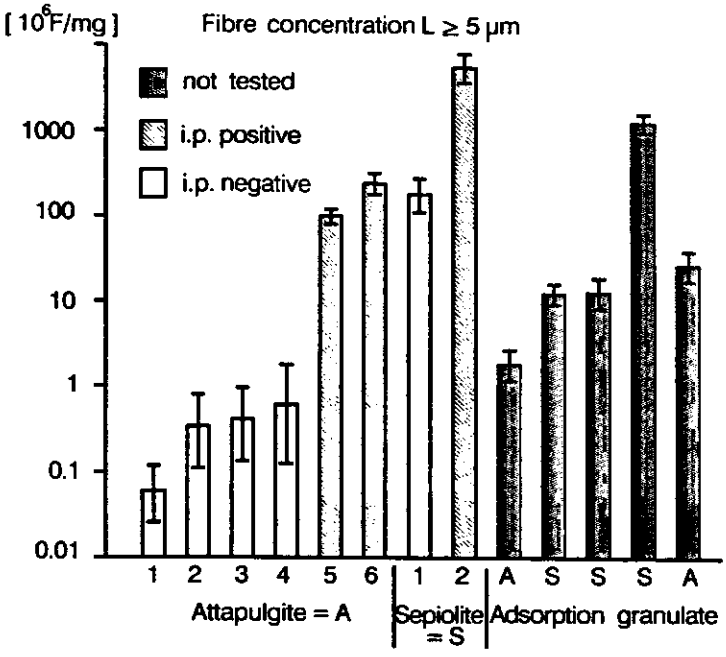


Figure 2. Number of fibres with a length of L ≥ 5 µm in 5 selected adsorption granulates as revealed by TEM at a magnification of 10,000x. Comparison of samples of attapulgite (Mormoiron 1, Lebria 2, Georgia 3, 4, Torrejon 5, Cacéres 6) and sepiolite (Spain 1, Finland 2) intraperitoneally or intrapleurally examined for carcinogenicity in rats, c.f.[4]

Table I

Causes of Death (ICD 9th Revision) 1950–1987

| | Official diagnosis (best information) | | Additional diagnosis |
|---|---|---|---|
| Malignant neoplasias | | | |
| Respiratory organs, intrathoracic organs (160–165) | | 59(58) | |
| lungs (162) | 50(52) | | |
| pleura (163) | 7(4) | | |
| larynx (161) | 1(1) | | |
| other (nose) | 1(1) | | |
| Organs of digestion, peritoneum (140–159) | | 58(59) | |
| stomach (151) | 34(35) | | 1 |
| intestine (152, 153) | 3(4) | | |
| rectum (154) | 4(4) | | |
| esophagus (150) | 2(2) | | |
| liver (155) | 1(1) | | |
| gallbladder (156) | 3(3) | | |
| pancreas (157) | 4(5) | | 1 |
| oral cavity and pharynx (140–149) | 4(4) | | |
| peritoneum (158) | 3(1) | | |
| Other | | 31(33) | |
| urinogenital organs (179–189) | 14(14) | | 1 |
| other locations | 17(19) | | |
| Primary location poorly designated | | 4(5) | |
| Neoplasias of unknown character (239) | | 1(0) | |
| Diseases of the respiratory organs (460–519) | | 32(33) | |
| chronic bronchitis (491) | 4(5) | | 3 |
| emphysema (492) | 6(6) | | 4 |
| asthma (493) | 8(9) | | 2 |
| tuberculosis (011, 012) | 6(5) | | - |
| pneumoconiosis (500–505) | 1(2) | | 8 |
| other chronic diseases | 0(0) | | 2 |
| pneumonia (480–486) | 4(4) | | 11 |
| other acute (infectious) diseases | 3(3) | | - |
| Diseases of the circulatory system (390–459) | | 203(201) | |
| myocardial infarction | 61(62) | | 2 |
| other ischemic heart diseases | 31(31) | | 9 |
| cor pulmonale | 7(6) | | 3 |
| other cardiac diseases | 39(37) | | 13 |
| diseases of cerebral vessels | 40(40) | | 6 |
| other circulatory diseases | 25(25) | | 25 |
| Diseases of the organs of digestion (520–579) | | 47(47) | |
| gastric and duodenal ulcer | 7(7) | | 6 |
| hepatic cirrhosis | 29(29) | | 1 |
| other | 11(11) | | - |
| Other diseases | | 27(25) | 11 |
| Accidents (E 800–949) | | 54(55) | 2 |
| Suicide and violent cause of death (E 950–999) | | 19(19) | - |
| Unknown causes of death | | 5(5) | |

still higher than the concentration found to be uneffective. The concentration of fibres with a length of $L \geq 5$ µm was clearly raised in a fifth adsorption granulate sample of sepiolite. Sepiolite from Spain is reported to be non carcinogenic.[6,7] The authors state that in their investigated products no fibres longer than 5 µm were found.[7] Yet, in a suspension of this sepiolite which was prepared from respirable dust to perform intrapleural injections, the proportion of fibres with a length of $L \geq 4$ µm was found to be 10.5%. In contrast, a concentration of 5.5% of fibres with a length $\geq 4$ µm was found in attapulgite from Torrejon which proved to cause mesotheliomas. In accordance with these findings, in Figure 2 the content of fibres with a length $L \geq 5$ µm in Spanish sepiolite tested by Wagner was rather high ($180 \cdot 10^6$ F/mg); whereas a Finnish sample containing $5500 \cdot 10^6$ fibres/mg longer than 5 µm and also anthophyllite was found to cause mesothelioma. (Pott et al., unpublished).

In summary, the concentration of fine fibres longer than 5 µm in adsorption granulates composed of attapulgite and/or sepiolite was lower with a factor of 4 to 100 in the majority of the samples than concentrations significantly carcinogenic in injection experiments. Only one animal experiment with a relatively high number of long sepiolite fibres shows a lower carcinogenic effect than experiments with long attapulgite fibres. However, the main component of adsorption granulates often resembles attapulgite as shown by analytical tests or it cannot be excluded as minor component or impurity. Thus, it is requested that the physical properties and biological effects of fibrous clays are investigated before deposits are handled.[7] In particular, the carcinogenic effects of the adsorption granulate of sepiolite with $1240 \cdot 10^6$ fibres per mg longer than 5 µm has to be tested in an animal experiment by intraperitoneal injection. Furthermore, the persistency of these long fibres has to be studied by intratracheal tests.[3]

Table II

Lung Cancer Mortality 1950–1986: Observed (O), Expected (E); Standardized Mortality Rate (SMR=O/E) with 95% Confidence Interval (95% c.i.)

| Lung cancer (ICD 162) | O | E | SMR (95% c.i.) | p |
|---|---|---|---|---|
| Total | 49 | 28,50 | 1,72 (1,21-2,57) | < 0,01 |
| Total smoker-adjusted | 49 | 47,04 | 1,04 (0,79-1,41) | n.s. |
| ≤ 25 F/ml year | 25 | 12,80 | 1,95 (1,17-3,74) | < 0,01 |
| ≤ 25 smoker-adjusted | 25 | 19,91 | 1,26 (0,83-1,95) | n.s. |
| > 25 F/ml year | 24 | 15,04 | 1,60 (1,01-2,96) | < 0,05 |
| > 25 smoker-adjusted | 24 | 26,16 | 0,96 (0,64-1,43) | n.s. |

Table III

Crocidolite Exposure of 4 Mesotheliomas (verified by autopsy) and Controls Matched for Sex, Age and Time of First Employment and Duration of Employment

| | high | high/medium | medium | negligible | unknown |
|---|---|---|---|---|---|
| mesothelioma | xx | x | x | | |
| lung cancer (without asbestosis) | | x | x | xx | |
| non-malignant respiratory disease | | x | | xx | x |
| cardiovascular disease | x | | xx | x | |
| alive (1987) | | | x | xxx | |

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# CARCINOGENIC, MUTAGENIC AND FIBROGENIC EFFECTS OF FLY ASHES

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## ABSTRACT

Studies of working environment in power plants and experimental studies aiming at the determination of the carcinogenic and fibrogenic effects of fly ashes produced during hard coal combustion, were carried on.

