MODERN WORK PROTECTION WITH THE SHOTCRETE CONSTRUCTION METHOD UNDER OVERPRESSURE

DIETHELM GOENNER, Prof. Dipl.-Ing.
TIEFBAU-BERUFSGENOSSENSCHAFT, MUNICH

The shotcreting method currently accounts for 90% of all urban traffic tunnels in the Federal Republic of Germany erected in the mining method of construction. Pressurized shotcreting has gained considerable importance in inner-urban tunnel construction during the past years. Examples in our country are encountered principally in the construction of the Munich subway system.

Driving underground cavities is accomplished under difficult working conditions and involves great health and accident hazards (Figure 1). The causes being:

- High dust development in shotcreting, rock excavating and conveying operations,
- poor air and visibility conditions,
- unfavourable climatic conditions,
- narrow, largely closed spaces,
- long handling distances for excavated and construction materials in long-extended, cramped spaces,
- frequently unforeseeable irregularities of the rock,
- piecework as well as schedule and cost pressure,
- parallelism of various operations performed in most closely restricted space.

The same health and accident hazards also apply for the workers engaged in shotcreting under overpressure.

The Compressed Air Ordinance stipulates that working chambers must be kept free from odours, as well as harmful gases, vapours and dusts (Figure 2).

Working methods involving intensive dust development, such as e.g. tunnelling with road headers without dust exhaust systems, are prohibited for work in compressed air just like the employment of internal combustion engines. Adherence to the maximum workplace concentration and technical standard concentration values for harmful substances in the breathing air must be assured. This can only be accomplished by means of constant control measurements of the inhaled air.

The technological problems had been solved. Concerning problems of labour medicine, studies were made to provide information on whether harmful mineral dust under overpressure constitutes a greater health hazard than atmospheric pressure.

The Medical Institute for Environmental Hygiene at the Düsseldorf University (Prof. Schlipköter) was thereupon commissioned by TBG to conduct appropriate animal experiments. In these preliminary studies it was found that the inhalation of dust containing quartz at an overpressure of 1.5 bar for a period of 6 months leads to increased typical quartz-induced lesions of mediastinal lymph nodes.

Further on it could be observed in the test of pulmonary function that for compressed air workers the flow-volume curve decreases significantly with increasing dust concentration:

> The test results indicate that with the measured high dust concentrations late damages to the respiratory tracts are possible.

More results on this deterioration of the pulmonary function tested after 1 working day and after 2 years will be published shortly in British JOURNAL OF OCCUPATIONAL HEALTH by Prof. Dr. Kessel, collaborator of Prof. Fruhmann, Munich University.

In view of these alarming findings, the following three institutions had been commissioned to perform further-going studies on primates under conditions similar to those prevailing at construction sites parallel to human medicine tests of compressed air workers at Munich subway construction sites:

- Med. Institute for Environmental Hygiene at the Düsseldorf University (Prof. Schlipköter)
- Institute for Surgical Research at the Munich University (Prof. Brendel)
- Institute and O.P.D. for Occupational Medicine of the Munich University (Prof. Fruhmann).

Interim results of these medical tests will be presented subsequently by Dr. Rosenbruch, Düsseldorf University and Dr. Krombach, Munich University. The Studiengesellschaft für unterirdische Verkehrsanlagen, STUVA, Köln, has assumed the technical management of the test programme and the medicine tests of compressed air workers were carried out by Munich University, Prof. Fruhmann.

Maximum workplace concentration values constitute a basis for assessing the questionableness or harmlessness of con-

No.	Work sector	Number of accidents	severe	fatal
1	Thrust-boring equipment	39	21	—
2+3	Tunnelling machines	17	6	1
4+5	Excavation work	8	3	—
6	Drilling and blasting	20	10	1
7	Falling stone, collapsing material	57	25	1
8	Sheeting, stabilization	27	13	—
9	Shotcreting work	76	10	—
10	Finishing (concrete + formwork)	32	13	—
11	Lining segments	11	6	—
12	Loading, mucking	16	8	—
13	(Underground) tracklaying	4	1	—
14	Materials handling	51	24	1
15	Welding + electr. work	11	3	—
16	Fitting	22	8	—
17	Toxic gases + vapours	7	5	—
18+19	Shaft construction + miscellaneous	31	14	1
	Total	425	173	5
	≙	(100 %)	(41 %)	(1.5%)

Figure 1. Accident analysis—tunneling, 2 years comparison.

centrations of noncarcinogenic working media appearing in the air at the workplace in the form of gas, vapour or suspended matter. Maximum workplace concentration values are scientifically founded, but apply only to pure substances. Since pollutant mixtures are the rule at the workplace, an orientation aid will be needed for the safety measures to be taken in modern work protection. This requires a pragmatic and generally applicable valuation concept. The Committee for Harmful Substances established by the Minister of Labour and Social Order in the Federal Republic of Germany has been engaged for some time in the valuation of pollutant mixtures in the air at the workplace with significant cooperation of the industrial insurance societies.

Re § 4 General requirements

1. Keep working chambers clean and free from odours as well as harmful gases, vapours and dusts

2. Ventilation

Supply 0.5 m^3/minute of fresh air per worker into the working chambers

Figure 2. Compressed air ordinance.

The valuation index of a substance mixture is the totalized value of pollutant indices J_i with the individual J_i being the quotient resulting from the concentration C_i established for the individual pollutant in the air at the workplace and the associated maximum workplace concentration (here MAK) value (Figure 3).

$$I = \frac{C_1}{MAK_1} + \frac{C_2}{MAK_2} + \ldots \frac{C_n}{MAK_n}$$

The valuation process is limited to those components of a substance mixture, for which parallel biological action can be supposed or not excluded with appropriate concentration in the breathing air. Since in compressed air work usually a lower air exchange and thus also a lower pollutant dilution occur than in tunnelling work under atmospheric pressure, particularly stringent requirements must be imposed on the quality of the breathing air. This is true all the more for pressurized shotcreting. Shotcrete dust normally must be classified under the category of siliciferous fine dust (Figure 4).

Therefore shotcrete dust is hazardous to health under two aspects:

- The dust may contain fine mineral dusts containing in some cases considerable amounts of quartz, which are added with the sand, the accelerating agents and the filler, and

$$I = \frac{C_1}{MAK_1} + \frac{C_2}{MAK_2} + \ldots \frac{C_n}{MAK_n}$$

$$= \sum_{i=1}^{n} \frac{C_i}{MAK_i} = \sum_{i=1} I_i$$

I ... Evaluation index

C... Average concentration

MAK .. Maximum workplace concentration

I ... Totalized value

I = 1 Limiting value for substance mixture

Figure 3. Evaluation of substance mixtures in the air at the workplace.

- due to their high alkalinity with a pH-value around 13, all accelerating agents must be regarded as caustic substances (Figure 5).

Additional dust sources, such as excavation and transportation e.g. contribute correspondingly to the silicon content of the total amount of fine dust.

The research project on shotcreting under overpressure is intended to clarify whether the current pollutant limits provide adequate health protection also for work under overpressure or whether more stringent requirements are necessary for the quality of the breathing air. We must make every effort to counter in time a silicosis hazard such as former generations experienced in mining, the consequences of which are still felt today.

As a rule following technical dust-combatting measures may contribute to the desired result (Figure 6).

1. Use of a liquid setting accelerator.
2. In case of powdered accelerators, selection of the one with the lowest possible respirable fine content and the lowest possible amount of quartz in the fine content. Accelerators without quartz are to be preferred.
3. Selection of sand without quartz, wherever possible, with a low respirable fine portion.
4. Fillers without quartz.
5. Addition and portioning of powdered accelerators in a closed system.
6. Addition of an adhesive to reduce dust.
7. For the shotcreting equipment:

Quartz content (% by weight)	Max. workplace concentration (mg/m^3)	Reference concentration
$Q < 1$	6.0	General dust limit (fine dust limit)
$1 \leq Q \leq 3.75$	4.0	Fine dust containing quartz
$Q > 3.75$	0.15	Fine quartz dust

Figure 4. Maximum workplace concentration limits for inert and siliciferous fine dust.

- selection of a low-dust shotcrete method
- lowest possible delivery lengths
- selection of the proper jet type, regular machine maintenance
- selection of a favourable hose diameter
- prewetting when adding adhesive
- proper inherent moisture of the design mix.

At some workplaces, such as e.g. the spray jet, technical dust protection measures alone will not be made available and also used.

The development of a dust protection helmet which combines protection of respiration, head, eye and face and also hearing was the result of a research project undertaken by the Tiefbau-Berufsgenosen-schaft.

Pressurized shotcreting is feasible only in combination with modern work protection.

