INTER-READER VARIABILITY AMONG READERS USING ILO 1971 AND 1980 CLASSIFICATIONS OF THE PNEUMOCONIOSES

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INTRODUCTION

The Coal Workers' X-ray Surveillance Program is a program mandated by the Occupational Safety and Health Act of 1969, which provides the opportunity for every working underground coal miner to have a chest radiograph taken at least once every five years.[1] One major objective of the program is to notify miners that their chest X-rays show signs of coal workers' pneumoconiosis (CWP), thereby allowing them the right to transfer to a low dust work environment. Broader objectives under the program provide for X-ray of all newly hired miners. Every participating miner is notified in writing of his X-ray status. The program is administered by NIOSH, Morgantown, WV.

Since the program inception in 1970, over 250,000 films have been processed. A diagram of the processing scheme is presented in Figure 1. Focusing on the X-ray processing scheme, it should be noted that a first reading is done at a facility close to the coal mine, usually by an 'A' reader. The films are then sent to NIOSH and distributed to 'B' readers selected on the basis of a randomized computer program. Thus all second readings are done by 'B' readers. The two results are summarized by a computer algorithm. If no consensus occurs, a third reading is requested from a 'B' reader. In order to become an 'A' reader, a person is required to correctly classify six X-rays from his/her own file, based on agreement with an expert panel. To become certified as a 'B' reader an examination given by NIOSH must be passed.

Reader variability between and within 'A' and 'B' readers has been an issue of concern since it reflects on the accuracy and consistency of data used for the transfer option, and on the perception of the program held by coal miners. In addition, it affects the ability to evaluate radiographic changes in individual coal miners, as well as overall estimates of prevalence and incidence over time.

Attfield documented inter-reader variability between and among 'A' and 'B' readers in a study in 1984.[2] The study was motivated by concerns brought to the attention of NIOSH, that first readings might be biased toward reading less abnormality since the choice of facility, and thus film reader, was made by the coal company. Findings of his study indicated that first readers, in fact, read higher levels of abnormality than second readers, but of more importance to this analysis, the study revealed high levels of variability, both between and among 'A' and 'B' readers.

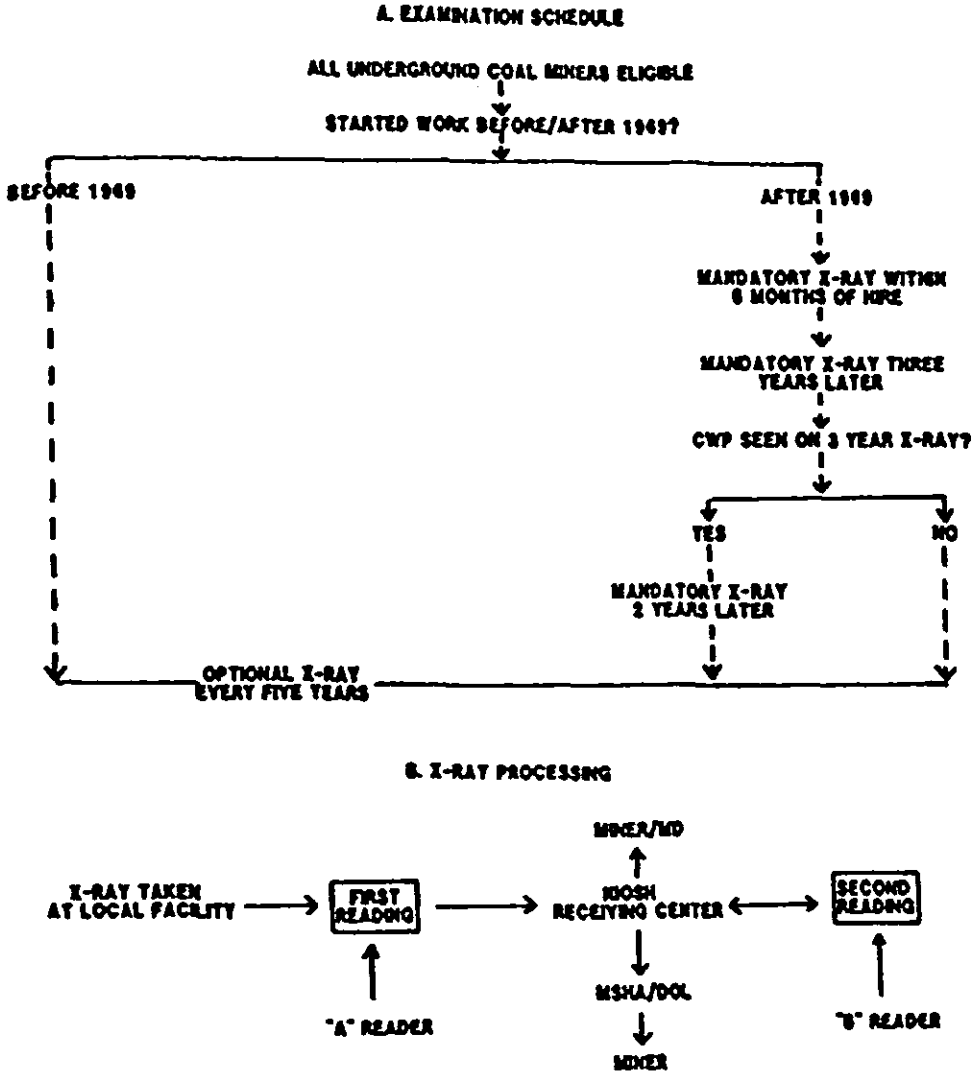
The chest X-rays reviewed by the Attfield study were taken in the CWXSP between 1978 and 1981. These films were classified using the 1971 ILO classification scheme.[3] During 1981 the use of the 1980 ILO classification was introduced into the program.[4] The change in classification required film readers to assess the overall profusion as well as the type of opacities in a different manner. Figure 2 presents an extracted section from each classification related to opacity type and profusion. In the 1971 scheme each reader assessed separately, and in a sequential manner the profusion of rounded, irregular, and combined opacities. The 1980 scheme requires the reader to specify the overall profusion, and then designate which opacity type, rounded or irregular, appears dominant.

This analysis was undertaken to determine if the change in classification affected the levels of reader variability noted previously. In order to do this, readings of chest X-rays evaluated by first and second readers on the 1980 ILO classification of the pneumoconioses, were compared to those read in the Attfield study. Comparisons between first and second readers for each classification were made for the profusion of opacities read, the types of opacities read, and within each reader group, for the range of prevalence of abnormal opacities which they reported.

METHODS

X-rays of readable quality for miners with 10 or more years of underground mining tenure were selected from films read during 1981–1987, by the 1980 ILO classification. These restrictions led to selection of a sample of 10,249 X-rays comparable to the 14,886 read during 1978–1981 by the 1971 classification. The tenure restriction of ten years eliminates the large weighting toward normal readers seen in miners with less than 10 years tenure. Only the first and second reading were considered for each film. General variability between and among first and second readers under each classification were compared in three ways:

1. Agreement based on profusion of small opacities for all first and second readers in each ILO group.
2. Agreement based on type of small opacities for all first and second readers in each group.
3. Range of variation within each group of readers based on prevalence of opacities, determined by the difference in highest and lowest ranked readers.



Broad processing scheme for X-rays in the CWXSP.

Tabulation of small opacities was based on the 12 point scale, ranging from category 0/− to 3/+. In all cases 0/− was combined with 0/0. These were combined to a 4 point major category scale in some cases. Prevalence comparisons were based on profusion of opacities, category 0/1 or greater.

Agreement was computed using the simple crude percentage. Since it seems reasonable to expect that the simple agreement of readings on the same films would be influenced by the underlying distribution of films, the kappa statistic[5] was used to adjust for the amount of agreement one would expect simply by chance. Kappa represents the excess agreement, having adjusted for expected chance agreement. It is computed as:

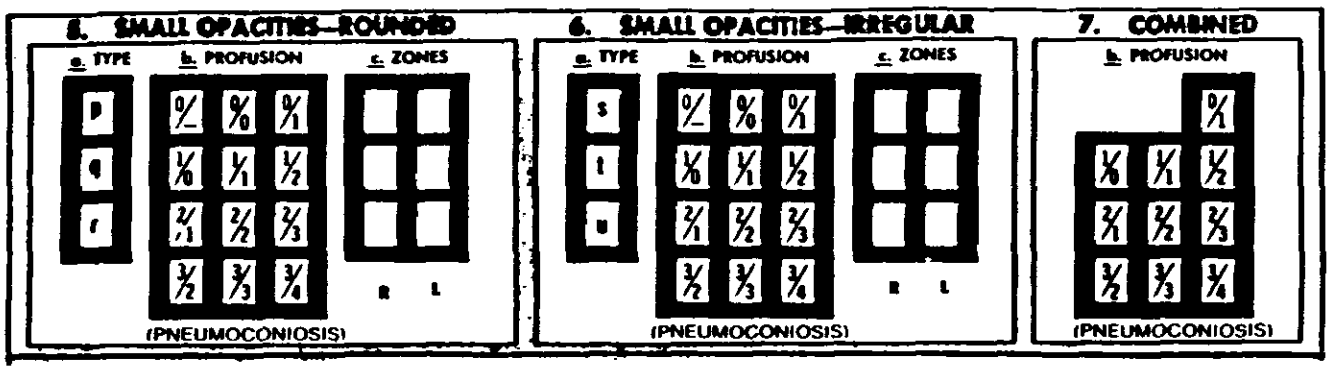
$$\text{kappa} = PC - PE / 1 - PE$$

where PC is crude agreement, PE is expected agreement, derived from marginal computations as in the chi-square test. A value of 0 represents no agreement, other than chance, while a value of 1 represents complete agreement.