Total dusts concentration at workplaces varied between 0.5 and 32 mg/m$^3$ and respirable fraction concentrations ranged from 0.3 to 0.9 mg/m$^3$.

The dust contained quartz and mullite, radioactive elements $K^{40}$, $Ra^{226}$, $Th^{228}$ trace elements / mainly Ba, Cr, Pb and Zn/, polycyclic aromatic hydrocarbons—benzene—soluble fraction /0.002 μg/mg. Free crystalline silica content was 9.6% and the content of fibrous dusts reached 50 × 10$^3$ fb/mg. In about 43% of rats, after intraperitoneal administration of 20 mg of dust, cancers, including 2 cases of mesothelioma malignum peritoneum, developed. Statistically significant increase of sister chromatide exchange frequency when compared with the control group, was found by means of SCE test /in vitro/ in human blood lymphocytes, already after the administration of 10 μg/ml of dust.

Fibrogenic effect indices, lung weight and hydroxyproline in lungs were significantly higher than in the control group.

The results obtained indicate that exposure to fly dusts may be associated with the risk of cancer and pneumoconiosis development.

No Paper provided.

# THE DEPENDENCE OF THE BIOLOGICAL EFFECTS IN RATS ON THE PHYSICAL CHARACTERISTIC VALUES OF INTRATRACHEALLY TESTED DUSTS

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To study the effect of the physical properties of the coal mine dusts on their specific harmfulness, four fractions of different size distributions (B, C, D, E) from four total airborne coal mine dusts (TF 1, TF 3, TF 4 and TF 5) sampled on filters[1] were characterized by means of physical methods in their dispersed state.[2]

Due to the discrepancy of sizes of coal and the different minerals, the composition of the fractions sometimes deviates considerably between each other. For the same dust, the proportion of coal generally tends to increase with particle size while the proportion of ash diminishes. By contrast, particularly in dusts high in minerals the quartz percentage rises with the coarseness of the dust.[1]

50 mg of each dust sample were applicated intratracheally in rats by a single instillation as a suspension in 0.5 ml of saline solution. 12 months after instillation the rats were killed and the total amount of hydroxyproline[3] and lipids[4] as well as the dust mass in lungs and lymph nodes[5] were determined and compared with the physically determined values (Table I).

Table I

Results of the Mineral, Physical and Biochemical Analysis of the Tested Airborne Coal Dust Samples

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| TF 1 | B | 90,8 | 24,6 | 1,31 | 0,50 | 14,36 | 10,59 | 102,22 | 56 | 0,4 |
| | C | 92,3 | 19,0 | 1,70 | 0,64 | 17,80 | 10,49 | 93,97 | 49 | 1,0 |
| | D | 91,8 | 19,4 | 2,24 | 0,85 | 13,70 | 9,86 | 90,90 | 50 | 1,8 |
| | E | 92,6 | 11,6 | 3,18 | 1,21 | 15,03 | 9,76 | 75,86 | 49 | 7,9 |
| TF 3 | B | 23,3 | 2,4 | 0,98 | 0,63 | 3,72 | 8,29 | 86,51 | 63 | 0,5 |
| | C | 27,8 | 2,6 | 1,46 | 0,92 | 4,70 | 8,15 | 65,56 | 62 | 1,3 |
| | D | 30,8 | 2,8 | 1,81 | 1,12 | 4,12 | 9,15 | 74,37 | 51 | 1,9 |
| TF 4 | B | 72,1 | 20,0 | 1,13 | 0,53 | 14,32 | 8,97 | 87,34 | 70 | 0,4 |
| | C | 74,8 | 19,3 | 1,38 | 0,63 | 12,14 | 9,71 | 101,53 | 58 | 1,3 |
| | D | 77,8 | 18,3 | 1,80 | 0,80 | 11,31 | 9,95 | 98,72 | 52 | 2,1 |
| | E | 82,8 | 13,2 | 2,12 | 0,91 | 14,35 | 9,34 | 70,47 | 55 | 6,4 |
| TF 5 | B | 81,4 | 28,9 | 0,89 | 0,35 | 20,11 | 9,74 | 102,50 | 58 | 1,0 |
| | C | 84,2 | 28,9 | 1,32 | 0,56 | 13,34 | 9,08 | 87,32 | 60 | 1,6 |
| | D | 86,2 | 25,9 | 1,87 | 0,77 | 10,43 | 8,74 | 77,07 | 48 | 2,5 |
| | E | 89,1 | 15,4 | 2,72 | 1,11 | 10,70 | 8,57 | 61,70 | 50 | 7,5 |

1 = ash content (wt-%), results of IR-spectroscopy (1)
2 = quartz content (wt-%), results of IR spectroscopy (1)
3 = minimum outer surface per volume ($m^2/cm^3$)
4 = minimum outer surface per mass ($m^2/g$)
5 = surface structure number
6 = total hydroxiproline (mg/lung)
7 = total lipids (mg/lung)
8 = dust mass in lungs (% of dose)
9 = dust mass in the lymph nodes (% of dose)

The fibrogenity of the applied dusts was dependent only on their surface properties, i.e., on the surface structure number ($r_s$ = 0.66— Figure 1), the total lipids correlated with the minimal outer surface area per mass ($r_s$ = −0.76— Figure 2), the elimination of applicated dust from the lung, and the penetration of dust in the lymph nodes respectivelly were dependent on their minimal outer surface area per volume ($r_s$ = −0.78—Figure 3 and $r_s$ = 0.88—Figure 4).

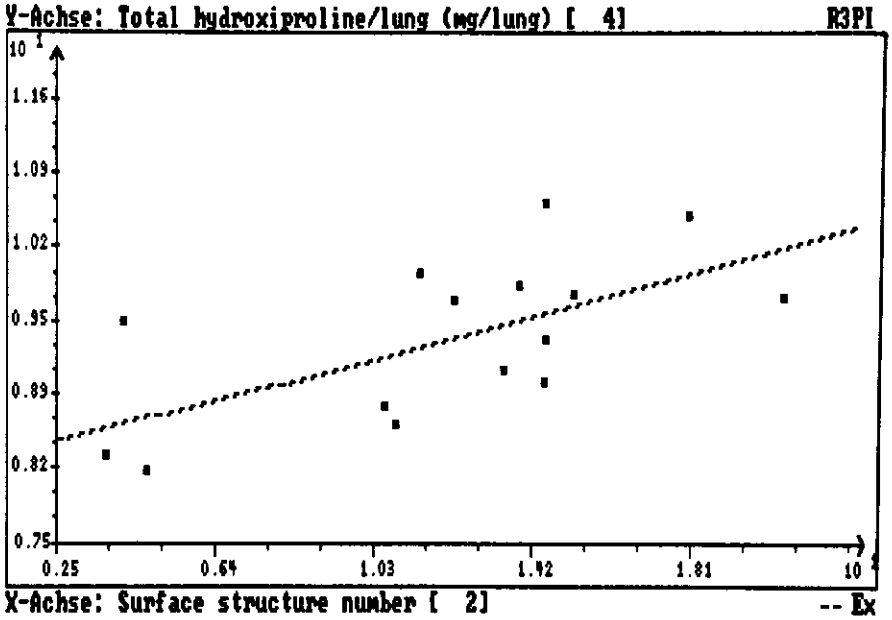


Figure 1. Relationship between the surface structure number and total hydroxyproline (mg/lung).