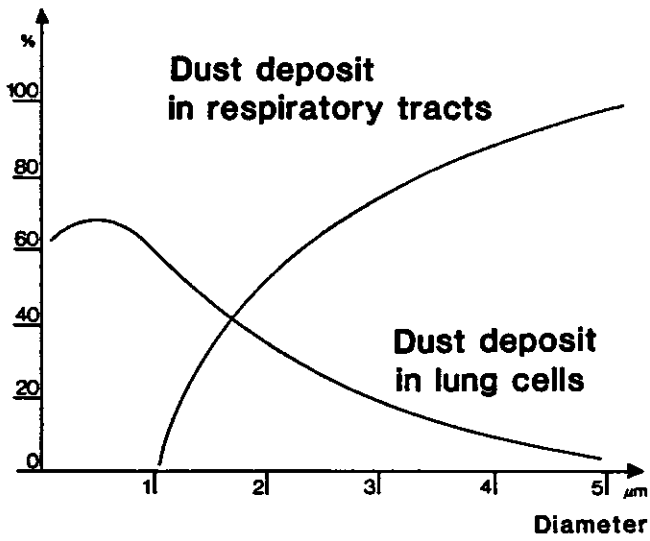

Figure 5. Schematic representation of dust deposit in the lung and in the respiratory tracts (acc. Winkler).

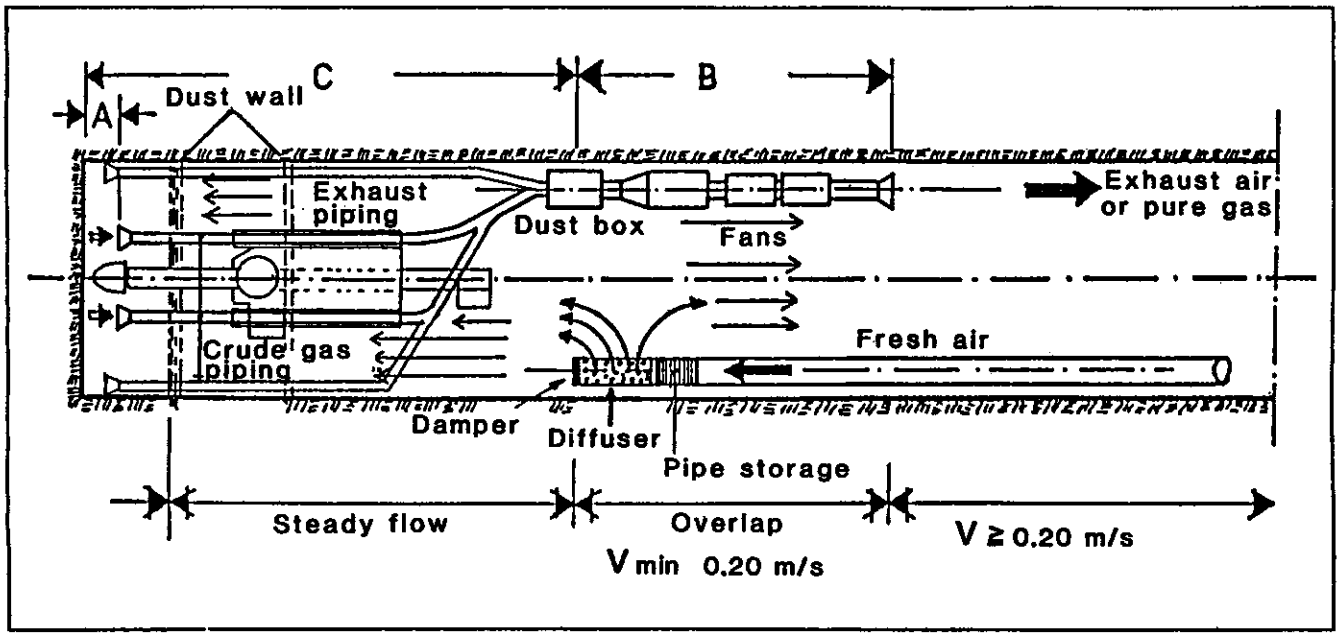

Figure 6. Principle of dust removal from a road header.

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

M. ROSENBRUCH* • M. Kouros* • F. Krombach†

*Medical Institute of Environmental Hygiene
†Düsseldorf, and Institute for Surgical Research
University of Munich (Großhadern), Fed. Rep. Germany

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 (5mg/m^3) and normal pressure (1.0 bar)
Group III	(n=4)	quartz dust DQ-12 (5mg/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 H_2O). 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.

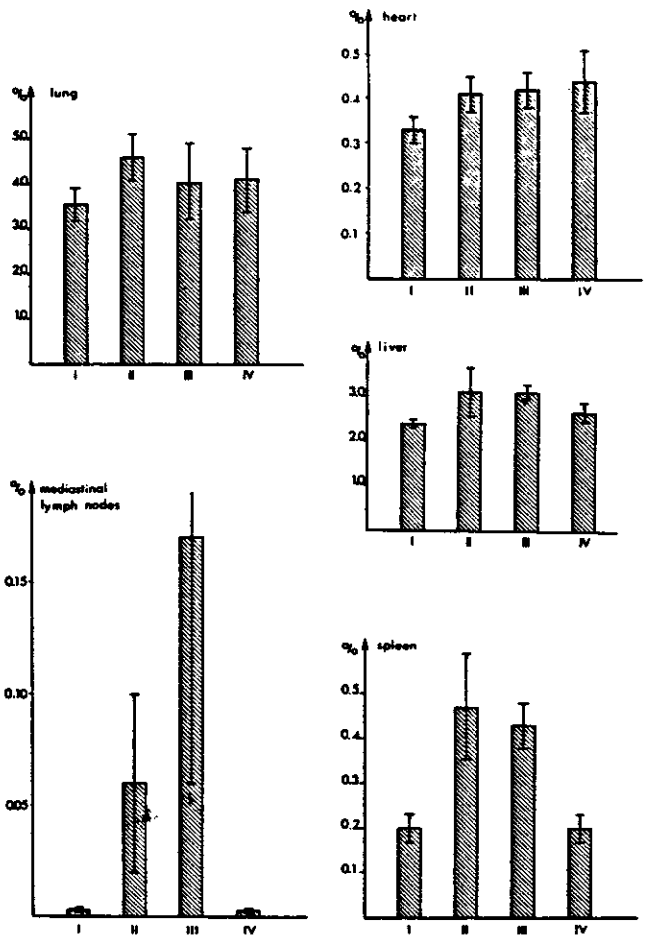

Figure 1. Relative organ weights of heart, lung, liver, spleen, and mediastinal lymph nodes, determined at necropsy, after intratracheal instillation and perfusion.

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 perivascularly. Due to intratracheal instillation, almost all intraalveolar cells are washed out. Many transformed alveolar pneumocytes II can be seen at the border of fibroses (Figure 3a). Using polarized light, bifringent 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.

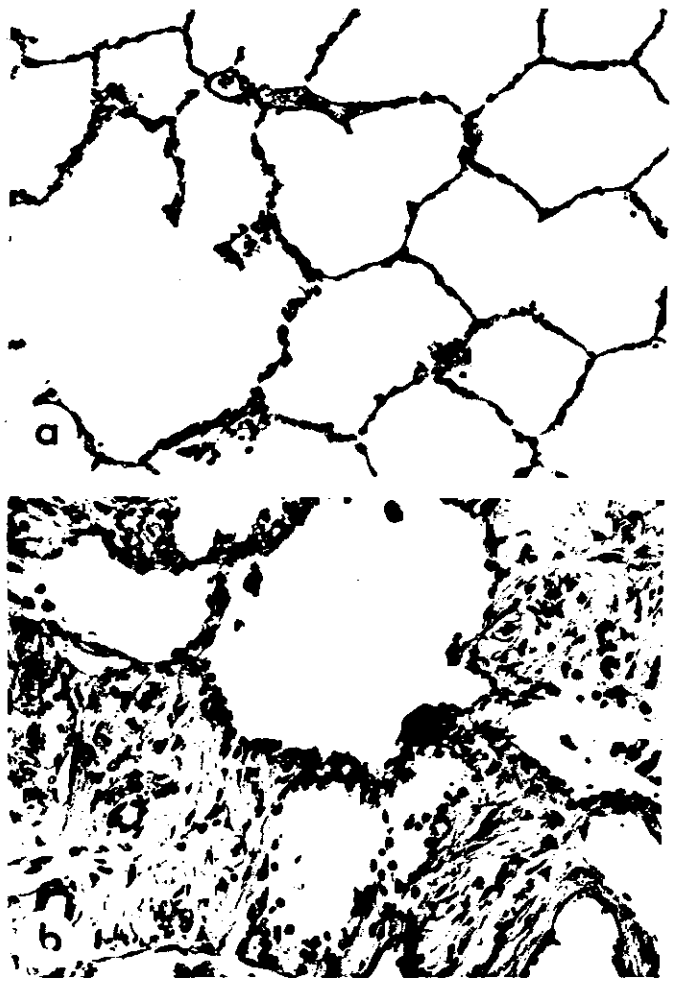

Figure 2. Lung histopathology after 26 months of inhalation:
a. Normal lung structure in a control animal (group I);
b. extended fibrosis in dust exposed animal (group II); H&E staining, obj. 10x.