For comparison of the two classification systems, the readings from the 1971 ILO were converted to a similar format to the 1980 ILO. A more detailed description is given elsewhere in these proceedings.[6] Briefly, for PRIMARY TYPE, if profusions for both rounded and irregular opacities were recorded by the 1971 ILO:

1. The larger of the two profusions was taken as the single profusion and the PRIMARY type was set to the type with the larger profusion.
2. If profusions were equal for each type the PRIMARY type was randomly assigned.

1971 Classification of Small Opacities



1980 Classification of Small Opacities

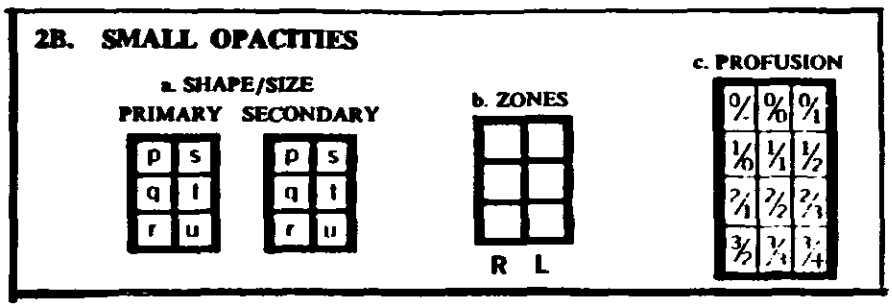


Figure 2

For the above situation SECONDARY TYPE was assigned as follows:

1. The SECONDARY type was set to the smaller of the profusion types.
2. The SECONDARY type was assigned the type not assigned to PRIMARY where profusions were equal.

If only one profusion type was recorded, that profusion was assigned to both PRIMARY and SECONDARY type.

RESULTS

Profusion of Opacities

Tabulations from the 1980 ILO group showed that 188 first readers and 20 second readers participated in evaluating the 10,249 X-rays. These values are close to the reported 196 first readers for the 1971 ILO, but indicate a decrease of 39% from the 33 second readers reported. Under 1980 ILO classification both first and second readers reported lower prevalence of category 0/1+ CWP, 12% for first and 4% for second readers. By the 1971 ILO classification scheme these values were 27% and 22% respectively. The lower prevalences under the 1980 ILO may be a reflection of an actual decrease in CWP, however, the differences between first and second readings shows a slight increase from 5% to 8% between the classification schemes. Figure 3 displays agreement between first and second readers further broken down into categories of the 12 point scale. Increases in reader agreement are seen in category 0/0 from 93 to 97% and in category 0/1 from 91 to 98%. Using the 1980 scheme decreases in agreement were evident in categories 1/0 and 1/2, from 30 to 27% and 30 to 16% respectively.

The agreement between first and second readers on the same films are presented in Table I. Overall crude agreement within major categories increased from 78% to 87% from the 1978 to the 1980 group. This overall increase is most likely due to the higher proportion of normal X-rays seen in the 1980 group. However the kappa statistic which adjusts for chance agreement is virtually unchanged. Further-

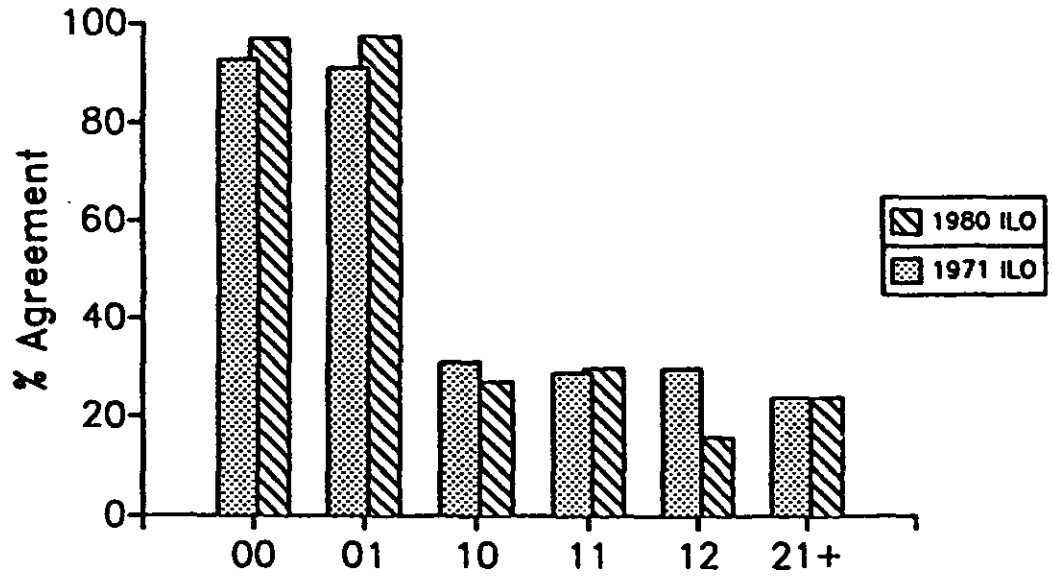


Figure 3. Agreement in profusion between reader 1 and reader 2 by minor category (agreement ± 1 minor category).

Table I

Agreement between 1st and 2nd Readers within Major Categories

1971 ILO		Reader 2			
		CAT 0	CAT 1	CAT 2+	TOTAL
Reader 1	CAT 0	10,793	927	30	11,750
	CAT 1	1,705	690	105	2,500
	CAT 2+	215	192	229	636
	TOTAL	12,713	1,809	364	14,886

(10+ years mining experience required.)

PC = 78%

PE = 69.6%

Kappa = .29

1980 ILO		Reader 2			
		CAT 0	CAT 1	CAT 2+	TOTAL
Reader 1	CAT 0	8,669	336	9	9,014
	CAT 1	757	251	24	1,032
	CAT 2+	82	56	65	203
	TOTAL	9,508	643	98	10,249

PC = 87%

PE = 82%

Kappa = .28

more the bias toward higher readings by first readers seen in the results using the 1971 ILO scheme continues to be seen. In the earlier study, 15% of readings classified as category 0 by the first readers were classified as category 1 or more by the second reader, while 8% of films classified as category 0 by the second reader were classified as category 1 or more by the first reader. This results in a 7% bias towards higher readings compared to the first readers. A similar calculation of findings from the 1980 classification group shows the bias reduced slightly to 5%. Both groupings show higher values in the lower diagonal elements than the comparable upper diagonal cells indicating that the change in classification scheme has not altered the tendency of first readers toward higher readings.

Type of Opacities

The agreement between readers in reading opacity type showed some parallel to profusion readings. On both classifications, first readers reported higher levels of rounded opacities as a percentage of all opacities than second readers, for primary type. The percentage for first readers increased slighty, from 74% to 75%, while that for second readers decreased from 63% to 56%. The difference in percentage between first and second readers showed a roughly 1.5 fold increase. A further breakdown into types of small opacities shown in Table II indicates that the change is due mostly to a shift in the second readers toward reading fewer rounded and more irregular type oapcities. Under both classification schemes first readers read the same percentages of rounded and irregular opacities, 74 and 26 percent respectively, although under the 1980 scheme a shift is seen towards reading more 's' and fewer 't' type irregular opacities. Second readers showed a much different pattern, with a large shift from fewer 'p' type rounded to more 't' type irregular opacities. This shift appears due to temporal changes in reading levels of rounded opacity types which have been occurring since 1978, discussed in more detail in Attfield, et al.,[6] rather than a change due specifically to the ILO classification scheme. The major point is that regardless of the underlying trends, the variability between first and second readers in the reporting of opacity types has not diminished under the 1980 ILO classification, but in fact has increased, compared to readings under the 1971 ILO scheme.

Table III presents data related to the final question, the range of variability among first and second readers. There was an overall decline in variability from 94% and 58% under the 1971 ILO to 68% and 31% under the 1980 ILO classification for first and second readers respectively. These reductions may in fact reflect actual levels of decrease in CWP, but the important element here is that first readers continue to read a range of disease at least twofold that of second readers.

DISCUSSION

The issue of variability in X-ray readings has implications, both as it relates to the CWXSP and to film readings in other research areas. In the CWXSP, the reduction of reader variability is of ongoing importance. One might expect that the change from the 1971 to the 1980 classification, given the focus on an overall evaluation of profusion of opacities, followed by a determination of primary and secondary opacity types, would increase agreement in readings between first and second readers.

Our findings indicate that this has not occurred. In summary, we found that first readers continue to read higher levels of prevalence of category 0/1+ opacities than second readers. The differences have increased rather than diminished. Reader agreement under the 1980 classification has increased in categories 0/0 and 0/1 only, decreasing in categories 1/0 and 1/2. Furthermore variability related to readings of small opacity types has increased. Among readers who read 50 or more films under each classification, the range of prevalence read by first readers as compared to second remains unacceptably high.

CONCLUSION

The findings of this study similar to those of Attfield[2] in-

Table II

Types of Opacities Reported as Primary Type for First and Second Readers

OPACITY TYPE	CLASSIFICATION			
	1971 ILO		1980 ILO	
	RDR1 (%)	RDR2 (%)	RDR1 (%)	RDR2 (%)
p	50.6	25.4	42.3	9.2
q	20.9	34.2	31.0	37.9
r	2.6	3.3	1.1	2.6
Total	74.0	62.9	74.4	49.7
s	14.2	19.7	18.1	19.7
t	11.0	16.7	7.1	30.4
u	0.8	0.7	0.3	0.3
Total	26.0	37.1	25.5	50.3

Table III

Range of Prevalence Category 0/1+ among First and Second Readers

ILO CLassification	First Reader	Second Reader
1971	1%–95%	3%–61%
1980	5%–68%	0%–31%

dicate that a quality control system is needed to minimize this problem in the CWXSP. This need exists in relation to both first and second readers. A system of feedback, notifying readers of their standing relative to all readers is one possibility. Specific details of the system could conceivably vary for 'A' and 'B' readers. A recently published paper documents unacceptably high reader variability in 'B' readers involved in asbestos medical surveillance.[7] The successful solution to this problem in the CWXSP might well serve as a model for other medical surveillance programs in which X-ray readers participate.