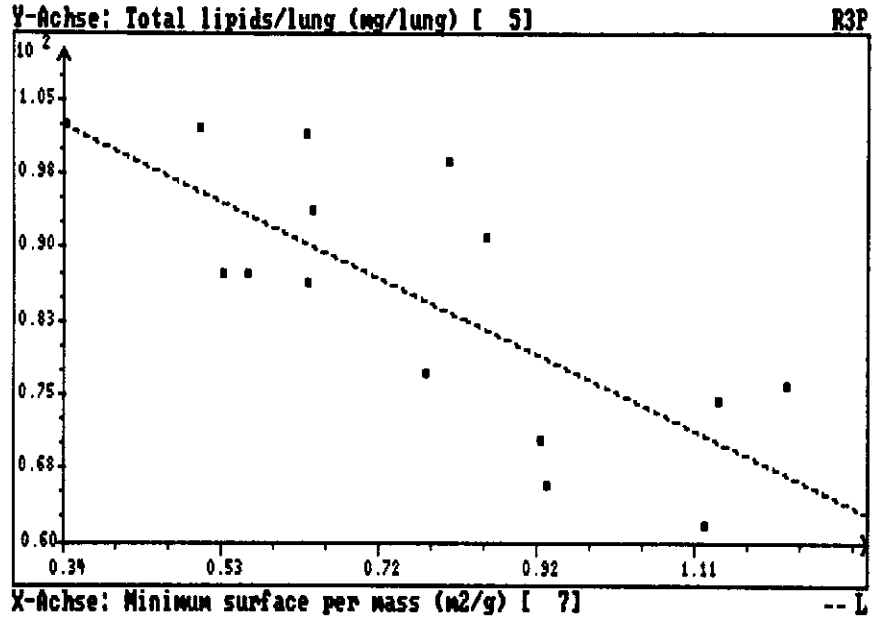


Figure 2. Relationship between the external minimum outer surface per mass ($m^2/g$) and total lipids (mg/lung).

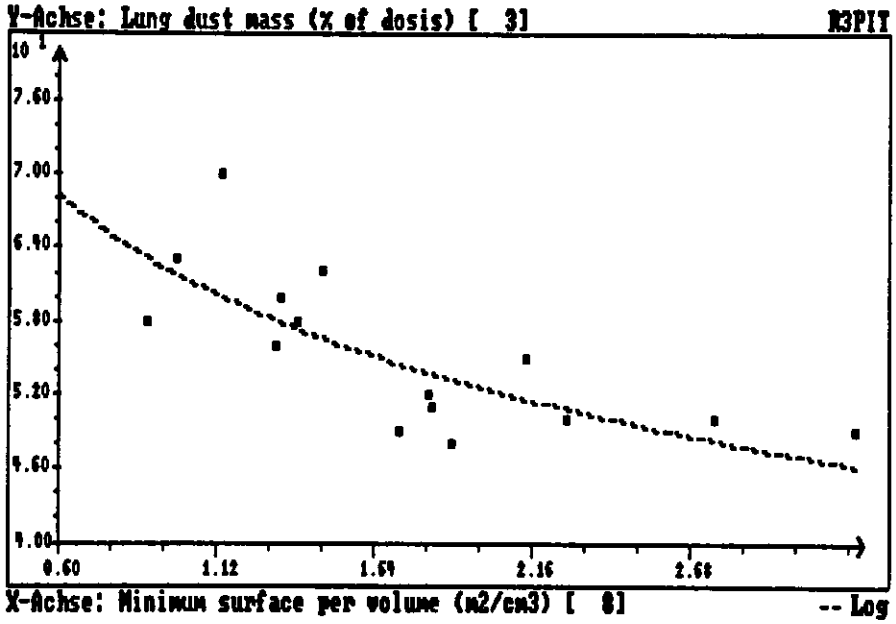


Figure 3. Relationship between external minimum surface per volume ($m^2/cm^3$) and the dust mass in the lung (% of dose).

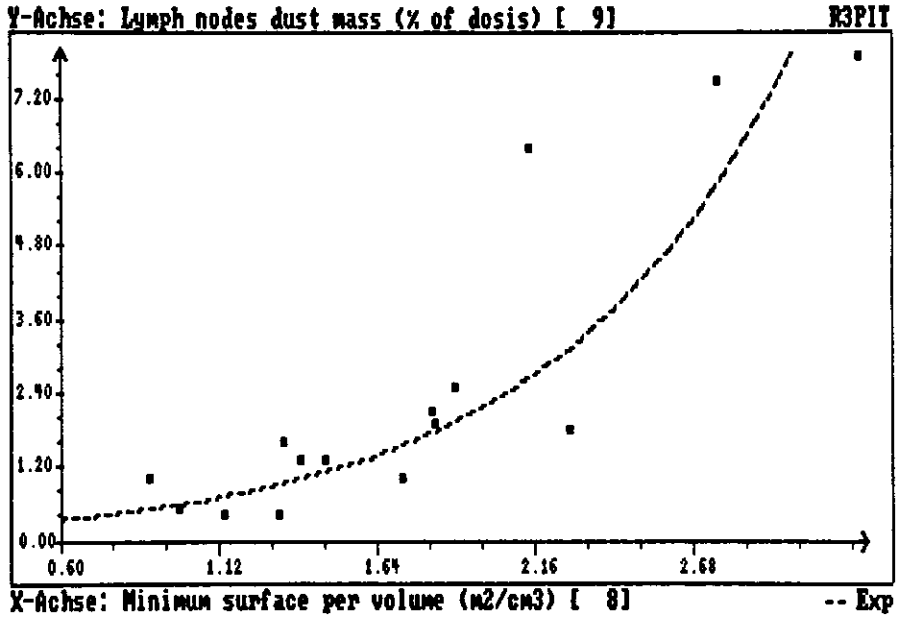


Figure 4. Relationship between external minimum surface per volume ($m^2/cm^3$) and the dust mass in the lymph nodes (% of dose).

According to the epidemiological studies, the results show that the activity of the surface gives a better indication of the fibrogenity of coal mine dust than the quartz content.

The different penetration rate of airborne coal mine dusts correlated not only with their minimum surface per volume but also with their cytotoxicity.[6] The results demonstrate that the cell cytotoxicity of coal mine dusts does not reflect the fibrogenity of these dusts.

On the other hand, the relationship between the physically determined parameters of tested dusts in their suspended states[7] and the corresponding biological effects were studied in Dörentruper quartz (DQ 12), natural anhydrite, titanium dioxide, McIntyre aluminium, polyvinylpyridine-N-oxide and cadmium sulphide instillated intratracheally separately or in mixture in rats (Table II). 3 months after intratracheally instillations the rats were killed, total hydroxyproline and total lipids were determined (Table II) and compared with the physically determined values in the dispersed states of the tested dusts.

The total hydroxiproline correlated again with the surface properties (i.e. with the coefficient of permeability) of the applicated dusts ($r_s = 0.79$—Figure 5), the total lipids in the lungs with the surface tension of the tested solution ($r_s = -0.87$—Figure 6).

The surface structure number as well as the coefficient of permeability represent the surface quality. The surface structure number represents the activity of the surface between the solid and the gas phase, the coefficient of permeability reflects the state of the surface between the suspension and the gas phase.