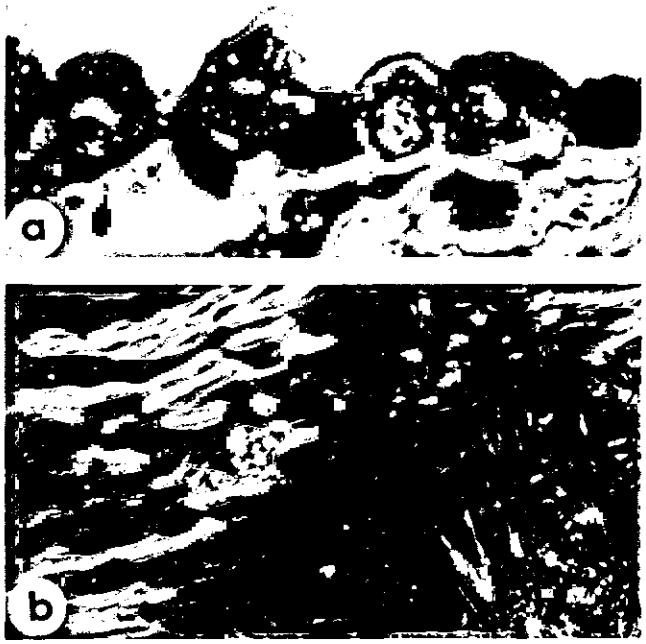

Figure 3. Detailed morphology of quartz induced lung alterations:
a. Pneumocytes typ II, with lamellar bodies in the cytoplasm, are lining alveolar surface; semithin section, methylene-bue staining, obj. 63x;
b. in polarized light, bifringent quartz particles as well as collagenous fibers are detectable; 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

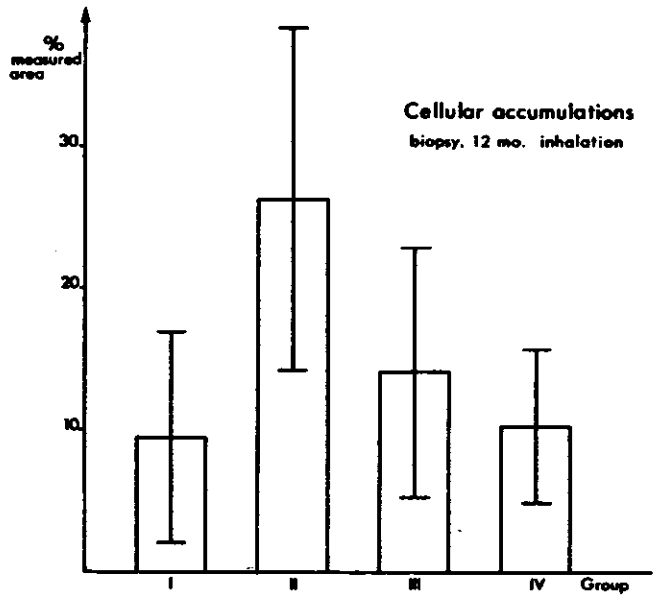

Figure 4. Percentage of cellular accumulations, corresponding to granulomas, in lung biopsies, taken after 12 months of inhalation; arithmetic mean and SEM.

(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,

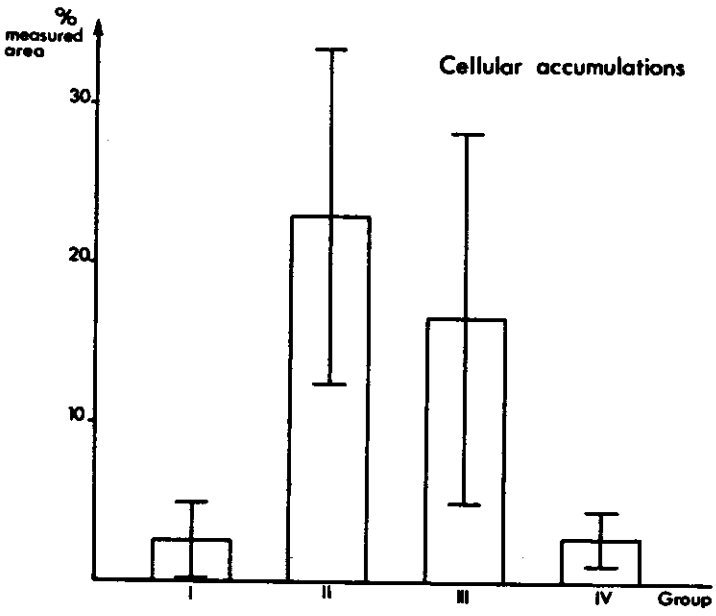

Figure 5. Percentage of cellular accumulations, corresponding to granulomas in the lungs, determined on 6 different locations after 26 months of inhalation; arithmetic mean and SEM.

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.

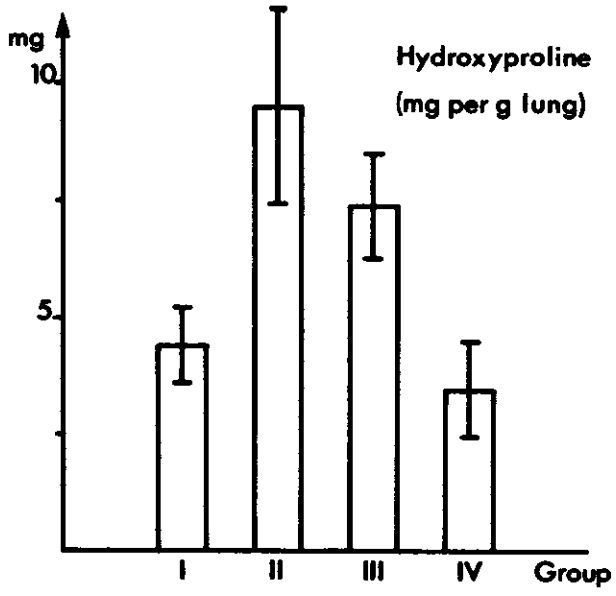

Figure 6. Hydroxyproline content of lung tissue.

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 case[8] 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 mesenterial lymph nodes (Figure 7).

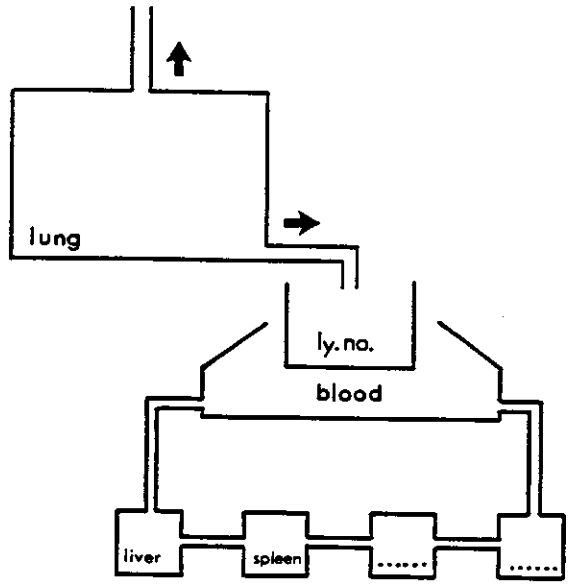

Figure 7. Pathogenesis of the lesions in various organs after inhalation of quartz dust.

Liver granulomas have been reported previously in rats, intravenously treated with silica[4] 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.