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EPIDEMIOLOGICAL METHODS DESIGNED TO ASSESS CROSS-SECTIONALLY AND LONGITUDINALLY THE RESPIRATORY HEALTH OF WORKERS EXPOSED TO CERAMIC FIBERS

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ABSTRACT

The purpose of this study is to evaluate the pulmonary health of workers occupationally exposed to refractory ceramic fibers (RCF) at seven facilities (three manufacturing sites) in North America. The primary objective is to conduct a 5-year longitudinal morbidity study of RCF exposed workers. Pulmonary symptomatology is being assessed through administration of the American Thoracic Society respiratory questionnaire. Pulmonary function changes are assessed through yearly administration of spirometry. RCF exposed workers receive chest radiographs every 3 years, interpreted independently by 3 certified "B" readers using the ILO Classification of Radiographs of the Pneumoconioses. A detailed occupational and environmental exposure questionnaire is administered to assess past and current occupational exposures and to develop an exposure profile. Occupational exposure is also assessed by examining historic industrial hygiene data and performing quarterly environmental sampling of each manufacturing site.

Included in this study are both former (n=668) and current (n=312) employees and an unexposed referent group. To date all current employees have been evaluated and a feasibility study is underway to evaluate referent populations. This paper will discuss the methodological framework of the study design. Issues to be discussed include cohort reconstruction, selection of an appropriate referent group, and the development, testing and reliability assessment of the occupational history questionnaire.

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MALIGNANT PLEURAL MESOTHELIOMA IN MONFALCONE, ITALY

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INTRODUCTION

The Trieste and Monfalcone shipyards, northeastern Italy, represents the most important shipbuilding installations in the Mediterranean area. A high incidence of asbestos-related pleural mesothelioma has been reported from the Trieste Province, the large majority of the patients having been exposed in the shipyard.[2,3,9] At the Hospital of Monfalcone an investigation into pleural mesothelioma has been under way since October 1979.[5,6,8] The Monfalcone Hospital serves as a small coastal area with a total population of about 60,000.

MATERIALS AND METHODS

Forty seven cases of malignant pleural mesothelioma were observed at the Monfalcone Hospital between October 1979 and March 1988. The diagnosis was based on (or confirmed by) necropsy findings in 37 cases. In a further 6 cases, mesothelioma was diagnosed on material obtained at pleurectomy. In the remaining cases the pathology diagnosis was performed on pleura needle biopsy specimens (2 cases), or by cytological examination of the pleural fluid (2 cases). Detailed lifetime occupational histories were obtained from the patients themselves or from their relatives by personal interviews. In 36 cases, seen at necropsy at our laboratory, the thoracic cavity was carefully examined for hyaline pleural plaques; these were classified into 3 classes: 1, mild; 2, moderate; and 3, severe. The small plaques (few centimeters in major diameter) were defined as class 1. Very large plaques, involving the major part of a hemithorax were classified as class 3. The intermediate conditions were labelled as class 2. In expressing the results, sometimes the terms small and large plaques are used, "small" corresponding to class 1, and "large" including classes 2 and 3. In necropsy cases isolation and quantitation of asbestos bodies were performed after chemical digestion of lung tissue.[24] Samples were obtained from the base of the right lung or from the left base, when the right was largely involved by the tumor.

RESULTS

The series included 40 men (age range 46–89 years) and 7 women (age range 48–89 years) (Table I).

The large majority of the male patients had been exposed to asbestos in the shipbuilding industry, Navy, Merchant Navy, and insulation (Tables II and III). Of the remaining three men, the first had been employed in the Monfalcone sodium carbonate factory, a workplace where asbestos exposure certainly occurred in the past;[7] the second had been exposed to a joiner and welder in a repair workshop; the third patient had worked for some years in a dye-house, where "fireproofed textile fabrics for naval furniture," were dyed. Of the women, five patients had been exposed to asbestos at home, having cleaned the work clothes of their relatives, employed in shipbuilding or in the chemical industry. One patient had a mixed (occupational and domestic) exposure. Concerning the woman with "negative" history, it should be noted that she had spent the first twenty years of her life in a coastal city; during that period she lived near a large dockyard.

The duration of the exposure to asbestos was generally over 20 years (Table IV); however some subjects had been exposed for shorter periods, one patient having worked as a painter in the shipyard for only a few months. Only 3 subjects had their first exposure after 1950 (Table V). The latency periods, defined as intervals between the presumable time of the first exposure to asbestos and the time of the diagnosis, ranged from 20 to 63 years (Table VI).

At necropsy the large majority of the patients showed hyaline pleural plaques (Table VII). Lung asbestos bodies ranged between 100 and about 10,000,000 per gram of dried tissue, with 21 subjects having more than 10,000 asbestos bodies/gram (Table VIII).

The most severe stigmata of asbestos exposure were found in the two insulators, the former of whom showed large pleural plaques and more than 4,000,000 asbestos bodies per gram of dried lung tissue; in the latter about 10,000,000 asbestos bodies/g were isolated and no plaques were seen. Of 25 shipyard workers seen at necropsy, 22 showed large pleural plaques; no plaques were observed in two subjects and small plaques in one. The majority of the shipyard workers, namely 12, showed asbestos body counts ranging between 10,000 and 100,000/g; higher amounts (100,000–1,000,000) were found in 6 cases, and figures less than 10,000 in 7. Some thousands of asbestos bodies/g were isolated in two sailors (4,000 and 7,000 respectively), the former with large and the latter with small pleural plaques. The textile industry worker showed 5,000 asbestos bodies/g and small plaques. Of 5 women with histories of domestic exposure, two showed large pleural plaques, and a further two small plaques. In this group, one patient had an unexpectedly high amount of asbestos bodies (90,000/g), the figures ranging between 100 and 6,000/g in the remaining cases. Finally the patient with "negative" history did not show pleural plaques; the amount of asbestos bodies in this case was 600/g.

The histological examination of lung tissues obtained at

Table I

Sex and Age Distribution in 47 Cases of Malignant Pleural Mesothelioma

Age (years)	Men	Women	Total
45 - 49	3	1	4
50 - 54	2	0	2
55 - 59	4	1	5
60 - 64	2	0	2
65 - 69	9	0	9
70 - 74	8	2	10
75 - 79	8	0	8
80 - 84	2	2	4
85 - 89	2	1	3
Total	40	7	47

Table II

Asbestos Exposures: Occupational Data

	No. of cases
MEN	
Shipbuilding industry	30
Navy and merchant navy	5
Insulation	2
Chemical industry	1
Construction industry	1
Textile industry	1
WOMEN	
Domestic exposure	5
Mixed exposure*	1
Negative history	1

* Occupational (shipyard) and domestic

Table III

Trades of the Shipyard Workers

	No. of cases		No. of cases
Painter	4	Calker	1
Plumber	4	Electrician	1
Shipwright	4	Laborers	1
Mechanic	3	Riveter	1
Carpenter	2	Sheet metal worker	1
Joiner	2	Various	2
Welder	2	Unknown	1
Worker	2		

Table IV

Duration of Asbestos Exposure

Years	Men	Women	Total
0 - 4	1	0	1
5 - 9	5	0	5
10 - 19	1	0	1
20 - 29	9	3	12
30 - 39	13	3	16
40 - 49	11	0	11
Total	40	6	46

Table V

First Exposure to Asbestos

Calendar years	Men	Women	Total
1920 - 1929	12	4	16
1930 - 1939	17	1	18
1940 - 1949	8	0	8
1950 - 1959	2	0	2
1960 - 1969	1	0	1
Undetermined	0	1	1
Total	40	6	46

Table VI

Latency Periods (First Exposure—Diagnosis)

Years	Men	Women	Total
20 - 29	3	0	3
30 - 39	3	0	3
40 - 49	13	0	13
50 - 59	16	4	20
60 - 69	5	1	6
Undetermined	0	1	1
Total	40	6	46

Table VII
Hyaline Pleural Plaques in 36 Necropsy Cases

Pleural Plaques	Men	Women	Total
Absent	3	2	5
Class 1	3	2	5
Class 2	5	1	6
Class 3	19	1	20
Total	30	6	36

Table VIII
Lung Asbestos Bodies in 36 Necropsy Cases

AB *	Men	Women	Total
2 - 3	0	2	2
3 - 4	10	3	13
4 - 5	12	1	13
5 - 6	6	0	6
6 - 7	2	0	2
Total	30	6	36

* Asbestos bodies, Log10/g dried tissue

necropsy revealed a variable degree of pulmonary asbestosis in 31 of 36 cases. Multiple tumors were observed in 7 neropsy cases. In particular three additional malignancies, beside mesothelioma, were found in a 77-year-old man: stomach adenocarcinoma, chronic lymphocytes leukemia, and prostate microelectronic. A further three men showed prostate microcarcinomas, and one man had chronic lymphocytes leukemia. Among women, NOS infiltrating breast carcinoma was associated with the mesothelioma in one case. Moreover another patient had been successfully treated for infiltrating breast carcinoma two years previously.