The total lipids were dependent on the hypothetical minimum surface per mass and were reflected by the different surface tension of the tested solution respectively. According to the thermodynamic low the surface tension tends to stop the surface of the phase (mass) in minimum it's energetic state and this tendency can be compared with the extrapolation to the minimum value of the surface in the solid matter. Therefore, the lipid effect of the tested dusts was produced by their influence on the surfactant system of the lungs and did not reflect the fibrogenity of dusts.

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Table II

Results of Physical and Biochemical Analysis of the Tested Dust Samples

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 35 mg anhydrite | 5,52 | 3,4 | 65 | 65,2 |
| 30 mg titanium dioxide | 5,19 | 3,3 | 73 | 59,2 |
| 30 mg $TiO_2$ + 5 mg quartz | 5,64 | 4,8 | 101 | 58,2 |
| 35 mg anhydrite + 5 mg quartz | 5,09 | 3,3 | 93 | 55,2 |
| 5 mg Dörentruper quartz | 8,78 | 5,9 | 192 | 53,2 |
| 2 mg Al + 11 mg quartz | 4,20 | 3,4 | 115 | 65,5 |
| 11 mg Al + 11 mg quartz | 3,95 | 3,7 | 152 | 59,5 |
| 20 mg Al + 11 mg quartz | 4,40 | 4,5 | 178 | 57,0 |
| 50 mg Al + 11 mg quartz | 4,35 | 5,7 | 153 | 60,0 |
| 2 mg PVNO + 11 mg quartz | 3,30 | 3,5 | 56 | 69,5 |
| 11 mg PVNO + 11 mg quartz | 3,90 | 4,1 | 56 | 70,0 |
| 2 mg PVNO + 20 mg CdS | 5,50 | 5,7 | 59 | 70,0 |
| 11 mg PVNO + 20 mg CdS | 5,55 | 6,2 | 59 | 69,0 |
| 20 mg PVNO + 20 mg CdS | 5,70 | 6,5 | 60 | 69,5 |
| control | | 3,3 | 41 | 71,2 |

1 = coefficient of permeability (joule/$cm^2$sec.$10^{-6}$)
2 = total hydroxiproline (mg/lung)
3 = total lipids (mg/lung)
4 = surface tension (erg/$cm^2$)

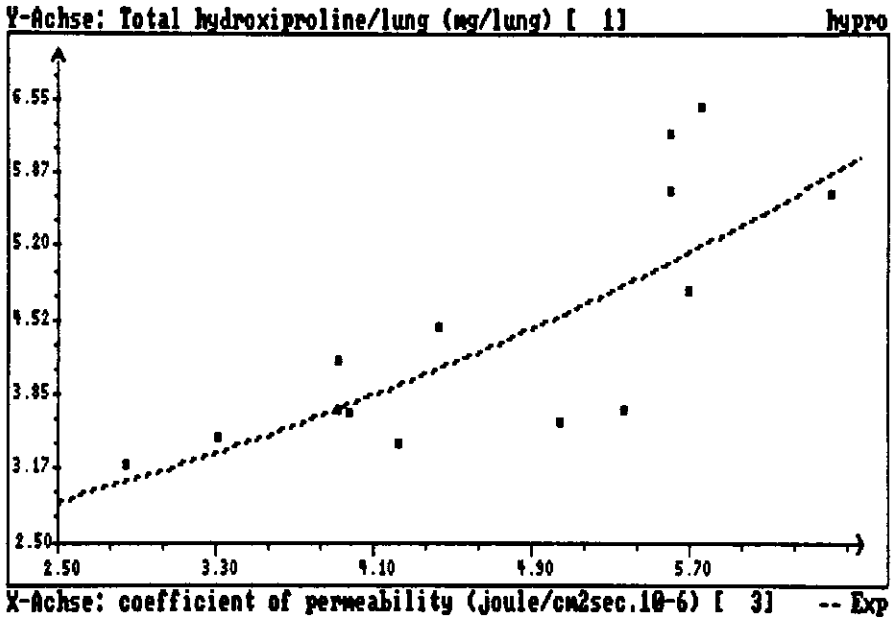


Figure 5. Relationship between the coefficient of permeability (joule/cm$^2$ sec • $10^{-6}$) and total hydroxiproline (mg/lung).

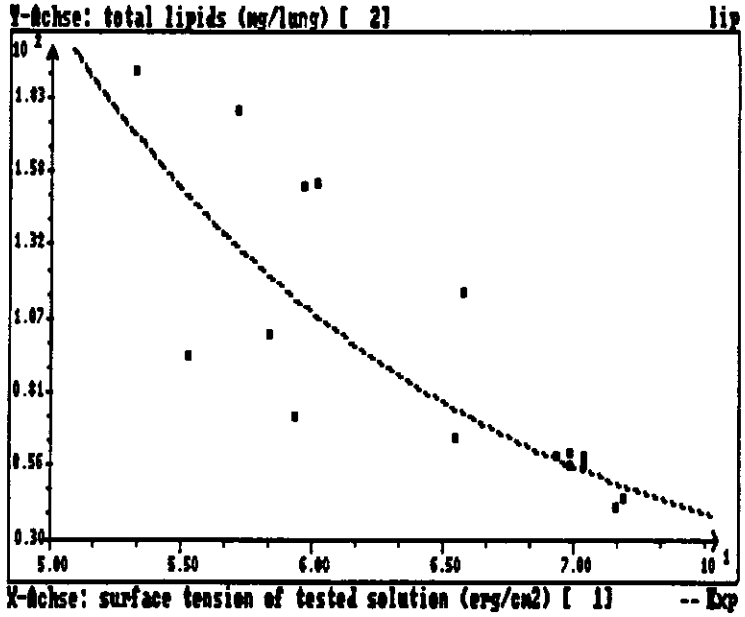


Figure 6. Relationship between the surface tension of tested solution (erg/cm$^2$) and total lipids (mg/lung).

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# A STUDY ON CHANGE OF TYPE I AND III COLLAGEN DURING FIBROSIS INDUCED BY SILICA AND WELDING FUME DUST

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## ABSTRACT

ELISA method was used to study the quantity and distribution of type I and III collagen in lungs of rats induced by silica and welding fume dust. The ratio of I/III collagen was obtained and tested for evaluation of the degree of fibrosis. On the 10th day after instillation of silica, I/III collagen ratio was lower than normal. After 20 days, it increased significantly and stayed at constant level since then. Similar type change of collagen was also observed from histological specimens. Increase of Type III collagen appeared in the early stage of fibrosis and Type I collagen increased more rapidly in the later stage.

In lungs of rats instilled with welding fume dust, Type III collagen increased predominantly until 180 days after instillation, while significant increase of Type I collagen was observed not until after 180 days. It induced a slower and milder fibrosis in the lung. Ratio of Type I/III collagen contents can be used to evaluate the degree of fibrosis.

## INTRODUCTION

The main characteristic of lung fibrosis is massive increase of interstitial collagen in the lung. The study of type change of lung collagen may be helpful to understand the process of fibrosis. In this study, ELISA method was applied to determine the contents and distribution of Type I and III collagen. The ratio of these two kinds of collagen was tested for evaluation of degree of fibrosis induced by silica and welding fume dust.

## MATERIALS AND METHODS

1. Rats

   Female Wistar rats were used. Body weights were about 200 g.

2. Dusts

   a. Quartz 95% of quartz with particle sizes smaller than 5 μm. Free silica content was about 97%. 50 mg of quartz were instilled to each rat intratracheally.
   b. Welding fume dust (Ji-507) 95% of the welding fume dust particles were smaller than 5 μm. Dosage of dust for each rat was 50 mg.

3. Preparation of Type I and III collagen and their antibodies. The procedures were the same as described in Reference 1.