REFERENCES

1. Carmichael, G.P., Targoff, C., Pintar, K., Lewin, K.J.: Hepatic Silicosis. *Am. J. Clin. Pathol.* 73:720-722 (1980).
2. Churg, A., Stevens, B.: Association of Lung Cancer and Airway Particle Concentration. *Environ. Res.* 45:58-63 (1988).
3. Heyder, J.: Studie of Particle Deposition and Clearance in Human. *Problems of Inhalatory Toxicity Studies;* Grosdanoff, P. et al. (Edts.); MMV Medizin Verlag München; bga-Schriften 5:155-180 (1984).
4. Kanta, J., Horsky, J., Kocarova, H., Tilser, I., Korolenko, T.A., Bartos, F.: Formation of Granulomas in Liver of Silica-treated Rats. *Br. J. Exp. Pathol.* 67:889-899 (1986).
5. Kessel, R., Holbach, M., Mauermayer, R., Praml, G.: Die Wirkung von Stäuben der Spritzbetonbauweise auf die Lungenfunktion. Ber. *Dtsch. Ges. Arbmed.* 27:511-514 (1987).
6. Krombach, F., Ronge, R., Hildemann, S., Burckhardt, D., Wanders, A., Allmeling, A., Hammer, C.: Bronchoalveolar Lavage in Monkeys Chronically Exposed to Silica, Compressed Air and Their Combination (Abstract). *Amer. Rev. Resp. Dis.* 137(4,Suppl):350 (1988).
7. Lum, H., Mitzner, W.: A Species Comparison of Alveolar Size and Surface Forces. *J. Appl. Physiol.* 62:1865-1871 (1987).
8. Masoaka, T., Akahori, F., Arai, S., Sakaguchi, K.: Effect of Paraquat on Plasma Fibronectin Serum Free Hydroxyproline, Serum Ceruloplasmin and Lung Collagen Content in Monkeys. *J. Tox. Sci.* 12:329-340 (1987).
9. Nasseri, M., Bücherl, E.S., Wolff, J.: Licht- und elektronenmikroskopische Untersuchungen über die Strukturveränderung der Lunge nach Einwirkung hohen Sauerstoffdruckes. *Virchows Arch. Path. Anat.* 342:190-198 (1967).
10. Pimentel, J.C., Menezes, A.P.: Pulmonary and Hepatic Granulomatous Disorders Due to the Inhalation of Cement and Mica Dusts. *Thorax* 33:219-227 (1978).
11. Stegemann, H.: Mikrobestimmung von Hydroxyproline mit Chloramin-T und p-Dimethyl-aminobenzaldehyd. *Z. Physiol. Chem.* 311:41-46 (1958).
12. Weibel, E.R.: Morphometry of the Respiratory Tract. *Problems of Inhalatory Toxicity Studies;* Grosdanoff, P. et al. (Edts.); MMV Medizin Verlag München, bga-Schriften 5:543-553 (1984).

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

CORRELATION OF BRONCHOALVEOLAR LAVAGE AND COMPUTED TOMOGRAPHY IN AN EXPERIMENTAL MODEL OF SILICOSIS*

F. KROMBACH • R. Rienmüller • E. Fiehl • S. Hidemann • R. Ronge • A. Wanders • D. Burkhardt • C. Hammer • W. Brendel

Institute for Surgical Research and Radiological Clinic
Klinikum Großhadern, University of Munich, Munich, F.R.G.

Modern tunnel construction involves health hazards such as quartz-dust and compressed air. Compressed air is used behind air-tight bulk-heads to control water seepage. Applying shotcrete lining methods, considerable amounts of quartz-containing respirable dust might be generated.[1] Thus shotcrete tunneling in a compressed air environment eventually exposes the underground workers to both silicogenic and hyperbaric conditions. In addition, exposure to compressed air always means exposure to hyperoxic conditions. Regarding the current concepts of the pathogenesis of silicosis,[2] the interactions between silica particles and alveolar macrophages might be altered by hyperoxia, finally leading to modifications in the development of silicotic lung fibrosis. Moreover, the deposition of respirable dust particles might be changed due to hyperbaric effects on lung function.[3]

The effects of short-term exposure on various parameters of lung function had been described in miners working with shotcrete in a compressed air environment.[4] Thus, the aim of our study was to investigate the individual and combined effects of long-term exposure to silica and compressed air in a non-human primate model. In a longitudinal study (26 months of exposure) we examined various parameters, using broncho-alveolar lavage (BAL) cytological and functional assessment of free lung cells, biochemical analyses of BAL fluid, lung function tests, radiological (X-ray, CT-scan) and pathohistological examinations as chief methods. This paper will focus on the correlation of cellular constituents of BAL and quantitative analysis of CT-scans of the lung.

METHODS

Animals

Thirteen months prior to the start of exposure, 28 cynomolgus monkeys (Macaca fascicularis; 4 male, 24 female) with a body weight of approximately 3–6 kg were separated into four groups. Previously, the animals had been kept in quarantine, dewormed, and tuberculin-tested. In exposure-free intervals, the animals were housed in spacious steel cages under natural daylight. A standard primate chop diet, additional fruit supplement, and tap water were supplied ad libitum.

*Supported in part by BMFT grant No. 01 VD 492/7.

Experimental Design

Following an acclimatization period of 6 months, control BAL was performed three times in each animal. After the start of exposure, BAL was carried out at intervals of 2 months. Open lung biopsies were taken 12 and 18 months after the start of exposure. After 26 months, the exposure was ceased. In the following two months, various lung function tests and radiological examinations (X-ray and CT-scan) were performed. Thereafter, the animals were sacrificed and the lungs were fixed by instillation of glutaraldehyde via the trachea under controlled hydrostatic pressure.[5]

Exposure Conditions

The four groups of animals received an intermittent inhalational exposure regimen of 8 hours/day and 5 days/week, except for public holidays and a 1-week rest following open lung biopsies. The animals were placed in open stainless steel cages, and the exposure took place in 7.5 m^3 capacity inhalational dust/pressure chambers. All test chambers featured controlled climatic conditions (25°C temperature, 70% relative humidity). One group of animals (quartz-exposed group) received a concentration of 5 mg/m^3 of DQ12 <5 µm (Dörentruper quartz) quartz-dust.[6] A second group (quartz/compressed air group) was exposed to a concentration of 5 mg/m^3 of DQ12 and additional hyperbaric conditions of 2.5 bar_a. A third group (compressed air group) was exposed to 2.5 bar_a only. A fourth group of animals (control group) was sham-exposed to clear normobaric air. The concentration of airborne respirable dust was measured with a TM digital µP photometer (OEB H. Hund GmbH, Wetzlar, FRG). The photometer reading was calibrated in terms of mass concentration of respirable dust by means of a gravimetric dust sampler.[7] In each test chamber, room temperature, humidity, pressure, and concentration of respirable dust were monitored and controlled continuously. Compression of the pressure chambers lasted 10–15 min. Decompression was initiated by a decompression step to 1.3 bar_a within 10 minutes, followed by decompression to 1.0 bar_a within 70 minutes.

Branchoalveolar Lavage

For BAL, the animals were anaesthetized with 15 mg/kg ketamine (Ketanest, Parke, Davis & Co., Munich, FRG) and

2 mg/kg xylacine (Rompun, Bayer, Leverkusen, FRG). With the animal in supine position, a flexible fiberoptic bronchoscope (BF P10, Olympus, Munich, FRG) was wedged into the main ronchus of the left lung. Following instillation of 100 ml of sterile 0.9% saline in aliquots of 20 ml, fluid was withdrawn, applying moderate suction. The lavage fluid was immediately filtered through sterile gauze, and the cells were pelleted at 300 g for 10 min. For some biochemical assays, the BAL supernate was examined in a fresh state. Otherwise, the supernate was aliquoted and stored at −70°C for further studies. In addition, possible bacterial contamination was assessed in each BAL sample. The BAL cells were washed twice and counted with a Coulter Counter. Cell viability was determined by the trypan blue exclusion technique. Cytocentrifuge smears served to identify the cellular populations stained with May-Grünwald-Giemsa, naphthyl acetate esterase and toluidine blue. Three hundred cells were counted, and the percentage of macrophages, lymphocytes, neutrophils, eosinophils and mast cells was determined.[8,9]

CT-Measurements

For CT-examinations, the animals were anaesthetized with 15 mg/kg ketamine and 2 mg/kg xylacine, and intubated. Immediately prior to each scan, the animals were hyperventilated to apnea and then scanned at a constant intratracheal pressure of 15 cm H_2O. CT-scans were performed in a Siemens Somatom DRH scanner (7s, 125 kV, 550 mAs). CT-sections were taken with a slice thickness of 1 mm at the level of the tracheal bifurcation, 5 cm cranially and 5 cm caudally, respectively. Lung areas of the thoracic scans were identified by using a modified ROI (region of interest)-method.[10] Starting from a manually specified line encircling each lung, contiguous pixels were analyzed. All pixels corresponding to chest wall, mediastinum and heart were eliminated based on their CT-numbers above −350 HU (Hounsfield units). Using histograms of CT-numbers, the mean CT-densities of both lungs were calculated in each animal.

Statistics

The results are expressed as means ± the standard error of the mean (x ± SEM). For data correlation, the Pearson correlation coefficient was calculated. The statistical comparison of group means was performed using One-Way Analysis of Variance and the Scheffé multiple comparison test. A p value <0.05 was considered to be statistically significant.

RESULTS

The exposure conditions were tolerated well by all animals. No signs of indisposition were observed during dust exposure, compression or decompression. The body weight of the animals kept stable and did not show any significant differences between the four test groups. However, during the entire observation period of 40 months, a total of seven animals was lost, due to severe fighting injuries or narcosis incidents respectively.

In all experimental groups, BAL cell viability and BAL fluid recovery did not change during the exposure period. However, total cell counts showed a bi-phasic profile, with a peak after 6 months followed by a decline and a final increase, starting after 18 months. The first peak of total cell counts was caused by increases of lymphocytes, macrophages, neutrophils and mast cells in that chronological order. In contrast, the final increase of total BAL cell counts was mainly due to a rise of neutrophils (Table I). Mean CT-densities were significantly ($p < 0.05$) augmented in both dust-exposed groups (Table II). There was perfect correlation of CT-densities, obtained after 27 months of exposure, and BAL total cell counts, obtained after 26 months ($r = 0.96$, $p < 0.001$).