DISCUSSION

In the epidemiology of malignant mesothelioma more serious difficulties are encountered than in the generality of other malignancies.[10] Recent investigations carried out in the U.S.A. show that the reliability of death certificates may be very scarce.[11,18,23] On the other hand, the histological diagnosis itself is not always reliable, the percentages of diagnoses confirmed by panels of expert pathologists being sometimes very low.[25] At any rate the high incidence of malignant pleural mesothelioma in shipyard areas belongs to the category of the well established facts in the geography of mesothelioma[3,9,11,12,16] The low numbers of mesotheliomas in some shipbuilding countries of Eastern Asia such as Japan,[21] Hong Kong,[17] and Singapore,[13] seem at variance with the above statement. However, it must be noted that in some of these regions industrialization is a recent process, so that a sufficient time may not have elapsed to allow the development of many mesotheliomas. Concerning Japan, certainly a long-established industrial power, the use of asbestos was minimum in this country until 1950, reaching important values only in the 1960's.[22]

In the Monfalcone area, the relationship with shipbuilding represents the most important characteristic of pleural mesothelioma, working in the shipyard accounting for the large majority of the cases. The risk is not confined to few trades. On the contrary, among shipyard workers practically all the occupational categories are involved, a fact already well documented.[3] In addition, "shipyard asbestos" is responsible for further cases, by inducing pollution of the domestic environment.

Asbestos exposure sufficient to induce the development of malignant mesothelioma may occur in a variety of workplaces, other than shipyards.[3,8,9,12,14,15,19,20] Maritime trades emerge as important occupations at risk in the present as well as in other mesothelioma series.[9] However, for

other occupations, the existence of a previous exposure to asbestos may be more difficult to determine. Some histories in the present series were judged as negative or uncertain at a first examination, and occupational asbestos exposure could be ascertained only be deeper inquiries. In fact the characteristics of a given workplace, as they were some forty-sixty years ago, are not simple to reconstruct, especially when this workplace is a small workshop. Obviously the objective data (pleural plaques, asbestos body amount, lung asbestosis) are of enormous importance in such a reconstruction.[7,8]

The dangers related to the large use of asbestos in the industrial world have not been sufficiently appreciated.[1] The data collected in Monfalcone as well as in other Western shipyard areas, have serious implications. Shipyard mesothelioma represents a particular "enhanced model" of asbestos-related malignancy. It is presumable that the situation which occurred in Western shipyard areas in the past has been repeated, although to a lesser extent, in a variety of workplaces during the last decades. This means that an epidemic of mesotheliomas might involve all the industrial world in the coming years. If large numbers of persons have been seriously exposed to asbestos in recent years, then all the possibilities of preventing the development of mesothelioma in such people should be explored.[4,5] In this context it is important that the mechanisms implied in the genesis of mesothelioma are clarified. In asbestos-related mesothelioma the main agent is well known. However, the other concurring factors, presumably playing some role in the genesis of the tumor, remain to be identified.[4,5] The role of environmental coveters such as nitrosamines have been hypothesized. On the other hand host factors have to be considered, and immune impairment, favored by asbestos itself and/or induced by other causes could be of critical importance. Such aspects should be included among the main objectives of the future research on mesothelioma.

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ATMOSPHERIC PAH CONCENTRATIONS DURING CHIMNEY SWEEPING

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ABSTRACT

Epidemiological investigations have confirmed the cancer risk to chimney sweeps as a result of their exposure to soot and tar whilst at work. These complex mixtures of substances, which are pyrolysis products of organic materials, contain, amongst other things, carcinogenic polycyclic aromatic hydrocarbons (PAH). In order to be able to estimate the health risk both air sampled from that inhaled by chimney sweeps during the "dirty work" and soot samples were analyzed for PAH. The air sampling at the chimney only included the cleaning process and excluded periods without dangerous expositions.

A total of 20 PAH were quantified by gas chromatography-mass spectrometry in the n=115 air samples and n=18 soot samples. These included benzo[b]fluoranthene, benzo[a]pyrene (BaP), chrysene, dibenz[a,h]anthracene and ideno[1,2,3-cd]pyrene, all of which have been unequivocally confirmed to be carcinogenic in animal experiments. The summed atmospheric concentration of these compounds depended on the type of fuel employed and averaged 2.27 $\mu g/m^3$ for oil fuel. If a mixture of oil and solid fuel was employed the concentration was 5.06 $\mu g/m^3$ and pure solid fuel heating yielded 5.08 $\mu g/m^3$. The air concentrations of BaP were 0.36, 0.83 and 0.82 $\mu g/m^3$ respectively. The soot samples recovered after employment of the three different fuel types were 10.50, 109.10 and 51.25 mg/kg BaP. The maximum total concentrations of the five carcinogenic PAH were 243.70, 691.06 and 213.94 mg/kg respectively.

The time-weighted, shift mean concentrations of 0.02 to 0.21 $\mu g/m^3$ benzo[a]pyrene obtained on 11 days form the basis for the industrial-medical estimation of risk.

No Paper provided.

ROLE OF MAST CELLS IN THE PATHOGENESIS OF SILICOSIS

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INTRODUCTION

Mast cells are bone marrow derived mononuclear cells containing vasoactive amines, proteoglycans and proteases within their specific granules. Under pathological conditions, mast cells play a central role in allergic inflammatory processes as well as in parasitic diseases, both in human and in animal individuals. Increased numbers of mast cells also occur under non-allergic conditions,[6] i.e. in inflammatory diseases of the intestine, rheumatoid synovitis, and fibrotic disorders of the skin, the lung and the nervous system. With respect to the lung, elevated levels of mast cells can be obtained by bronchalveolar lavage in asthmatic patients[12,17] and in patients with various fibrotic lung disorders,[5,13] including silicosis.[6]

The role of mast cells in silicosis is not well understood. We therefore investigated the effects of silica dust DQ 12 on rat mast cells *in vivo* and *in vitro*. In addition, we analyzed mast cell topography in transbronchial lung biopsies from three patients with anthracosilicosis.

MATERIAL AND METHODS

Silica Dust

Quartz dust DQ 12, particle size <5 µm, were used in all animal experiments. For the *in vitro*-studies a stem solution (100 µg/ml) in buffer (Hanks balanced salt solution) have been prepared briefly before use, as described elsewhere.[20] For intratracheal instillation quartz dust DQ 12 has been suspended in sterile 0.9% saline. The inhalation experiments have been performed using native quartz dust.

Animals

Female Lewis rats, 8 weeks old, SPF-state, were used throughout all experiments, if not otherwise stated.

In-vitro Experiments

Rat peritoneal cells containing about 16 percent of mast cells[20] were harvested according to the method of Uvnäs and Thon.[18] Triplicate samples of 10^6 cells per ml buffer were then incubated (37°C, 10 to 120 minutes) with 3—100 µg/ml quartz DQ 12. The reaction was terminated by the addition of icecold buffer. For light microscopical investigations cytocentrifuge preparations have been performed. The cells were fixed in formaline vapour and subsequently stained with the combined alcianblue-safranine sequence.

For electron microscopy cells were fixed with glutaraldehyde, postfixed with osmium tetroxide, dehydrated and embedded in Araldite. Ultrathin sections were investigated in a Philips 400T electron microscope.

Cell viability was determined by the use of the Eosin Y dye exclusion test.

Short-term Inhalation Experiments

Short-term inhalation experiments were performed in a chamber containing a rotating wheel, as described elsewhere.[2,4] Groups of 5–6 rats were exposed to 10 mg quartz DQ 12 per cbm air, 6 hours a day, for up to 28 days. After termination of the experiment bronchoalveolar lavage (BAL) according to the method of Brain and Frank[8] was undertaken. The BAL cells were either spinned down in a cytocentrifuge followed by fixation and staining as indicated above, or processed for electron microscopical analysis.

A second group of animals were fixed by instillation of 2% buffered glutaraldehyde (10 cm H_2O, 20 minutes). After removal of total lungs, the right middle lobe was cut into small pieces. After postfixation, dehydration and embedding ultrathin sections were analyzed in the electron microscope.

For the determination of lung histamine content, from a third group of animals the left lung was removed; its wet weight was determined. It was then cut into small pieces, mechanically homogenized and resuspended in 2% perchloric acid. After sonification and centrifugation the supernatant was assayed for histamine, using the fluorimetric autoanalizer technique. Histamine content was expressed as microgram per gram wet weight of the lung.

Intratracheal Instillation Experiments

Groups of 5 female Wistar rats, SPF state, were used in these experiments. The animals received a single dose of 40 mg of quartz DQ 12 in 0.5 ml saline by instillation. Control rats were instilled with saline only. After 8 weeks the lungs were fixed and prepared for light microscopical investigations. Mast cell analysis was performed under the light microscope. 8 µm sections were stained with alcianblue-safranine O and the number of mast cells was determined.

Statistics

For statistical analysis Student's t-test has been performed.

Patients

Transbronchial lung biopsies from three male patients with

anthracosilicosis were obtained by standardized procedures. All samples were then fixed and processed for electron microscopical investigations.