4. Method for determination of Type I and III collagen content in the lung. Rat lungs were dipped in acetone for 2 days, then dried and pulverized. To 25 mg of the dried lung powder, 5 ml of 0.5 mol acetic acid containing 5 ml of pepsin solution (2 mg/ml) were added and collagens were extracted for 24 hrs. The supernatants obtained after centrifugation were used for determination of Type I or III collagen contents with ELISA method.

5. Histological study of distribution of Type I and III collagen in the lung. Lung tissue slices were soaked in 1% peroxidate solution to inhibit the intrinsic peroxidase activity. After rinsing with saline, they were covered with collagen antiserum (Type I or III) and incubated at 37°C for 1 hr. Rinsed with phosphate buffered saline (PBS). The slices were then covered with hydrogen peroxidase labelled IgG at 37°C for 30 min. Washed with PBS again. The slices were dried, dehydrated and fixed and then were observed under the microscope to study the distribution of Type I and III collagen and their relative contents were determined by microscopic specrophotometric analysis.

## RESULTS

1. Changes of Type I and III collagen in silicotic rat lung.

   The contents of Type I and III collagen in silicotic rat lung were both increased continuously as the time prolonged after dusting. At 10 days after dusting the ratio was about 2 and kept at constant level until 90 days after dusting (Figure 1, Table I). Histological study of collagen fibers in the slices showed that after dusting, the alveolar septa and lung interstitial were all expanded and accumulated with Type I and III collagen. At 10 days after dusting with silica, there was mainly Type III collagen appearing in the lung, while at 20 days, there was mainly type I collagen present in the lung (Figure 1). This indicated that Type III collagen increased predominantly at early stage of silicosis and Type I collagen increased predominantly at later stage of silicosis.

Table I
Change of Type I and III Collagen Contents in Lungs of Silicotic Rats

| Group | Days after dusting | Collagen content (mg/g protein) | | I/III Ratio |
|---|---|---|---|---|
| | | Type I | TypeIII | |
| Normal | -- (6) | 38.9 + 6.2 | 28.2 + 4.6 | 1.38 |
| Silicotic | 10 (6) | 48.4 + 10.3 | 54.6 + 2.7** | 0.89 |
| | 20 (6) | 172.6 + 66.7** | 67.6 + 22.5* | 2.55 |
| | 30 (6) | 239.5 + 109.7* | 113.8 + 26.8** | 2.10 |
| | 60 (6) | 291.0 + 92.1** | 145.1 + 54.3** | 2.10 |
| | 90 (6) | 353.8 + 111.4** | 177.6 + 29.4** | 1.99 |

* $P < 0.01$, compared with the normal control
** $P < 0.001$, compared with the normal control
( ) Number of rats indicated in the parenthesis

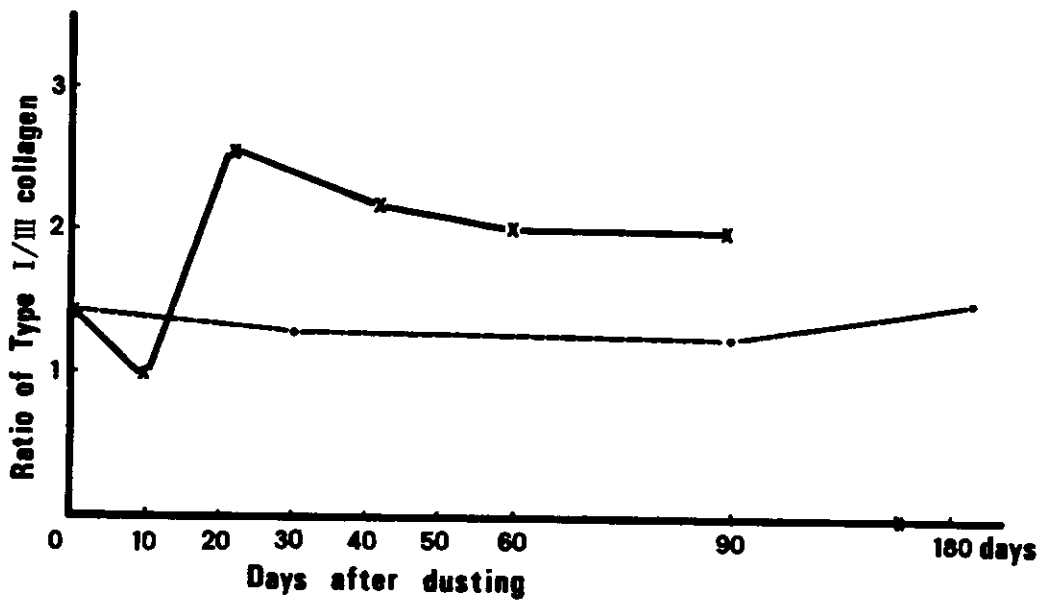


Figure 1. Ratio of Type I/III collagen in rat lung after dusting.
——— Silica
------ Welding fume dust

2. Change of Type I and III collagen in lungs of rats instilled with welding fume dust.

   At 30 days after installation with welding fume dust, the content of Type III collagen increased significantly, but significant increase of Type I collagen was not observed until 180 days after dusting (Table II). The ratios of I/III collagen in the lung decreased gradually within 90 days and were raised to nearly normal level at 180 days (Figure 1). The results showed that this kind of welding fume dust induced a slower and milder lung fibrosis as compared to silicosis. Histological observation confirmed this results (Figure 3).

## DISCUSSION

The increase of lung collagen was usually expressed by increase of hydroxyproline. In this paper, we used ELISA method to determine both Type I and Type III collagen. The privilege of this method is that collagen contents and change of type of collagen in the fibrotic process can be determined directly. Through comparison of Type I/III collagen ratio, the fibrogenic ability of various dusts can be demonstrated. By ELISA staining method the distribution of Type I or III collagen in the lung can be observed, while all other methods do not differentiate the collage types.

Table II

Change of Type I and III Collagen Contents in Rats Lung Instilled with Welding Fume Dust

| Group | Days after dusting | Collagen content (mg/g protein) | | I/III Ratio |
|---|---|---|---|---|
| | | Type I | Type III | |
| Normal | -- (6) | 38.9 + 6.2 | 28.1 + 4.63 | 1.38 |
| Silicotic | 30 (6) | 48.1 + 6.6 | 38.4 + 5.4 ** | 1.25 |
| | 90 (6) | 40.0 + 2.7 | 35.2 + 2.6 * | 1.13 |
| | 180 (6) | 69.0 + 21.5 * | 48.9 + 8.2 ** | 1.41 |

* $P < 0.05$, compared with the normal control

** $P < 0.01$, compared with the normal control

( ) Number of rats indicated in the parenthesis

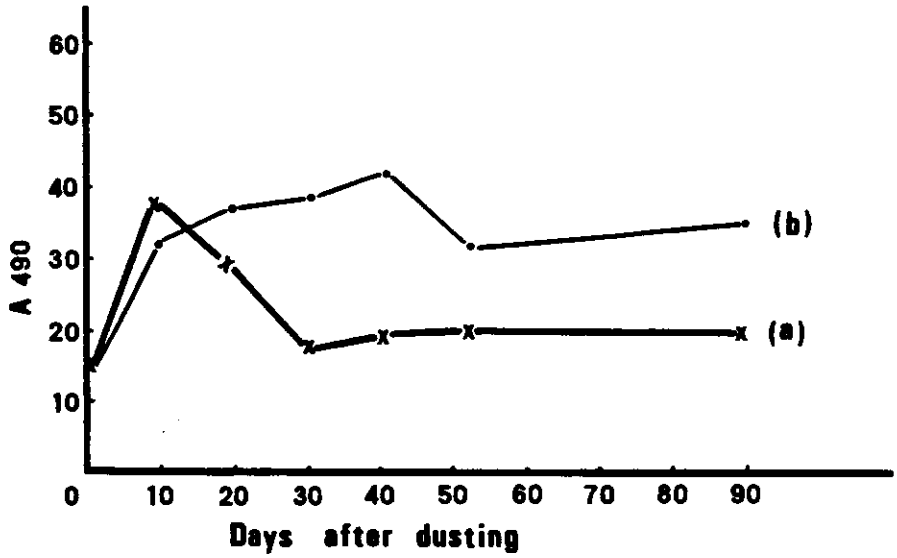


Figure 2. Microscopic spectrophotometric analysis of silicotic lung slices stained with ELISA method.
a. Type III collagen, b. Type I collagen