DISCUSSION

The data obtained so far suggest that hyberbaric conditions do not accelerate or intensify the manifestation of silicosis dramatically. In contrary, particular data indicate some kind of a "protective" or delaying effect of long-term intermittent hyperbaric exposure. However, final conclusions may be drawn only when all measured parameters (cellular and biochemical BAL constituents, X-ray, CT-scan, various tests of lung mechanics and pulmonary gas exchange, morphometric pathohistology) will be evaluated and correlated. In our experimental model of silicosis, the serial BAL offered excellent insights into both the kinetics and function of free lung cells. In combination with biochemical factors of the BAL fluid (e.g., proteins, phospholipids, enzymes, immunological mediators, fibrogenic activity) more precise informations on the development of silicosis in a primate model will be available.

Under standardized conditions, the quantitative analysis of CT-scans proved to be a sensitive tool for the non-invasive assessment of structural pulmonary alterations. In addition, the perfect correlations of BAL-data and CT-data warrant more sophisticated analyses of CT-histograms as well as further studies on the validity of BAL in pneumoconioses.

REFERENCES

1. Gönner D.: Modern Work Protection with the Shotcrete Construction Method Under Overpressure. *(published in this issue.)*
2. Davis, G.S.: Pathogenesis of Silicosis: Current Concepts and Hypotheses. *Lung* 164:139-154 (1986).
3. Van Liew, H.D.: Mechanical and Physical Factors in Lung Function during Work in Dense Environments. *Undersea Biomed. Res.* 10:255-265 (1983).
4. Kessel, R., Mauermayer, R., Praml, G., Redl, M.: Der Einfuß von Spritzbetonstäuben in Druckluft auf die Lungenfunktion. *Verh. Dtsch. Ges. Arbeitsmed.* 25:49-53 (1985).
5. Rosenbruch, M., Kouros, M., Krombach F.: Morphology and Morphometry in Cynomolgus Monkeys after 2 Years Inhalation of Quartz Under Normal and Excess Pressure *(published in this issue).*
6. Robock K.: Standard Quartz DQ12 <5 µm for Experimental Pneumoconiosis Research Projects in the Federal Republic of Germany. *Ann. Occup. Hyg.* 16:63-66 (1973).
7. Armbruster, L., Breuer, H., Gebhart, J., Neulinger, G.: Photometric Determination of Respirable Dust Concentration without Elutriation of Coarse Particles. *Part. Charact.* 1:96-101 (1984).
8. Krombach, F., König, G., Wanders, A., Lersch, C., Hammer, C.: Effect of Repeated Bronchoalveolar Lavage on Free Lung Cells and Peripheral Leukocytes. *Transplant Proc.*17:2134-2136 (1985).
9. Krombach, F., Ronge, R., Hildemann, S., Burkhardt, D., Wanders A., Allmeling A., Hammer, C.: Bronchoalveolar Lavage in Monkeys Chronically Exposed to Silica, Compressed Air and their Combination (Abstract). *Amer. Rev. Resp. Dis.* 137 (4, Suppl.): 350 (1988).
10. Rienmüller, R., Schätzl, M., Kalender, W., Fiehl, E., Krombach, F.: Quantitative CT-Untersuchungen der Lunge am Tierexperimentellen Modell der Silikose. *Fortschr. Röntgenstr.* 148:367-373 (1988).

Table I

BAL Fluid Recovery, Total and Differential BAL Cell Counts Prior to and During Exposure

	months	control	quartz pressure	quartz	pressure
fluid recovery (%)	0	64.3 ± 3.9	65.4 ± 3.9	75.1 ± 1.6	74.4 ± 1.0
	6	70.5 ± 2.4	74.0 ± 1.8	74.1 ± 2.1	68.0 ± 3.0
	12	77.o ± 0.9	77.0 ± 1.1	75.7 ± 2.1	78.6 ± 1.0
	18	72.8 ± 5.6	76.6 ± 2.6	76.0 ± 0.9	77.7 ± 1.6
	24	79.4 ± 1.9	79.4 ± 1.4	80.5 ± 1.6	82.4 ± 2.3
total cell counts (x 10^6)	0	16.3 ± 2.2	15.1 ± 2.7	14.1 ± 2.9	14.1 ± 2.8
	6	19.9 ± 2.2	56.4 ± 8.6*	59.0 ± 5.0*	8.3 ± 1.2
	12	7.9 ± 1.1	26.2 ± 4.4*	37.7 ± 3.2*	5.5 ± 0.7
	18	9.6 ± 2.4	49.3 ± 6.2*	34.4 ± 4.0*	6.5 ± 1.4
	24	14.1 ± 3.5	82.6 ± 11.1*	56.0 ± 6.0*	4.2 ± 1.1
macrophages (%)	0	87.2 ± 3.2	84.0 ± 2.7	93.3 ± 1.9	88.7 ± 3.9
	6	84.0 ± 2.6	74.4 ± 4.2	76.1 ± 2.9	87.6 ± 3.6
	12	85.8 ± 3.0	74.3 ± 2.6	77.8 ± 2.8	82.7 ± 4.4
	18	85.6 ± 4.2	68.3 ± 1.3*	74.8 ± 3.9	85.2 ± 3.6
	24	82.6 ± 6.4	65.0 ± 3.3	65.8 ± 4.3	86.0 ± 3.3
lymphocytes (%)	0	6.3 ± 2.0	5.1 ± 0.6	2.7 ± 0.8	4.1 ± 0.9
	6	9.3 ± 1.8	9.6 ± 1.4	8.3 ± 0.9	5.0 ± 1.0
	12	6.2 ± 1.9	9.1 ± 1.8	4.5 ± 1.2	6.6 ± 1.7
	18	7.2 ± 2.4	7.4 ± 1.3	5.4 ± 0.5	5.7 ± 1.4
	24	8.4 ± 2.0	10.9 ± 1.4	11.8 ± 2.3	6.2 ± 1.4
neutrophils (%)	0	2.5 ± 1.1	4.0 ± 1.7	2.1 ± 1.7	1.0 ± 0.4
	6	1.3 ± 0.5	6.4 ± 1.1	8.3 ± 2.7	0.4 ± 0.3
	12	0.7 ± 0.5	7.7 ± 1.3	9.0 ± 3.9*	0.1 ± 0.1
	18	1.0 ± 0.5	11.3 ± 1.6*	11.1 ± 4.0*	0.3 ± 0.2
	24	2.6 ± 1.5	16.0 ± 3.1*	9.5 ± 1.6	0.2 ± 0.2
mast cells (%)	0	1.8 ± 0.9	1.3 ± 0.7	1.0 ± 0.7	1.3 ± 1.1
	6	2.8 ± 1.5	3.7 ± 1.7	5.6 ± 1.2	3.1 ± 1.8
	12	5.3 ± 1.9	6.6 ± 2.1	6.0 ± 1.7	4.9 ± 1.5
	18	4.0 ± 1.4	11.9 ± 1.6*	7.8 ± 2.0	4.0 ± 1.8
	24	5.4 ± 2.8	7.6 ± 2.3	11.8 ± 3.8	4.2 ± 1.2

Data expressed as mean ± SEM.
* $p < 0.05$ vs. control

Table II

BAL Fluid Recovery, Total and Differential BAL Cell Counts, and Mean CT-Densities after 26 Months of Exposure.

	control (n = 5)	quartz (n = 7)	quartz pressure (n = 4)	pressure (n = 5)
fluid recovery (%)	79.8 ± 1.8	80.7 ± 1.4	80.8 ± 2.3	80.2 ± 1.4
total cell counts (x 10^6)	7.0 ± 0.9	79.6 ± 20.3*	61.4 ± 2.3	5.1 ± 1.8§
macrophages (%)	87.4 ± 4.4	69.8 ± 5.7*	78.5 ± 2.2	88.0 ± 1.6§
lymphocytes (%)	4.2 ± 1.6	5.5 ± 1.3	3.8 ± 0.8	5.4 ± 1.7
neutrophils (%)	0.6 ± 0.4	19.5 ± 7.6*	10.5 ± 2.7	0.2 ± 0.2
mast cells (%)	4.6 ± 2.9	5.3 ± 1.9	7.3 ± 2.9	2.4 ± 1.4
mean CT-density (HU)	-892.3 ± 8.5	-734.6 ± 27.5*	-800.0 ± 7.5	-908.6 ± 7.8§$

Data expressed as mean ± SEM.
* $p < 0.05$ vs. control group
§ $p < 0.05$ vs. quartz-exposed group
$ $p < 0.05$ vs. quartz/pressure-exposed group

ACKNOWLEDGMENTS: The authors would like to thank Ms. A.-M. Allmeling, Ms. S. Münzing and Ms. R. Schüler for perfect technical assistance.