RESULTS

Effect of Quartz DQ 12 on Rat Mast Cells *In Vitro*

The cytotoxic effect of quartz DQ 12 on rat peritoneal cells is time- and dose-dependent (Figure 1). At concentrations where a substantial amount of cells are still viable (12.5 µg/ml/10^6 cells) quartz DQ 12 induces a stimulatory action. Cell stimulation is proofed by the appearance of increased numbers of degranulated mast cells after incubation with low concentrations of quartz, but not at higher doses (Figure 2). During quartz-induced activation of mast cells a substantial amount of histamine is released into the incubation medium. Particle counts indicate that only 7 particles per cell (i.e. 12.5 µg/ml/10^6 cells) are responsible for cell stimulation, whereas 30 particles per cell (i.e. 50 µg/ml/10^6 cells) are already cytotoxic.[20]

Electron microscopical investigations show partially degranu-

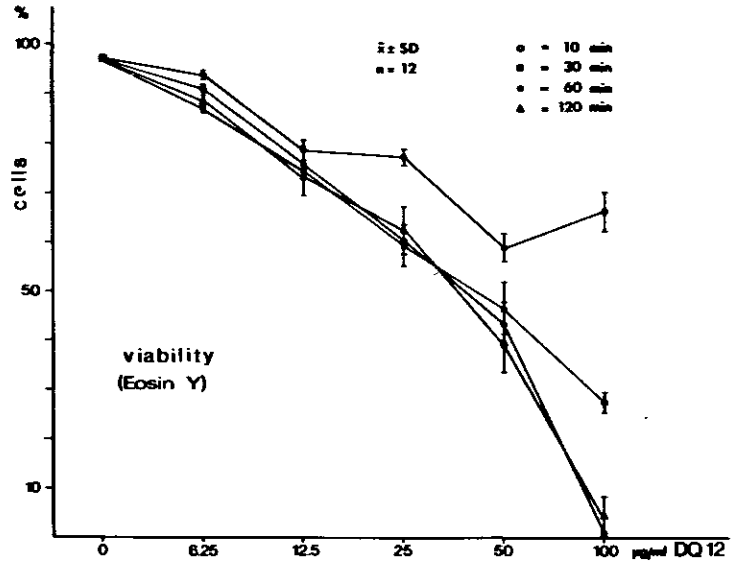

Figure 1. Dose- and time-dependent effect of quartz DQ 12 on the viability of rat peritoneal cells *in vitro*.

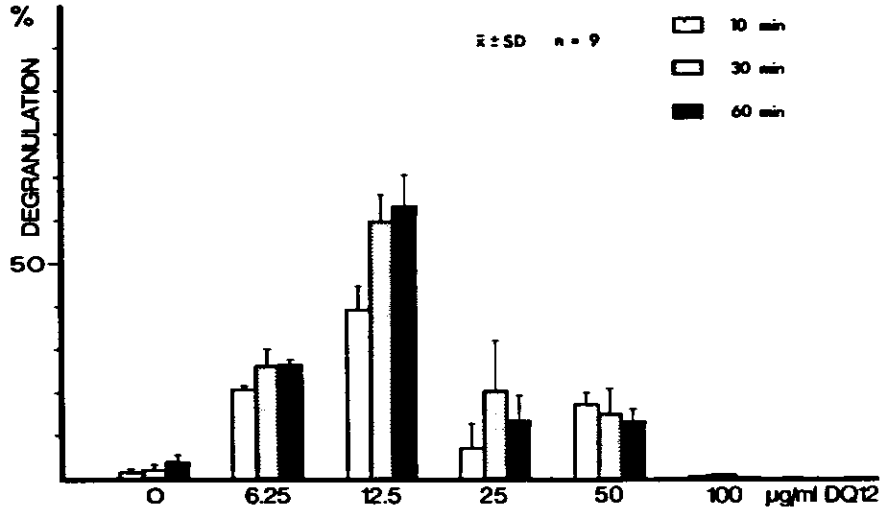

Figure 2. Dose- and time-dependent effect of quartz DQ 12 on the number of degranulated rat mast cells *in vitro*.

lated mast cells after incubation with low doses but not with high concentrations of quartz DQ 12 (Figure 3). Additionally, quartz-mediated mast cell/macrophage interactions are prominent, even under the conditions of the incubation method employed (shaking water bath). Phagocytosis of particles by mast cells could never be detected.

Effect of Short-term and Low Dose Inhalation of Quartz DQ 12 on Rat Lung Mast Cells

Early cellular events in rat lungs induced by quartz dust inhalation are characterized by an inflammatory response which has been described in detail elsewhere.[2,3,4] With respect to lung mast cells, intraepithelial mast cells occur as early as after an inhalation period of 4 days,[3] followed by the appearance of small numbers of mast cells in the bronchial lumen at day 8 and at day 14.[4] In addition, lung histamine content is significantly reduced at day 8 and remains at lower levels during the whole inhalation period (Figure 4). No increase in the number of parenchymal mast cells could be detected.

Number and Topography of Mast Cells in Silicotic Rat Lungs after Intratracheal Instillation of Quartz Particles

Instillation of 40 mg quartz DQ 12 into the trachea of rats results in lung fibrosis after 8—12 weeks. Initiation of fibrosis is accompanied by an 68.5% increase in the number of mast cells. In addition to mast cell hyperplasia, about 50% of these cells are localized interstitially (Figure 5), indicating mast cell redistribution. Most of the mast cells display safraninophilic granules. They therefore represent the connective tissue subtype, with respect to mast cell heterogeneity. Consequently, they are situated within bundles of collagen (Figure 6). Cells who are in close contact to fibroblasts display partial degranulation. Interactions between mast cells and dust-laden macrophages are frequently seen.

Occurrence of Mast Cells in Transbronchial Lung Biopsies Obtained from Silicotic Patients

Numerous mast cells are present in transbronchial lung

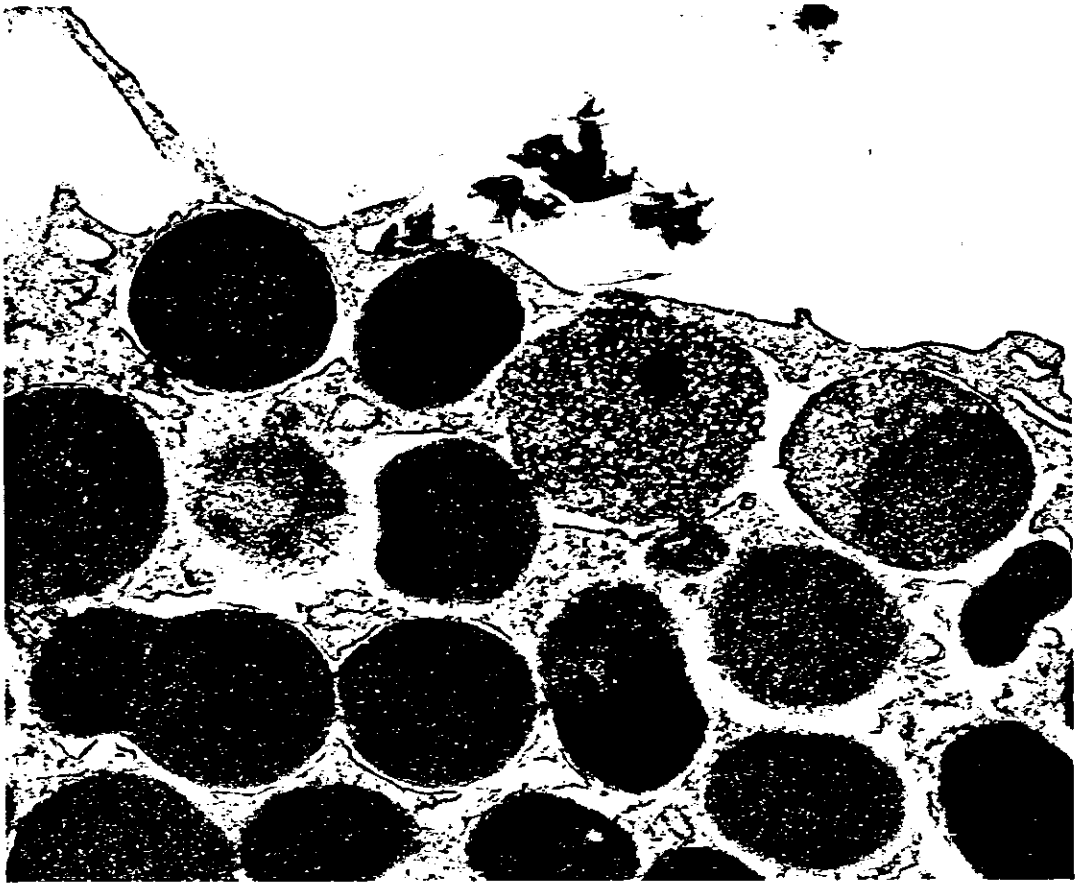

Figure 3. Electron micrograph of part of a mast cell undergoing degranulation induced by quartz DQ 12 *in vitro* (50 µg quartz DQ 12; 10 min.) Magn. x39000.

biopsies from silicotic patients (Figure 7). The cells display the species-specific whorls and scrolls within their granules. Exocytotic figures indicating mast cell degranulation are infrequently seen. If they occur they are always accompanied by fibroblast activation (Figure 8). Again, contacts between dust-laden macrophages and mast cells are visible (Figure 9). Examination of cells obtained by bronchoalveolar lavage reveal mast cells which are similar to cells seen in fibrotic lung areas and which are different from those obtained by bronchoalveolar lavage from asthmatic patients.[5]

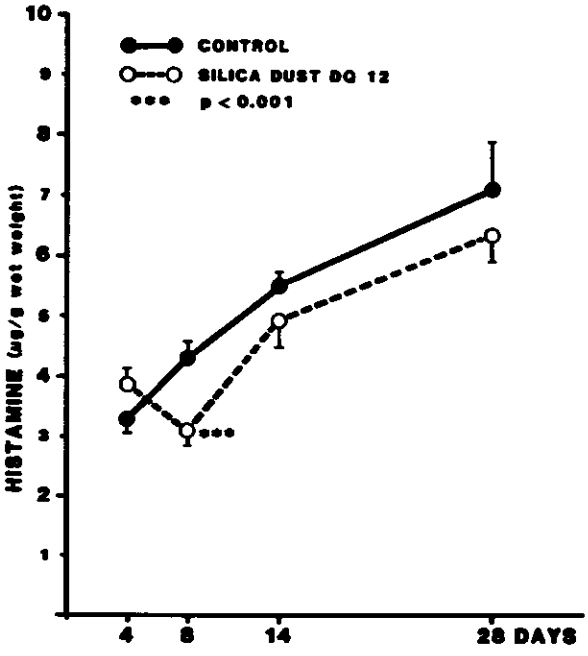

Figure 4. Effect of quartz dust inhalation (10 mg/cbm, 6 hrs per day) on total lung histamine content. Values are expressed as µg histamine per g wet weight of the lung.