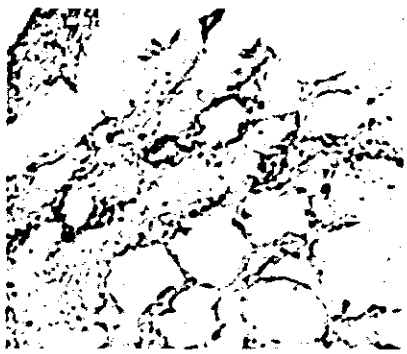

Figure 3. ELISA staining of normal lung (collagen Type I).

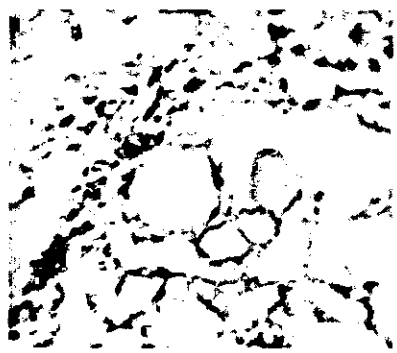

Figure 4. ELISA staining of normal lung (collagen Type III).

Figure 5. ELISA staining of $SiO_2$ dusting lung (1 month, collagen Type I).

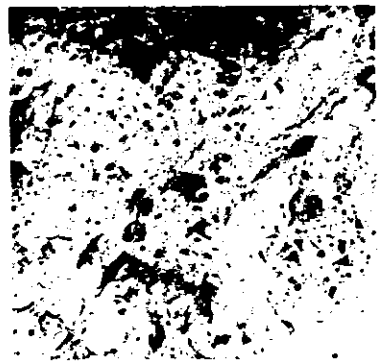

Figure 7. Welding fume dust lung specimen (ELISA staining, 3 month, collagen Type I).

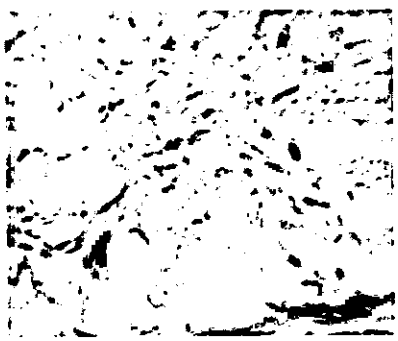

Figure 6. ELISA staining of $SiO_2$ dusting lung (1 month, collagen Type III).

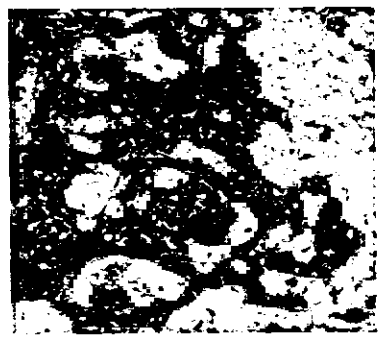

Figure 8. Welding fume dust lung specimen (ELISA staining, 3 month, collagen Type III).

Reiser[2] in his study reported that I/III ratio was constant during silicosis, but in our study, a sharp decrease of I/III ratio on 10th day after dusting and an obvious increase on the 20th day were observed which indicated that Type III collagen increased predominantly in the early stage of fibrosis. This fact is similar to those reported by Ganesh[3] in the study of adult respiratory distress syndrome.

Comparison of I/III ratio between rat lung dusted with silica and welding fume dust showed that the difference was significant. The ratio of I/III for welding fume dust was lower, the type change was not so obvious as those in silicotic fibrosis. It proved that I/III ratio can be used for evaluation of fibrogenicity of dusts.

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# THE DEPOSITION OF FIBERS AND SPHERES AT THE CARINA IN EXCISED LUNGS

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## INTRODUCTION

The deposition of dusts in a respiratory system is a function of the airflow characteristics and the aerodynamic behavior of the particles within the system. The deposition of spheres in the human respiratory track has been studied both empirically and theoretically.[1] Thus, several models exists for the deposition of compact particles in regions of the respiratory system. However, the information on the deposition of fibers is relatively lacking. The correspondence of the mathematical models available to predict the deposition of fibers in the human respiratory tract to the deposition models for compact particles suffer due to the limited empirical knowledge of fiber deposition available. Studying the comparative deposition of fibers and spheres at the carina can provide valuable insight into the deposition of fibers in the tracheo bronchial system. The aim of this study was to investigate the comparative deposition of fibers and spheres at the carina experimentally.

## EXPERIMENTAL METHODS

The basic principle of the experimental method is the assimilation of the natural breathing in an excised calf lung. To accomplish this end, the excised lungs were caused to inspire and expire by varying the pressure around them by placing them into a variable pressure apparatus consisting of a sealed chamber connected to a respiration pump. The pump controlled both the breathing rate and the tidal volume by systematically withdrawing and replacing air from the chamber housing. On inspiration the resultant decrease in pressure around the lung caused it to expand until the intra alveolar pressure equilibrated with the new pressure in the chamber. The pumps full cycle was complete when the withdrawn air was replaced returning the chamber to its original atmospheric pressure. Every experiment utilized 15 respirations per minute. Typically, the tidal volumes generated were between 400–500 cc.

Nearly monodisperse, size classified glass spheres and glass fibers prepared by using the method described by Esmen et al.[2] were used as the deposition material. The dust generator used in this experiment consisted of a dust reservoir, a clapper, and tubular delivery system. Before reaching the trachea air the stream was split in two by a copper bifurcation to provide for a sampling port. The sampling port was used to measure the airborne concentration of the particles during each experiment. This sampling rate was equal to the lung's tidal volume and was drawn simultaneously with lung inspiration.

The 29 pneumonia free calf lungs used in this experiment were obtained at the time of slaughter. After carefully excising the lung carcass, the surrounding tissues and organs were removed. The lung surface was rinsed and inspected for cuts and rips and the lungs were kept moist until the end of the experiment. In the final preparation, the trachea was cut about 18 cm above the carina and two ring clamps were placed on the trachea just above the right apical bronchi. An artificial tracheal extension was inserted into the trachea and secured by the ring clamps. The entire preparation was seated inside the variable pressure unit with the artificial trachea passing through a hole in the top of the chamber connected a leg of copper bifurcation. The lung, inspiring and expiring with the changes in chamber pressure was connected to a spirometer. The tidal volume was monitored for 3–5 minutes. The tidal volume usually stabilized within 1–2 minutes. The dust generator was started and synchronized such that a clap on the dust reservoir occurred simultaneously with the onset of inspiration. The exposure was about 20 minutes.