The study is part of the German BMFT grant No. 01 VD 492/7 ("Spritzbetonbauweise unter Druckluft: Tier- und arbeitsmedizinische Untersuchungen") and was projected in cooperation with the "TBG" (Tiefbau-Berufsgenossenschaft, Munich), the "STUVA" (Studiengesellschaft für unterirdische Verkehrsanlagen, Köln), the "Medizinisches Institut für Umwelthygiene der Universität Düsseldorf" (Director: Prof. Dr. H.W. Schlipköter), and the "Institut und Poliklinik für Arbeitsmedizin der Universität München" (Director: Prof. Dr. G. Fruhmann).

CORRELATION OF CHEST FILM AND LUNG FUNCTION ANALYSIS IN PATIENTS WITH SILICOSIS

H. OTTO • W. Jansen

Sittardsberger Allee, D-4100 Duisburg, FRG
Former Affiliation: Knappschaftskrankenhaus Essen, FRG

INTRODUCTION

Numerous studies treating the subject on the correlation between X-rays of a patient with silicosis and a pulmonary function test can be found in today's literature. However, only a mere correlation between the two methods of examination could be demonstrated. The possible reason for this may be the inaccurate description of the X-rays which in all current publications was conducted using the ancient silicosis classification. For this reason we investigated a sizable patient collective with mixed dust silicosis for reasons of correlation and, in the process applied the new precise ILO-classification of 1980 in it's expanded version. In the course of this, the attempt was made to draw up a sum index which will register the crucial parameters of the chest X-ray, in order to establish the relation to the pulmonary function.

MATERIAL AND METHODS

Our material consisting of 283 miners was to be studied in a survey at our hospital. In addition to the clinical examinations, chest X-rays p.a. and lateral were made according to the technique suggested by the ILO. The coding was effected by two examiners employing the ILO-classification. In case of deviations a renewed classification was jointly carried out. The pulmonary function analysis involved the measurement of the vital capacity, the resistance, the intrathoracal gas volumina (ITGV) as well as the blood gas analysis.

The obtained data was statistically worked out applying the Chi-Square-Test and the regression analysis.

RESULTS

In Figure 1 it can be seen that 40% of the experimentees showed no signs of large opacity, in 20% an A-shadow, in 30% a B-shadow and in 10% a C-shadow was evident. The analysis indicating on which side the opacity was located revealed type A to always be single sided, type B to be mostly single sided and type C to be predominantly both sided ($p = 0.001$). Figure 2 demonstrates the distribution of the individual categories of profusion.

Sections S1 and S2 are predominant, thus groups 0/1 to 2/3. A similar distribution can be found in the size of the small rounded opacities, although hereby the categories p/p to p/q are dominant; 20% show larger spotty shadows. There was a correlation between the formation of small rounded opacities, inasmuch as large opacities turned up significantly more often when more formations of small rounded opacities were apparent in the pulmonary fields.

In the correlation of radio-morphological variation to the bronchial resistance, a significant augmentation in resistance was observed while the size of large opacities increased ($p = 0.05$). Most characteristic was the effect in B- and C-type shadows (Figure 4). At first, the number of large opacities, the size of small rounded opacities, the category of profusion as well as the distribution over the 6 pulmonary fields seemed to be of no importance. We then divided the patient collective into simple and complicated CWP. A positive correlation between the category of profusion and an increased bronchial resistance in patients without large opacities was found (Figure 5). Thus, the dominating factor influencing the resistance is to be found in the formation of large opacities.

There are many reasons for the lacking influence of the formation of small rounded opacities on the resistance. Following extensive epidemiological investigations Ulmer and Reichel have found the obstruction not to be an immediate result of pneumoconosis, but rather a result of the increased incidence of bronchial catarrhs in persons suffering from silicosis. An additional critical factor is the loss of elastic fibers which again will lead to small airways disease (Legg et al.). From a purely qualitative point of view, this lack of correlation between radiomorphologic and pneumofunctional analytical findings is clearly described in observations made by Marek. He conducted comparative examinations between miners, arc welders and persons exposed to asbestos and found concrete differences between size, form and number of pneumoconiotic small rounded opacities, however, there was no correlation between X-rays and progressive pneumofunctional disorders.

The vital capacity shows a clear and significant correlation to the size of the large opacity (Figure 6). In the same manner a statistically significant relationship to the number of large lesions was observed.

With increasing size of small rounded nodes, a statistically proven decrease in vital capacity was detected, while the number of small rounded opacities expressed in category of profusion showed no relation to the vital capacity, at most, a tremendous reduction of VC after an increasing category of profusion could be observed ($p = 0.073$). The influence of the vital capacity by the large opacity's size may at first be

explained by a decrease of intact tissue, on the other hand, however, the formation of an emphysema also plays a certain part. Yet, it must be considered that this fibrotic reaction in anthracosilicosis which leads to an increase of restrictive functional disorders occurs much less than in asbestosis (Ruckley). The lack of correlation to the formation of small rounded opacities could possibly be due to the fact that the small spotty shadows are often surrounded by an emphysema leading to a significant functional cut back, however, the X-ray picture will give cause for a much too low classification. At all events, the perinodular emphysema, however, shows no correlation to the silicosis degree (Hieber).

The comparison between type of lesion, the number of large opacities and the distribution over the pneumatic fields revealed no correlation whatsoever to the ITGV. Merely an increasing category of profusion indicated a trend towards an increase of ITGV ($p = 0.12$). This fact can be explained through observations made by Worth and Smidt, suggesting that already an early state of silicosis will lead to a significant bronchiolitis with bronchiolectasis as well as a dynamic bronchiolostenosis with a constructive formation of an emphysema. These alterations are radiomorphologically not ascertainable, yet they have an enormous influence on the pulmonary function and hereby especially on the ITGV. Furthermore, it should be considered, that an increase in the ITGV occurs with every chronic exposure to dust, thus making the disruption in pulmonary function silicosis unspecific (Ulmer).

The blood gas analyses exclusively demonstrate a dependency to the number of large opacities; an increase in number causes a statistically significant increase in the carbondioxide pressure as well as a decrease in the oxygen pressure, whereby the latter occurs only when there are bilateral lesions. The missing correlation to the formation of small rounded nodes may be due to the fact that in the case of an anthracosilcosis a fibrotic reaction happens only following an extended exposure, which then may lead to a disruption in the gaseous interchange.

We chose two of the 24 supplementary symbols available in the ILO-classification to investigate for a possible correlation to the pulmonary function. We selected the symbols em and tb to be the most profitable expecting a most likely relationship between morphology and function. The symbol em was coded in a total of 159 (56%) experimentees (Figure 7). The explanation for this large number is that the symbol em is not clearly explained in the ILO-classification and that the radiological definition is unclear seeing that no less than 36 radiologic symbols are described which are compatible with an empyhsema. Beyond this, the critical examination of the single symptoms is generally impossible since those structures of small rounded nodes and large opacities which are of interest are being superimposed. Nevertheless, in a medium or severe increase of ITGV a significantly more frequent coding of the symbol em was received.

The symbol tb was applied to 68 (24%) experimentees. No correlation to the pulmonary function analysis was evident in this group. The reason for this is the fact that a coding of the symbol tb happens most often when there are inactive apical localizations of pulmonary tuberculosis present which are of no importance to the pulmonary function.

In order to achieve an exact correlation of several radiological parameters with one single pulmonary functional analytical parameter each, a score for the radiological findings was introduced. The large opacities includinq their size, the small rounded opacities in size and distribution as well as the allocation over the pulmonary fields were given a value between 1 and 3, merely the location of large opacities received only half a point value, because no correlation could be obtained in previous single results. With an increasing sum index a significant boost in resistance ($p = 0.006$) as well as an important decrease in vital capacity was noted ($p = 0.0395$).

This result signifies that our originally stated assumption holds true, namely of a positive correlation between radiomorphologically comprehensible parameters, which can be metrically documented using symbols according to the ILO-classification. This conformity is of statistical significance in a large patient collective. Yet, the attempt to project the results on to the individual experiment turned out insufficient and uncertain because in the isolated case drawing to a safe conclusion from the X-rays to the anticipated pulmonary functions is impossible.

CONCLUSION

1. Severe silicosis with small rounded opacities having a category of profusion of 2/3–3/3 as well as large opacities of type B and C occure significantly more along with an increase of resistance. The number of lesions, as well as the size of small rounded opacities and their distribution over the six pulmonary fields have no influence on this functional parameter.

2. The size of large opacities and their number as well as the size of the formation of small rounded nodes significantly correlates with the vital capacity and vice versa.

3. An evaluation of the ITGV on the basis of radiologically comprehensible parameters is only possible in modestly severe and severe augmentations of the ITGV. Here the symbol em was coded much more frequently.

4. A correlation between X-rays and blood gas analysis exists only if several large opacities are apparent in both lungs and this holds true for hypercapnia as well as for a diminution in oxygen pressure.