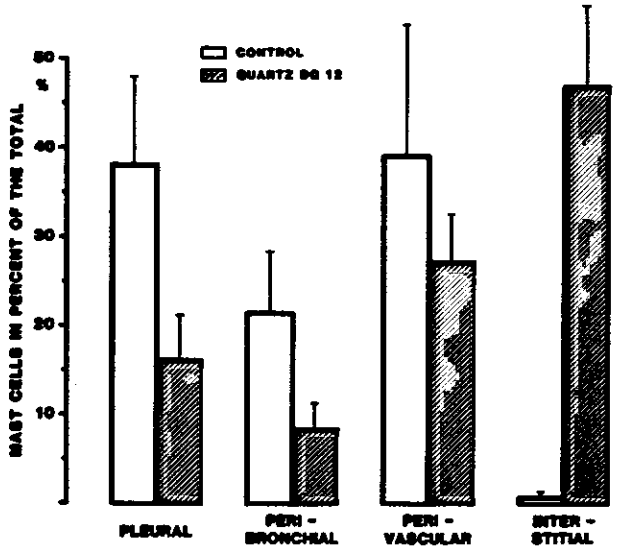

Figure 5. Distribution of mast cells in the rat lung 8 weeks after a single intratracheal dose (40 mg) of quartz DQ 12.

DISCUSSION AND CONCLUSIONS

This paper describes the occurrence, topography and functional behaviour of lung mast cells in response to cytotoxic quartz particles. The results obtained show that mast cells participate in the generation of silicotic pulmonary fibrosis. As is evident from our *in vitro* and *in vivo* experiments in the rat, mast cells are clearly involved in both, the initial inflammatory process and in fibrogenesis. Since continuous inflammation is an important link between inhaled quartz particles and the development of fibrosis in the lung,[7] mast cells are supposed to be one important element influencing and modulating this process. It is well known that mast cells release a variety of chemically active mediators of inflammation including diverse chemotactic factors upon appropriate stimuli.[14] Macrophages have been reported to release products with histamine liberating activity[16] as is true for oxygen radicals.[11] Histamine itself is able to potentiate the phagocytic activity of alveolar macrophages.[10] For this functional interaction between mast cells and macrophages in the lung the morphological equivalent is demonstrated in this paper. The phenomenon is not only relevant in the pathogenesis of experimental silicosis but is also present in transbronchial lung biopsies from silicotic patients. Therefore, mast cell—macrophage cooperation seems to be a general mechanism involved in the pathogenesis of silicosis.

The role of mast cells within fibrotic lung areas is not well understood. Mast cells can activate fibroblasts to divide,[15] and mast cell granules have been shown to affect fibroblast functions.[1] Fibroblasts in reverse are needed for the differentiation, development and granule synthesis of connective tissue mast cells.[9] Additionally, mast cells are able to influence some extracellular components of the connective tissue itself.[1] Furthermore, mast cell hyperplasia has been reported to occur in lung parenchyma of chronically hypoxic rats.[19] Therefore, mast cells can influence a variety of parameters which are necessary for and involved in fibrogenesis induced by toxic silica particles.

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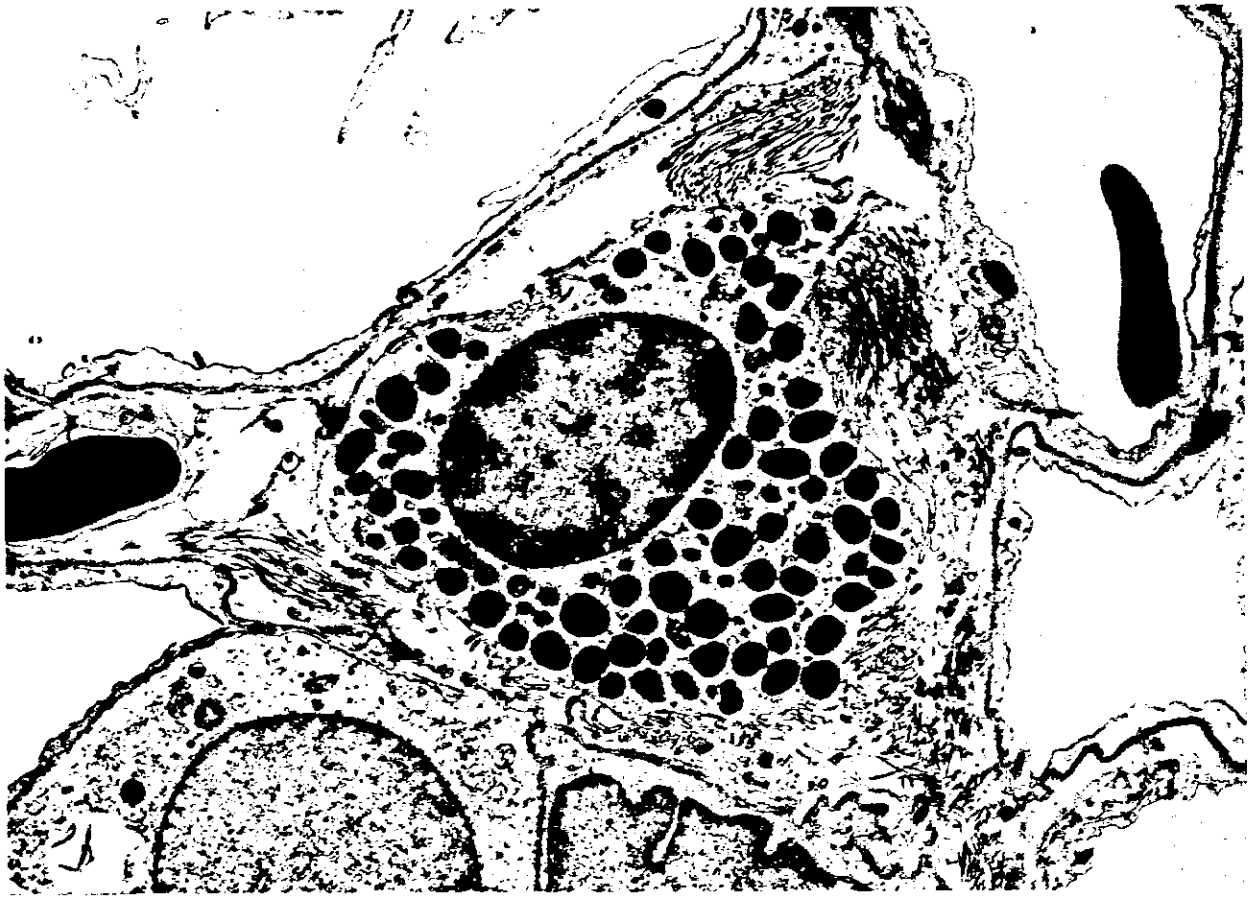

Figure 6. Rat lung mast cell surrounded by bundles of collagen fibres 8 weeks after intratracheal application of 40 mg quartz DQ 12. Electron micrograph. Magn. x9000.

Figure 7. Human mast cell lying within the interstitium of a fibrotic lung area from a silicotic patient. Transbronchial biopsy. Electron micrograph. Magn. x17000.