The experimental section was separated from the rest of the lung by carefully cutting away the surrounding parenchymal tissues and then cutting the bronchi about one inch distal to the carina. This portion was carefully cleaned of adhering fat and parenchymal tissue and frozen. In order to minimize particle translocation, all subsequent cutting was performed on the frozen tissue. The trachea was sliced into two sections for analysis of deposited particles. The first slice was made just under the right apical bronchi. The second section which included the carina was taken after slicing 1.5 cm posterior to the carinal plane. The removal of the particles from the tissue was achieved by sonication and subsequent ashing. The ashed material was redeposited on a filter for analysis. The filters were viewed under cross polarization. If the total number of spheres or fibers deposited on the filter was less than about 2000, then the entire filter was viewed and all particles counted. Generally, an analysis of 1 $cm^2$ was sufficient. Some of the particles were lost during the transfer and processing operations. A calibration was performed to delineate the lost fraction.

## RESULTS AND DISCUSSION

A list of the experimental parameters are presented in Table

Table I

The Experimental Conditions and Parameters

| Lung | Size | | | Tidal Volume | Tracheal Velocity | Stokes' Number(*) | |
|---|---|---|---|---|---|---|---|
| | Sphere Dia. | Fiber Dia. | Length | | | | |
| | um | um | um | liter | cm/sec | Sphere | Fiber |
| 12 | 24.5 | 10.9 | 50 | 0.330 | 34 | 0.126 | 0.054 |
| 13 | 24.5 | 10.9 | 50 | 0.355 | 36 | 0.133 | 0.057 |
| 15 | 24.5 | 10.9 | 50 | 0.355 | 55 | 0.232 | 0.104 |
| 16 | 24.5 | 10.9 | 50 | 0.330 | 77 | 0.396 | 0.185 |
| 17 | 24.5 | 10.9 | 50 | 0.380 | 55 | 0.243 | 0.104 |
| 18 | 24.5 | 10.9 | 50 | 0.430 | 68 | 0.315 | 0.134 |
| 19 | 24.5 | 10.9 | 50 | 0.330 | 93 | 0.574 | 0.245 |
| 21 | 24.5 | 10.9 | 50 | 0.380 | 45 | 0.181 | 0.082 |
| 22 | 24.5 | 10.9 | 50 | 0.430 | 72 | 0.333 | 0.146 |
| 30 | 12.5 | 6.4 | 48 | 0.400 | 58 | 0.063 | 0.051 |
| 32 | 12.5 | 6.4 | 48 | 0.475 | 134 | 0.202 | 0.164 |
| 34 | 12.5 | 6.4 | 48 | 0.550 | 103 | 0.124 | 0.101 |
| 36 | 12.5 | 6.4 | 48 | 0.575 | 87 | 0.095 | 0.078 |
| 38 | 12.5 | 6.4 | 48 | 0.500 | 98 | 0.124 | 0.101 |
| 39 | 12.5 | 6.4 | 48 | 0.525 | 111 | 0.141 | 0.115 |
| 40 | 12.5 | 6.4 | 48 | 0.475 | 65 | 0.068 | 0.055 |
| 41 | 17.2 | 9.1 | 50 | 0.505 | 85 | 0.199 | 0.143 |
| 42 | 17.2 | 9.1 | 50 | 0.565 | 111 | 0.282 | 0.202 |
| 43 | 17.2 | 9.1 | 50 | 0.485 | 58 | 0.110 | 0.079 |
| 44 | 17.2 | 9.1 | 50 | 0.500 | 69 | 0.143 | 0.103 |
| 45 | 17.2 | 9.1 | 50 | 0.505 | 82 | 0.187 | 0.134 |
| 46 | 17.2 | 9.1 | 50 | 0.525 | 70 | 0.128 | 0.092 |
| 47 | 17.2 | 9.1 | 50 | 0.555 | 88 | 0.183 | 0.131 |
| 48 | 17.2 | 9.1 | 50 | 0.570 | 101 | 0.243 | 0.174 |
| 49 | 17.2 | 9.1 | 50 | 0.525 | 81 | 0.176 | 0.126 |
| 50 | 17.2 | 9.1 | 50 | 0.525 | 92 | 0.210 | 0.151 |
| 51 | 17.2 | 9.1 | 50 | 0.515 | 86 | 0.196 | 0.141 |
| 55 | 12.5 | 6.4 | 48 | 0.560 | 123 | 0.165 | 0.134 |
| 56 | 12.5 | 6.4 | 48 | 0.575 | 96 | 0.119 | 0.097 |

(*) The Stokes' numbers for the fiber diameters Df and aspect ratio B is calculated by the use of impactive diameter Di using the formula (3):

$$Di = Df\,(1+0.013(\ln B)^3\,(0.71 + 0.91 \ln B)^{1/2}$$

calculated using impaction diameter formulation developed by Burke and Esmen.[3] The graphical representation of the deposition efficiency as a function of tracheal velocity for the lowest group of Stokes' number particles is shown in Figure 1. These results indicate that a consistent deposition occurs at low velocities; seemingly independent of the velocity. This may be explained by theory developed by Harris,[4] who predicted such an effect would occur due to interception. As the tracheal velocity increases, the slope of the curve changes rapidly, indicating that a critical point was reached permitting the impaction to facilitate deposition rapidly.

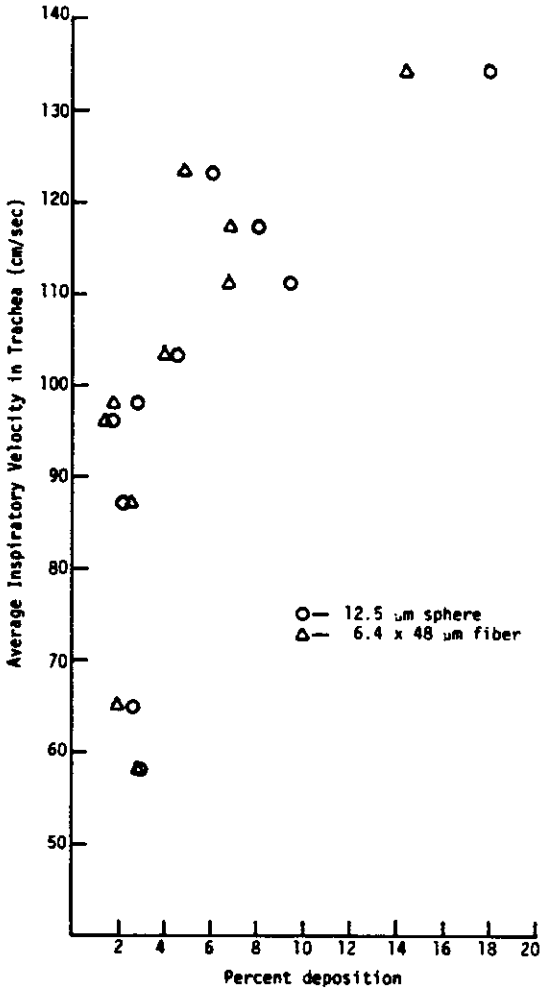


Figure 1. Deposition of fibers and spheres as influenced by inspiratory velocity.

The critical point was investigated by incorporating the air flow characteristics with particle physical parameters and observing the depositional efficiencies as a function of Stokes number. Such a graph of all results is provided in Figure 2. In this figure, data of impactive deposition as observed by Johnston and Muir[5] and Landahl and Herrmann[6] are also included.