5. By the establishment of a score, which takes into account all radioparameters including their various importance, a statistically significant correlation to resistance and vital capacity was found in the total collective. In the isolated case, however, the evaluation of the pulmonary function by means of radiological parameters is not reliable enough, thus making the use of such a score when applied to expert interrogations not sufficiently adequate.

REFERENCES

1. Hieber, E.M.: Klinisch-epidemiologische Untersuchungen an Steinkohlenbergarbeitern zur Frage der Häufigkeit von chronischer Bronchitis und Lungenemphysem. *Prax. Pneumol.* 34:32-41 (1980).

2. Legg, S.J.: Lung mechanics in relation to radiographic categorie of coal workers simple pneumoconiosis. *Brit. J. Indust. Med.* 40:28-33 (1983).
3. Marek, K.: Lung function in different types of pneumoconiosis. *Z. Erkrank. Atm.-Org.* 163:270-272 (1985).
4. Ruckley, V.A.: Comparison of radiographic appearances with associated pathology and lung dust content in a group of coal workers. *Brit. J. Indust. Med.* 41:459-467 (1984).
5. Seaton, A.: Coal and the lung. *Thorax* 38:241-243 (1983).
6. Ulmer, W.T.: Die Begutachtung der Silicose. *Prax. Pneumol.* 31:144-152 (1977).
7. Worth, G., Smid, U.: Lungenverstaubung-Staublungen. *Pneumologie* 151:185-200 (1975).

ACKNOWLEDGMENT: We thank Ms. S. Erckmann for the translation into English.

EVALUATION OF RESPIRATORY HAZARDS BY LUNG FUNCTION INVESTIGATIONS

W. T. ULMER • H. P. Hoffarth • B. Höltmann • D. Schött

University Hospital "Bergmannsheil Bochum"
Bochum/FRG

ABSTRACT

There are many different methods available for lung function measurements. In epidemiological studies and for the control of workers exposed to different hazards we compared the efficiency of the most available methods. The most useful method is the measurement of airway resistance and of thoracic gas volume by bodyplethysmography. This method is very sensitive and very specific. It does not depend on the cooperation of the measured persons. The predicted values are very precise and reliable. We also use for special questions the 1-concentration-metacholine-challenge-test. This test enables to differentiate between normal bronchoreagibility and hyperreabibility. After different short-term types of exposure, a hyperreagibility of the bronchial system could be detected. Early signs of changes will be found by these methods, and different exposure types results in typical functional changes.

No Paper provided.

SISTER CHROMATID EXCHANGE FREQUENCY AND CHROMOSOMAL ABERRATIONS IN ASBESTOS FACTORY WORKERS

QAMAR RAHMAN, Ph.D. • F. Nahid,* M.Sc. • A. K. Jain,* Ph.D • S. S. Ayarwal,* M.D.

Industrial Toxicology Research Centre, Post Box No. 80
Lucknow-226 001, India
*ICMR Centre for Advanced Research in Genetics, Dept. of Medicine
K.G's Medical College, Lucknow—226 003, India

INTRODUCTION

Asbestos, belongs to a group of naturally occurring magnesium silicate fibrous substances. It has a wide variety of uses in modern society. It is considered to be a carcinogenic for several organ systems.[13] Past study reveals that asbestos exposed workers have an increased risk to develop mesothelioma, bronchogenic carcinoma and other cancers.[3,4] It has also been found to have mutagenic properties. Sincock and Seabright[14] first demonstrated that Chinese hamster ovary cells exposed to chrysotile and crocidolite asbestos showed the occurrence of chromatid and chromosomal changes. It has also been reported that chrysotile produced chromosomal aberrations in cultured Syrian hamster embryo cells in dose related manner.[8]

Chrysotile (UICC) variety has already been reported to induce chromosomal abnormalities in cultured human lymphocytes.[17] Rom et al.[12] found that asbestos workers had an elevated mean sister chromatid exchange (SCE) rate compared to that of controls. In another study slightly higher incidence of chromosomal aberrations in asbestos exposed factory workers was observed.[16]

An Indian variety of chrysotile asbestos has also been found to induce chromosomal aberrations and sister chromatid exchange in Chinese hamster ovarian cells in vitro.[1,2]

In India extensive use of asbestos in various occupations leading to an increased risk of asbestos exposure to workers demands a critical evaluation of asbestos dust. Therefore, present work was undertaken to evaluate the cytogenetic effects of asbestos dust on workers in an asbestos cement factory. This preliminary report may be helpful to determine genotoxic effects of asbestos dust exposure on human population.

MATERIALS AND METHODS

22 factory workers as well as 12 controls were studied to assess the frequency of sister chromatid exchange and the incidence of chromosomal aberrations. The controls were of similar age, sex, having similar habits and socio-economic status. All subjects were carefully examined and detailed clinical history was taken. No subject had taken any drug for at least two months prior the sampling of peripheral blood. All subjects were divided in four groups; asbestos exposed smokers, asbestos exposed non-smokers, control smokers and control non-smokers. The mean duration of exposure was 12.0 years.

From each subject, peripheral venous blood was collected in a heparinized tube under sterilized conditions. Whole blood lymphocyte culture was done in RPMI-1640 medium supplemented with fetal calf serum (20%), L-glutamine (0.03%), penicillin (100 I U/ml), Streptomycin (100 µg/ml), Phytohemagglutinin-M (3%), 5'-bromo-2'-deoxyuridine (5 µg/ml), and blood (0.3 ml). Culture vials were wrapped with aluminium foil and incubated at 37°C in 5% CO_2 atmosphere.

Cultures were harvested at 48 hours to study chromosomal aberrations and at 72 hours for SCE analysis. 3 hours prior to harvesting colchicine (0.1 µg/ml) treatment was given to arrest the cells in metaphase. Centrifugation of the culture was done at 1200 rpm for 10 minutes. Supernatant was discarded and pellets were resuspended in hypotonic solution (0.075 M KCl) and incubated at 37°C for 20 minutes. Again centrifugation of the material was done for 10 minutes at 1200 rpm, the supernatant was discarded and pellets were fixed in fresh and chilled fixative methanol and acetic acid (3:1). Slides were prepared by flame dry method. For differential staining slides were stained with Hoechst 33258 (50 µg/ml) for 15 minutes and then exposed to bright sunlight for 2 hours in the presence of 2 SSC followed by staining with Giemsa (4%) in phosphate buffer (pH 6.8). Coded slides were scored to avoid scoring bias. 100 well spread metaphases were scored for chromosomal aberrations and 50 well spread metaphases with good differentiation were scored for SCE analysis for each subject. Students' 't' test was used for statistical analysis.

RESULTS AND DISCUSSION

In the present study all the subjects were of similar age group. It is evident from Table I that the aberrant metaphase percentage was significantly higher ($p < 0.01$) in exposed smoker and exposed non-smoker groups in comparison to their respective control. Chromosomal aberrations were of chromatid gap and break type (Figure 1). Mitotic index was low in both the groups but not significantly. In both the exposed groups mean SCE/cell was elevated significantly ($p < 0.001$) without affecting mitotic index and cell cycle

Table I

Chromosomal Aberrations in Asbestos Factory Workers
The Values Represent Mean ± SD *(P / 0.01)

Subjects	Age (yr) Mean ± S.D.	Duration of exposure (yr) Mean±S.D.	Chromosomal aberrations				
			No. of cells scored	Mitotic index Mean±S.D.	Aberrant Metaphase (%) Mean±S.D.	Chromatid gap (%) Mean±S.D.	Chromatid break (%) Mean±S.D.
Exposed smokers (n=11)	34.1±2.4	12.0±0.3	1100	2.14±0.82	4.09±1.51*	2.05±1.20	2.04±1.21
Exposed non smokers (n=11)	34.0±2.3	12.0±0.4	1100	2.90±0.71	3.54±1.21*	1.76±1.00	1.78±0.9
Control smokers (n=6)	33.1±1.9	0	600	3.90±0.98	1.50±0.54	0.60±0.5	0.90±0.7
Control non smokers (n=6)	34.0±2.0	0	600	3.94±0.25	1.30±0.81	0.50±0.4	0.80±0.7

n = Number of subject

* p / 0.01

kinetics (Table II). It indicates that asbestos exposure may induce undesirable genetic damage in occupational populations.