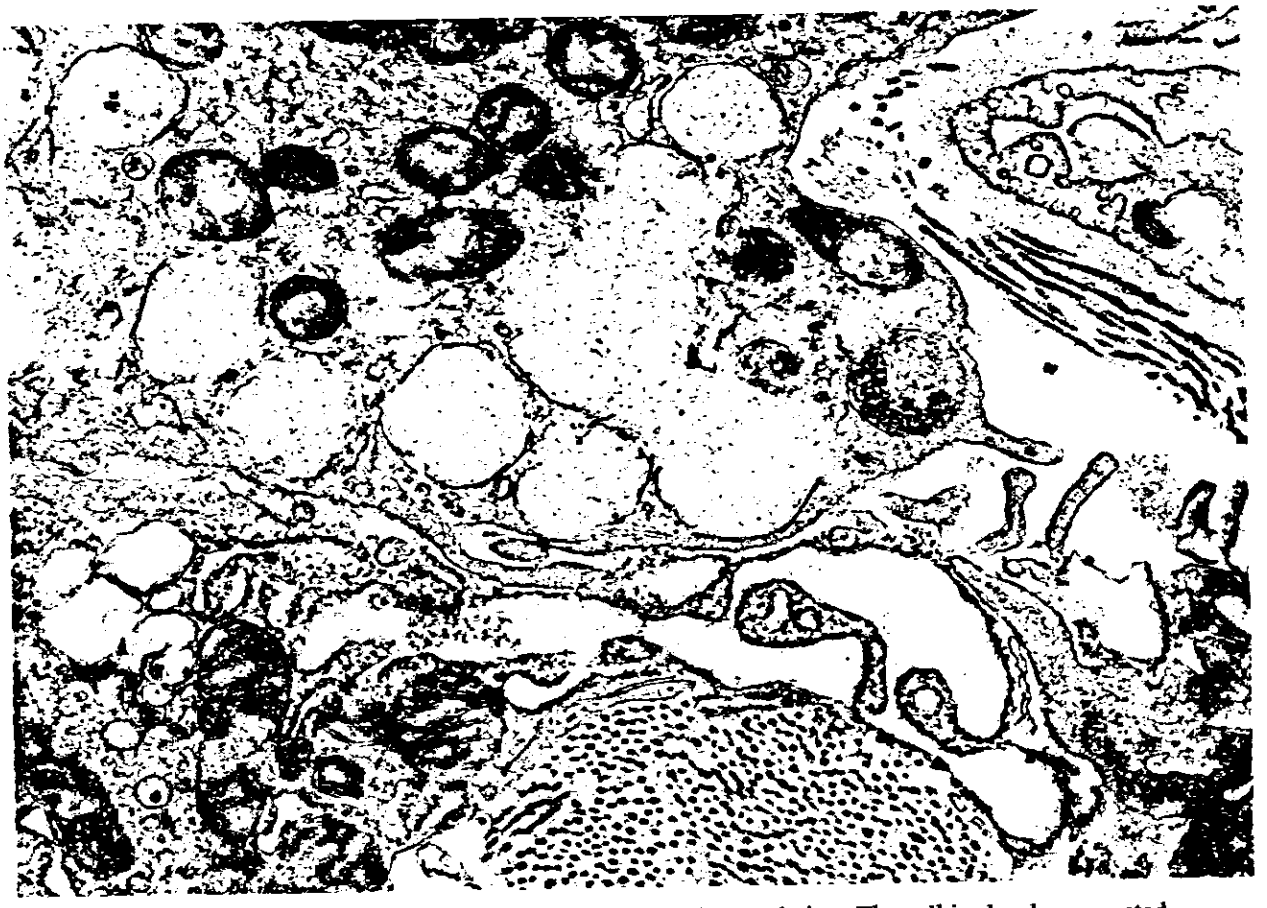

Figure 8. Part of a parenchymal mast cell undergoing degranulation. The cell is closely connected to a fibrocyte. Transbronchial lung biopsy. Electron micrograph. Magn. x28000.

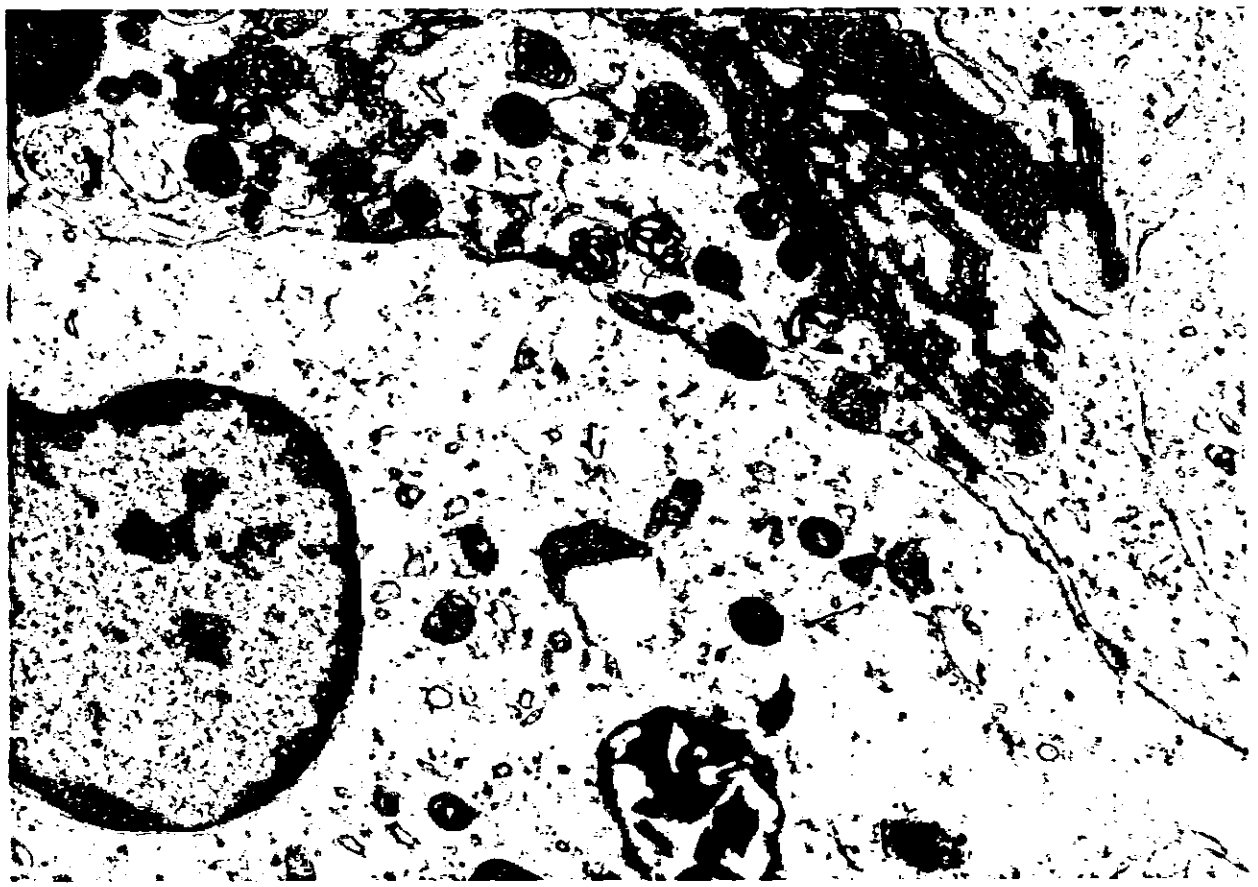

Figure 9. Interaction between a mast cell and a dust-containing macrophages in lung parenchyma of a silicotic patient. Transsbronchial biopsy. Electron micrograph. Magn. x17000.

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AUTOIMMUNITY PHENOMENA AND ALTERATIONS OF HUMORAL IMMUNOLOGICAL RESPONSES IN SILICOTIC PATIENTS

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INTRODUCTION

In the course of silicosis a variety of humoral alterations may occur.[1,2,3,6,10-14] So far it is not known, if these humoral alterations are the cause or the consequence of the disease. The most important humoral alterations occurring in silicosis can be divided into three groups: 1) Parameters indicating a stimulation of macrophages: lysozyme, angiotensin-converting enzyme (ACE), fibronectin; 2) Effects on humoral immunity: immunoglobulins, complement, response to antigens; 3) Autoimmunity phenomena: antinuclear antibodies (ANA), circulating immune complexes (CIC), rheumatoid factors.

In our investigations on silicotic patients the following aspects were of particular interest:

1. Are serological changes, from which an influence of quartz-containing dust on the immune system and specific macrophage functions can be concluded, detectable in miners and other silicotic patients?
2. Are such changes detectable only when radiologically manifest silicosis exists or are they already detectable in exposed persons without silicosis?
3. Is there an influence of the quality of quartz containing dust on immunological disturbances?

MATERIALS AND METHODS

In this context, we studied four groups: 34 miners who had radiologically manifest fibrosis following exposure for 15 years and 40 miners who showed no radiological signs of silicosis even after exposure for more than 30 years. In addition 48 stone masons and quarriers were under investigation. For comparison a group of 25 non-exposed men was included.[7,8]

The following parameters were studied in serum or plasma: lysozyme, angiotensin-converting enzyme (ACE), fibronectin, the complement component C_3c, immunoglobulins of classes IgG, IgA, IgE and IgM, circulating immune complexes (C_3, Clq, IgG, IgA, IgM bound in the complexes were detected here), and antinuclear antibodies (ANA). The methods used have been described previously in detail.[5,8]

RESULTS AND DISCUSSION

Lysozyme, ACE and fibronectin which indicate macrophage stimulation (for review s.8) revealed significantly higher values in coal mine workers with and without pneumoconiosis in comparison to the control group. (Table I)

It has to be emphasized that the significantly increased values

Table I

Parameters Indicating Macrophage Stimulation in Silicotic Patients

	COAL MINE WORKERS		CONTROL
	with silicosis	without silicosis	
Lysozyme (mg/l)	8.25(±3.19)[a] (n=34)	6.75(±1.56)[a] (n=39)	5.00(±1.73) (n=25)
ACE (U/l)	391.71(±99.98)[a] (n=34)	367.69(±84.77)[b] (n=39)	297.84(±77.17) (n=25)
Fibronectin (mg/l)	65.47(±14.06)[a] (n=14)	58.84(±6.57)[a] (n=5)	38.42(±6.54) (n=25)

in paranthesis standard deviation, n=number of cases

Significantly increased over control a ($p \leq 0.001$; students t-test)
b ($p \leq 0.01$)

of these parameters were already detected in coal mine workers without radiological signs of fibrosis.

The determination of immunoglobulins of classes IgE and IgM showed no differences between the four groups. Also the concentration of the immunoglobulins of classes IgG and IgA was unchanged in miners with or without pneumoconiosis in comparison to control. In contrast, concentrations of IgG and IgA in sera of silicotic stone masons and quarriers were significantly increased over the values of the control group. Such variable findings concerning the IgG and IgA levels in sera of silicotic patients have also been reported by other authors. Beside data which are not striking[11] increases in the immunoglobulin classes IgG and IgA were reported.[1,4] An important component of non-specific humoral immune response is the complement, which gets activated in cascade fashion by the classical or alternative pathway. In this process the complement component C_3 plays a central role, since it is involved in both pathways. Furthermore, of all complement components in serum, C_3 shows the highest concentration. Results of evaluation of the complement component C_3 in sera of the four groups were the following: coal miners with silicosis and silicotic stone masons and quarriers revealed the highest values. The differences to the control group were highly significant ($p \leq 0.001$).