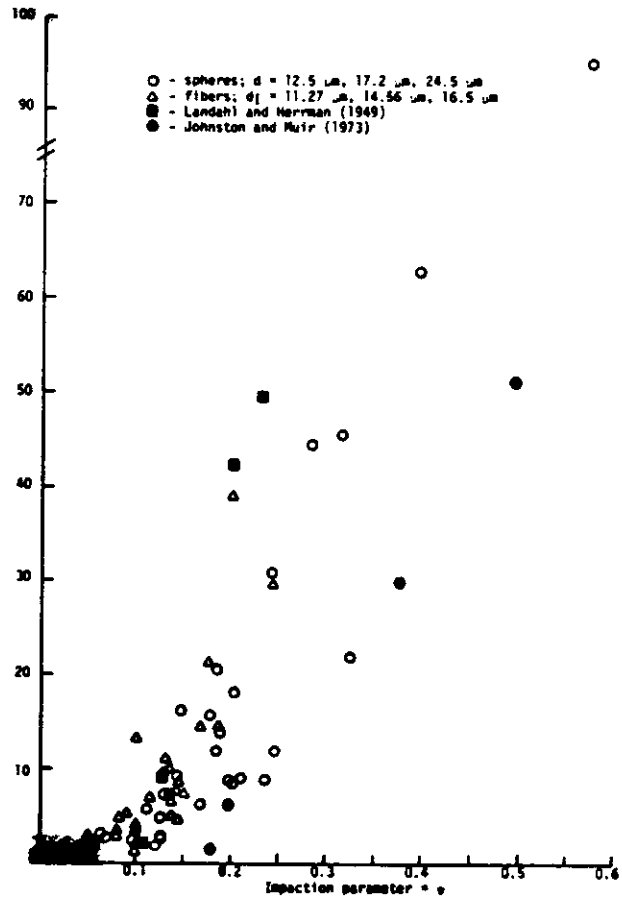


Figure 2. Deposition of fibers and spheres as a function of Stokes' number.

Clearly, during the experiments, impaction was occurring simultaneously with particle removal by other mechanisms. By the use of current theories, estimates of depositional efficiency due to the other mechanisms may be made. Harris noted that interception is an effective removal mechanism in the tracheobronchial region for fibers as small as 10 μm in length. If interception were significant in these experiments then one would expect a uniform shift to the left on the deposition curve (Figures 1 and 2); because, the amount removed would be independent of all parameters except airway and particle size. The results shown in Figure 1 suggests that the interception would account for 2–4 percent deposition.

Similarly, if sedimentation were a significant mode of particle removal the shift would not be uniform. Rather it would be biased in favor of those experiments involving large particle sizes and low average tracheal velocities. Review of the data does not provide any evidence of such bias. Harris' equation for settling in turbulent flow suggests that only 2 percent of the largest fibers which experienced the lowest average tracheal velocity would settle at the carina.[4]

If the impaction efficiency for round jets impinging upon an impaction surface perpendicular to the flow axis is taken to be 0.5 for Stokes' number about 0.25,[7] then the results suggest that the impaction efficiency observed is significantly less than what would be expected. In fact a recent theoretical work on impactive deposition of fibers this deviation was also noted.[8] There is strong evidence indicating that interception, and sedimentation may be augmented by secondary flow patterns that develop downstream from a bifurcation. Schroter and Sudlow[9] identified these flows as occurring both on inspiration and expiration. On inspiration a pair of vortices develop in each daughter branch. They are strong enough to complete one helical cycle within three diameters downstream. Under this condition, by the rotation of the fibers, the impaction efficiency is expected to drop significantly. In the experiments reported here, the enhancement of interception and sedimentation is not expected to play an important role, since the contribution of these mechanisms to deposition is relatively low. In contrast any change in the impaction efficiency would be fully reflected on the observations. In addition to the secondary flows developed during inhalation, on expiration a set of four vortices are generated in the parent airway ahead of the bifurcation. The effect of this air pattern on deposition has not been investigated. However, it is reasonable to assume that, using the rationale suggested for the secondary airflow patterns which develop on inspiration, deposition would be further enhanced by interception and sedimentation, and further diminished by impaction. It should be noted that no attempt was made to control the branching angle of the bronchi in this study. The angle is fixed by the rigid cartilaginous structure for the first 0.5–1.0 cm from the carinal ridge. Estimates of the branching angle in these calf lungs appeared to correlate well with reported branching angles in the human lung. Thus the use of the Stokes' number for fibers and compact particles in the estimation of impactive deposition in the human lung should be reasonable.

## CONCLUSIONS

Mathematical modeling, animal exposure, airway simulator and human exposure experiments have been employed to predict the deposition of compact particles in the human respiratory system. The depositional probabilities of spheres is modeled by relation to an associated aerodynamic equivalent diameter with reasonable accuracy. In the estimation of impaction potential fibers, Figure 3 suggests that there is no discernible difference in the deposition of fibers and spheres as a function of Stokes number when the actual diameter is employed for the spheres and the empirical impaction diameter is employed for the fibers.

This implies that not only can the impaction diameter be employed as a viable predictor of impaction, but one may estimate the series of fiber parameters that comprise the smallest fibers removable in the lung by this mechanism. That is to say that for every fiber diameter there will correspond a length that will represent the smallest fiber of that diameter that will be removed by impaction early on.

Weibel[10] has provided an exhaustive description of lung architecture developed from airway casts. Using his information and assuming that given a particle size, shape and density impaction is governed only by the airway radius and average conveyance velocity, an impaction index may be calculated as a ratio of the average velocity to airway radius. Such an impaction index for the first ten generations of human respiratory tract with liter/sec airflow is given in Table II. Clearly this potential reaches its maximum in the third through sixth generation with the fifth generation theoretically possessing the largest capability. We may visualize the first five generations as successive impaction stages, each stage being capable of removing successively smaller particle sizes. The lower limit of removal by this mechanism is then related to the 5 characteristics of the final stage. For instance, a unit density fiber 4.4 μm in diameter

Table II

Impaction Index for the First Ten Generations in the Human Lung

| Generation | Velocity | Radius | Impaction Index |
|---|---|---|---|
| | cm/sec | cm | 1/sec |
| 0 (trachea) | 393 | 0.900 | 437 |
| 1 | 427 | 0.610 | 700 |
| 2 | 462 | 0.415 | 1113 |
| 3 | 507 | 0.280 | 1810 |
| 4 | 392 | 0.225 | 1742 |
| 5 | 325 | 0.175 | 1857 |
| 6 | 254 | 0.140 | 1814 |
| 7 | 188 | 0.115 | 1634 |
| 8 | 144 | 0.093 | 1548 |
| 9 | 105 | 0.077 | 1364 |
| 10 | 74 | 0.065 | 1138 |

would have to be almost 90 μm long to afford complete removal. The fiber size parameters decrease with an increase in the tidal volume. Thus, at a 1450 cc tidal volume, the fiber would only have to possess an impaction diameter of 10.2 μm. This criteria would be satisfied by a unit density fiber 4.1 μm in diameter and 82 μm in length. If we further assume a fiber to have a density equal to 2.5 gm/cc (asbestos or fiberglass), the lower size limit for impaction in the lung (T.V. = 1450 cc) becomes a 3.6 × 29 μm fiber. Gross et al.[11] has observed that fibers present in the lungs of fibrous glass workers at autopsy are rarely in excess of 3.5 μm in diameter with an average length of 27 μm. This suggests that impaction initially prescreens those fiber sizes within the first six or so generations effectively and those fibers which pass this prescreening are then available for removal by sedimentation, diffusion and interception in the finer airways.

The method presented for studying particle deposition at the carina in excised lungs can be utilized to investigate the influence of sedimentation, interception and diffusion on fiber removal. Altering the physical properties of the particles and/or the tracheal velocities should provide suitable conditions to derive empirical relationships defining the interplay of particle and airflow characteristics and removal efficiencies via these other deposition mechanisms. For example, by choosing several large fiber sizes of equal impactive potential but of dramatically different length, the deposition enhancement by interception may be investigated.

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