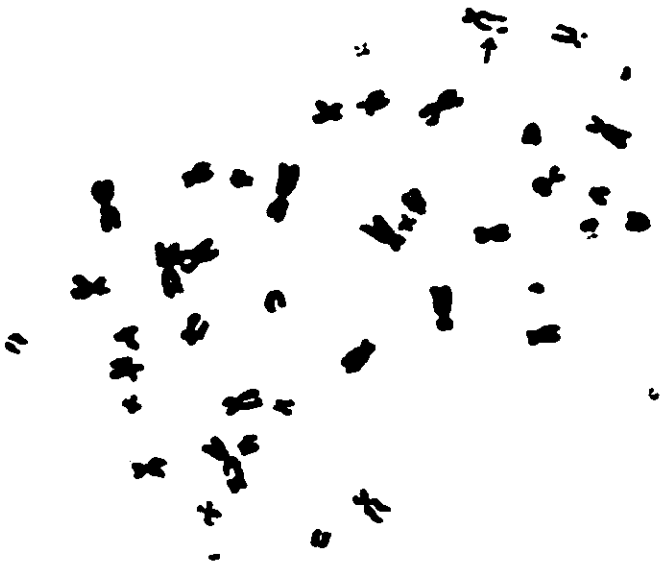

Figure 1. Cell with chromatid break in an exposed non-smoker subject.

The higher SCE/cell (Figure 2) in asbestos exposed smokers (8.16±0.45) in comparison to exposed non-smokers (6.63±0.50) (p / 0.001) may be due to synergistic action of asbestos and smoking (Table III). The results are in agreement with other workers.[6,12] The variation in the magnitude of chromosomal aberrations among both exposed smoker (4.09±1.51) and exposed non-smoker groups (3.54±1.21) was not significantly different. The elevation of SCE rate due to cigarette smoking is in accordance with the earlier reports.[7,10] The highest SCE frequency was observed in exposed smokers and lowest in control non-smokers. These results are analogous to lung cancer risk among insulators where the vast majority of cases occur in those insulators who smoke. The exact mechanism involved in the production of SCE is not well established. However, SCE analysis has been adopted as sensitive indicator of genetic damage.[11] The higher incidence of asbestosis in asbestos exposed smoker group (72.2%) in comparison to asbestos exposed non-smokers group (27.2%) (Table III) suggests that smoking may act synergistically to enhance asbestosis in the smoker group.

Marked variation in SCE frequencies have also been reported among individuals with several different types of cancer.[5,9,18] In addition, increase in SCE level has reportedly been found in cohort studies in those individuals who are at a higher risk of cancer due to occupational or environmental exposure to a wide variety of both mutagens as well as carcinogens.[15] We conclude that the evaluation of SCE per cell and higher chromosomal aberrations may point out the

Table II

Sister Chromatid Exchange Frequency in Asbestos Factory Workers
The values are expressed as mean ± SD *(P / 0.001).

Subjects	Age (yr) Mean ± S.D.	Duration of exposure(yr) Mean ± S.D.	Sister chromatid exchange						
			No.of cells Scored	Mitotic index Mean±S.D.	SCE/cell Mean±S.D.	SCE range	Cell cycle kinetics (%) Ist	IInd	IIIrd
Exposed smokers (n=11)	34.1±2.4	12.0±0.3	550	5.39± 0.72	8.16± 0.45*	3-16	30.39± 6.30	55.98± 5.53	12.50± 4.11
Exposed non smoker (n=11)	34.0±2.3	12.0±0.4	550	5.44± 0.60	6.63± 0.50*	3-12	29.20± 6.63	57.45± 9.62	10.15± 1.61
Control smokers (n=6)	33.1±1.9	0	300	5.80± 0.78	5.73± 0.16	3-9	33.64± 4.32	57.86± 3.34	8.74± 1.72
Control non smokers (n=6)	34.0±2.0	0	300	5.86± 0.80	3.61± 0.14	3-6	33.48± 5.18	56.44± 4.03	10.05± 3.47

n = Number of subject

* p / 0.001

Figure 2. Cell showing sister chromatid exchanges in an exposed smoker subject.

risk of developing cancer in asbestos exposed workers. However, further study is required to establish the fact that higher SCE and chromosomal aberrations are associated with development of lung cancer in asbestos exposed workers.

REFERENCES

1. Babu, K.A., Lakkad, B.C., Nigam, S.K., Bhatt, D.K.,Karnik, A.B., Thakore, K.N., Kashyap, S.K., Chatterjee, S.K.: *In vitro* cytological and cytogenetic effects of an Indian variety of chrysotile asbestos. *Environ. Res.*, 21:416-422. (1980).
2. Babu, K.A., Nigam, S.K., Lakked, B.C., Bhatt, D.K., Karnik, A.B., Thakore, K.N., Kashyap, S.K., Chatterjee, S.K.: Effect of chrysotile asbestos (AP-1) on sister chromatid exchange in Chinese hamster ovary cells. *Environ. Res.* 24:325-329. (1981).
3. Becklake, M.R.: Asbestos related diseases of the lungs & other organs; their epidemiology and implications for clinical practice. *Am. Rev. Respir. Dis.* 114:187-227. (1976).
4. Craighead, J.E., Mossman, B.T.: The pathogenesis of asbestos associated diseases. *N. Engl. J. Med.* 306:1446-1455. (1982).
5. *Differentiation and neoplasm.* Vol II pp. 93-101—R.G. Mackinell, M.A. Berardino and M. Blumenfeld et al. eds. Springer-verlag, Berlin. (1981).
6. Kelsey, K.T., Christiani, D.C., Little, J.B.: Enhancement of benzo(a)pyrene-induced sister chromatid exchanges in lymphocytes from cigarette smokers occupationally exposed to asbestos. *JNCI* 77:321-327. (1986).
7. Lambert, B., Lindblad, A., Nordenskjold, M., Werelius, B.: Increased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas.* 88:147-149. (1978)
8. Lavappa, K.S., Fu, M.M., Epstein, S.S.: Cytogenetic studies on chrysotile asbestos. *Environ. Res.* 10:165-173. (1975).

Table III

Chromosomal Aberrations, Sister Chromatid Exchange, Frequency and Incidence of Asbestosis in Asbestos Factory Workers
The values represent mean ± SD *(P / 0.001).

	Exposed non smokers					Exposed smokers			
S.No.	Chromosomal aberration (%)	SCE/Cell mean±S.D.	Asbestosis	Incidence of asbestosis (%)	S.No.	Chromosomal aberration (%)	SCE/Cell mean±S.D.	Asbestosis	Incidence of asbestosis (%)
1.	2	5.88±2.13	A		1.	4	8.34±1.36	A	
2.	5	6.56±2.17	A		2.	3	8.10±1.57	P	
3.	4	7.26±1.73	P		3.	3	7.52±1.56	A	
4.	4	7.15±2.20	P		4.	5	8.38±2.15	P	
5.	3	6.08±1.95	A		5.	3	8,52±1.68	A	
6.	6	7.16±1.55	P	27.2	6.	2	8.66±1.93	P	72.7
7.	4	7.04±2.90	A		7.	3	8.12±2.42	P	
8.	3	6.10±2.30	A		8.	7	8.70±1.18	P	
9.	3	6.98±1.34	A		9.	5	7.58±1.67	P	
10.	3	6.40±1.62	A		10.	6	8.48±1.57	P	
11.	2	6.36±1.74	A		11.	4	7.46±2.40	P	
Mean± S.D.	3.54±1.21	6.63±0.50				4.09±1.51	8.16±0.45*		

P = Present
A = Absent
* = (P/ 0.001)

9. Livingston, G.K., Cannon, L.A., Bishop, D.T. et al: Sister chromatid exchanges: Variation by age, sex, smoking and breast cancer status. *Cancer Genet. Cytogent.* 9:289-299. (1983).
10. Murthy, P.B.: Frequency of sister chromatid exchanges in cigarette smokers. *Hum. Genet.* 52:343-345. (1979).
11. Perry, P., Evans, H.J.: Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 258:121-125. (1975).
12. Rom, W.N., Livingston, G.K., Casey, K.R., Wood, S.D., Egger, M.J., Chiu, G.L., Jerominski, L.: Sister chromatid exchange frequency in asbestos workers *JNCI.* 70:45-48. (1983).
13. Rom, W.N., Palmer, P.E.S.: The spectrum of asbestos related diseases. *West.* J. Med. 121:10-20. (1974).
14. Sincock, A., Seabright, M.: Induction of chromosome changes in Chinese hamster cells by exposure to asbestos fibres. *Nature* 257:56-58. (1975).
15. Sorsa, M.: *Monitoring of sister chromatid exchange and micronuclei as biological endpoints.* IARC Sci Publ. No. 59. pp. 339-350—Lyon, France. (1984).
16. Srb, V.E., Kucova, J.M. Musil: Testing genotoxic activity in exposure to asbestos. 1. Cytogenetic examination of lymphocytes of human peripheral blood. *Proc. Lek.* 36:175-178. (1984).
17. Valerio, F., DeFerrari, M., Ottaggio, L., Repetto, E., Santi, L.: *Cytogenetic effects of Rhodesion chrysotile on human lymphocytes in vitro.* IARC Sci. Publ. No. 30. pp. 485-489. Lyon, France. (1980).
18. Wiencke, J.K., Vosika, J., Johnson, P. et al: Differential induction of sister chromatid exchange by chemical carcinogens in lymphocytes cultured from patients with solid tumors. *Pharmacology.* 24:67-73 (1982).