It is especially remarkable that also coal miners without clinical signs of pneumoconiosis possessed high values of C_3, significantly different from controls ($p \leq 0.001$).

Of importance is the occurrence of circulating immune complexes (CIC) in sera of the four groups. For detection of immunoglobulins bound in these immune complexes we used the method of polyethylene glycol-precipitation and the radial immunodiffusion for subsequent determination of IgG, IgA and IgM.

Silicotic patients revealed significantly higher values of CIC than the non-exposed control group. It is remarkable that a significant increase of circulating immune complexes was already detected in coal miners without clinical signs of silicosis. Formation of immune complexes activates complement by the classical pathway. On the contrary, activation by the alternative pathway is not dependent on the presence of an antigen-antibody-reaction. Specifically, the latter one is activated by certain substances, such as bacterial endotoxins, macrophage enzyme elastase, and also by quartz dust.

It has to be emphasized that we found remarkable high concentrations of C_3 in sera of silicotic patients despite the fact that high concentrations of circulating immune complexes were present, which are known to bind and consume C_3. Results suggest that quartz dust induces activation of complement by the alternative pathway leading to increased values of C_3 in sera.[8,14]

An important sign of autoimmunity phenomena is the occurrence of antinuclear antibodies (ANA) beside the presence of circulating immune complexes (CIC). For detection of ANA we used the method of indirect immunofluorescence. The kidney cell line CV-1 from Cercopithecus aethiops has been employed as the substrate. In this study 114 sera of non-exposed persons served as a control. In sera of non-exposed

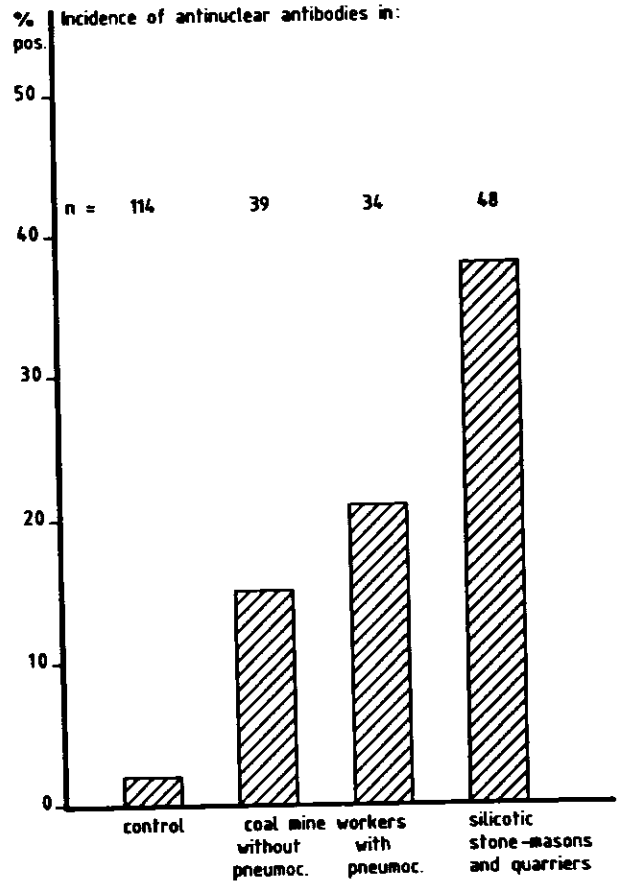

Figure 1. Incidence of antinuclear antibodies.

persons ANA were found in 2% of cases. In contrast, persons exposed to quartz-containing dusts are showing a remarkably higher incidence of ANA. So we found in sera of coal mine workers without pneumoconiosis ANA in 15% of cases, while in sera of coal mine workers with pneumoconiosis ANA were found in 21%. A nearly doubled incidence of ANA of 38% was found in sera of stone masons and quarriers (Figure 1). It is known that pattern of immunofluorescence of cell nuclei can be membranous, homogeneous, speckled and nucleolar depending on the reaction with the corresponding antigen. The pattern is schematically shown on Figure 2. Although all patterns of nuclear immunofluorescence were observed in silicotic patients, preferentially the type of homogeneous and speckled immunofluorescence was seen. As an example, in Figure 3 pattern of homogeneous immunofluorescence observed with ANA-containing serum from a silicotic patient is shown. As demonstrated by detection of ANA, the quality of quartz-containing dust has a great influence on the occurrence of immunological disturbances. The determination of all above described parameters is demonstrating almost the same effect: the alterations do not only occur when radiologically manifest silicosis exists, but are sometimes already found in miners without radiological fibrosis. These findings illustrate that quartz-containing dusts stimulate not only those functions of the macrophages which induce fibrosis,[16] but

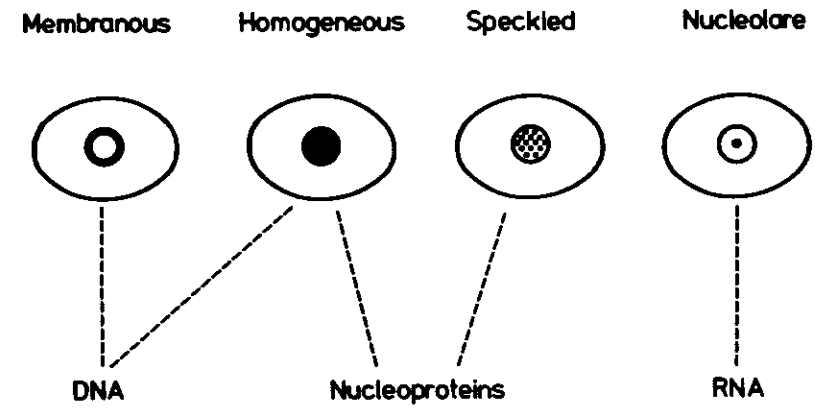

Figure 2. Pattern of immunofluorescence of cell nuclei and corresponding antigen in presence of anti-nuclear antibodies.

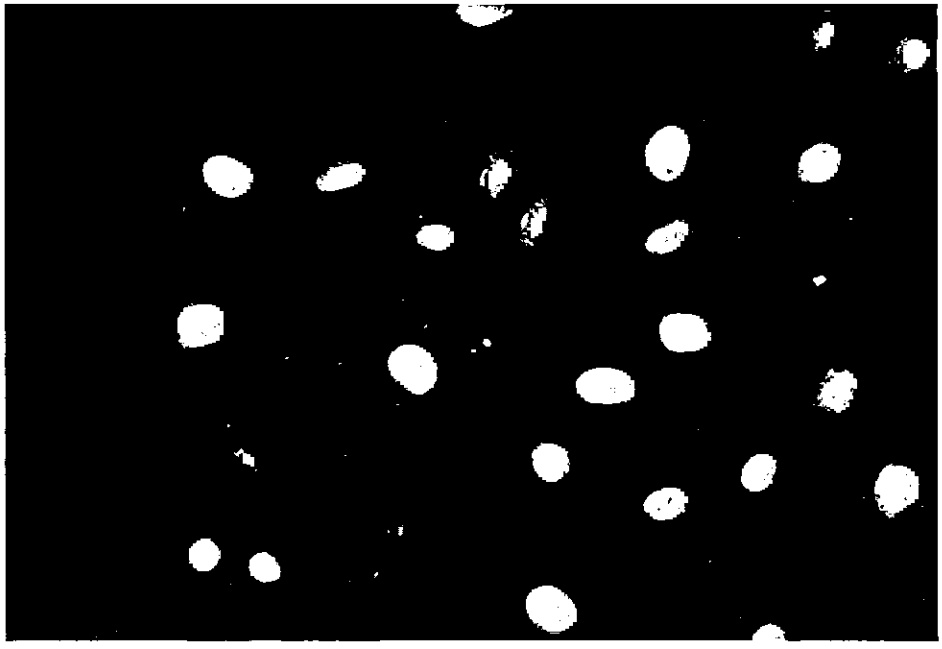

Figure 3. Pattern of homogeneous immunoflurescence of ANA.

at the same time the immunoregulatory functions and that disturbances in the latter functions are detectable before fibrosis or independently thereof.

Important recent findings demonstrate a cross-reactivity of anti-DNA antibodies with proteoglycans, cardiolipin and other phospholipids as well as with the intermediate filament vimentin.[3,9,15] These reports allow a new view on autoimmunity in silicotic patients.

SUMMARY AND CONCLUSIONS

1. In silicotic patients the following humoral disturbances and autoimmunity phenomena are found:

   a. Indicators for macrophage stimulation, such as lysozyme, angiotensin-converting-enzyme (ACE), and fibronectin are increased.

   b. Effects on humoral immunity are seen by an increase of complement component C_3 and in part by an increase of immunoglobulins IgG and IgA.

   Autoimmunity phenomena are shown by occurrence of antinuclear antibodies and of circulating immune complexes, containing C_3, IgG, IgA and IgM.

2. The above mentioned alterations do not only occur when manifest silicosis exists, but are also found in miners without radiologically fibrosis. These findings illustrate that quartz-containing dusts stimulate not only those functions of the macrophages which are involved in fibrosis, but also lead to immunoregulatory dysfunctions which are detectable before incidence or independently of a clinical manifest fibrosis.

3. As demonstrated by incidence of ANA, the quality of quartz-containing dust has a great influence on the manifestation of autoimmunity phenomena.

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