PATHOLOGY CLASSIFICATION AND GRADING SCHEMATA FOR SILICOSIS

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ABSTRACT

In 1985, the National Institute for Occupational Safety and Health established a committee to review the pathology of silicosis and the silicate-associated lung diseases, and to develop a classification and pathology grading system for epidemiological studies. The committee considered a number of different schemata developed in the USA and abroad. However, we were unsuccessful in devising a satisfactory approach which would permit pathology grading for correlative studies. An outline of the various proposed grading systems will be presented, and the shortcoming and problems in their utilization and broad application will be discussed.

No Paper provided.

MICROBIAL CONTAMINANTS OF STORED TIMBER AS POTENTIAL RESPIRATORY HAZARDS FOR SAWMILL WORKERS

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INTRODUCTION

Occupational exposure to wood dust may be a cause of respiratory diseases such as hypersensitivity pneumonitis (allergic alveolitis),[7,12,23,26] asthma[8] and chronic obstructive lung disease (COLD).[2] The etiology of these diseases is not fully known and both the allergenic and/or toxic constituents of wood tissue itself and the substances produced by microorganisms developing in wood have been suggested as potential agents.[8,10,22,27] Many species of allergenic and/or toxic molds developing on wood (*Alternaria tenuis, Aspergillus fumigatus, Cryptostroma corticale, Mucor spp., Paecilomyces spp., Penicillium spp., Rhizopus spp.)* have been described as causative agents of pulmonary diseases in woodworkers.[6,7,8,12,23,24,25,26] The role of bacterial factors in wood-associated diseases was studied to a lesser extent.[10,22] It has been reported that woodworkers may be exposed to notable amounts of gram-negative bacteria and endotoxin.[1,4,25]

The aim of this study was to extend the knowledge of the potential respiratory risk of woodworkers to wood-inhabiting microorganisms by quantitative and qualitative determination of the microflora of stored timber logs scheduled for processing in a sawmill.

MATERIAL AND METHODS

Two series of microbiological wood samples were taken in August and October of the year 1987 from timber logs stored on the lumber yard at a sawmill in Kingwood, West Virginia. The logs had been stored for a period of 4–6 weeks and did not show any apparent signs of decay. At each sampling time, samples were taken from a log of each of the following species: American basswood (*Tilia americana L.*), black cherry (Prunus serotina Ehrh.), black locust (*Robinia pseudoaccacia L.*), red oak (*Quercus coccinea Muenchh.*), soft maple (*Acer saccharinum L.*) and white poplar (Populus alba L.). From each log, one sample was taken from the heartwood (by boring from the transverse section), one from the sapwood (by boring from the transverse section) and one from the bark (by centripetal boring).

The wood samples were collected with a novel "drill and collect" device (model #2) for quantification of microorganisms in wood.[5] This is a manually operated drilling device in which a combined action of a twist boring bit and a spring-containing mobile ring collects the pulverized wood into a sterile flask attached beneath the bit in a one-step sterile process. The wood surface to be sampled was first sterilized by wiping with 70% propanol and "Clorox" (a commercial 5.25% sodium hypochlorite solution) and then an average sample was taken by multiple boring (5–7 times) in a circle up to 3 cm in diameter.

The concentrations of bacteria and fungi in the wood samples were determined by dilution plating. Aliquots of 200 mg of each sample were suspended in 20 ml of sterile phosphate buffered saline (Sigma Chemical Co., St. Louis, MO) containing 0.1% (v/v) Tween 80 (Fisher Scientific Co., Fair Lawn, NJ) and, after vigorous shaking, serial 10-fold dilutions were made up to 10^{-6}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following agar media: (i) sheep blood agar for total aerobic bacteria, (ii) eosin methylene blue agar (EMB agar; Difco Lab., Detroit, MI) for gram-negative bacteria, (iii) half-strength tryptic soya agar (Difco) for thermophilic actinomycetes, (iv) rose bengal streptomycin agar (RBS) for total fungi, and (v) yeast malt agar for yeasts. The blood agar and EMB plates were incubated for 48 hrs at 35°C, the tryptic soya plates for 120 hrs at 55°C, and the RBS and yeast malt plates for 96 hrs at 28°C.

Following incubation, bacterial colonies were counted and differentiated on the basis of colony morphology, Gram reaction, and biochemical reactions. The gram-positive isolates were identified according to Bergey's Manual.[21] The gram-negative isolates were identified with the API^{R} Systems 20 E (for enterobacteria) and NFT (for non-fermenting bacteria) (API Analytab Products, Plainview, NY), using supplementary biochemical tests selected according to Bergey's Manual[14] and API^{R} Systems recommendations. Mold colonies were counted and differentiated on the basis of morphological properties. Representative yeast colonies were isolated and differentiated on the basis of morphological and biochemical properties.[13] Final results for microbial concentrations were reported in terms of the colony forming units (cfu) in one gram of ground wood.

For endotoxin determination, 100 mg portions of the wood samples were extracted with 5 ml of sterile non-pyrogenic water (Travenol Laboratories Inc., Deerfield, IL) by rocking for 60 min. at room temperature. The suspension was centrifuged at 1000 g for 10 minutes to remove particulate debris, and the supernatant fluid was separated for further

analysis. Quantification of gram-negative bacterial endotoxin content was performed in duplicate by a quantitative chromogenic modification of the Limulus amebocyte lysate test (QCL-1000; Whittaker Bioproducts, Walkersville, MA). Results were reported in terms of Endotoxin Units (EU) in one gram of ground wood.

The Students' t-test for matched pairs, test for linear regression and chi-square test were used for statistical evaluation of the results.

RESULTS

The concentration of microorganisms and endotoxin varied significantly with the kind of wood examined ($P<0.001$). As shown in Figures 1–6, the highest levels of bacteria, fungi and endotoxin were found in the wood samples from logs of American basswood and black locust (10^3–10^8 cfu/gm, 10^2–10^7 cfu/gm and 10^4–10^6 EU/gm, respectively). The levels were lower in the logs of soft maple and black cherry (0–10^6 cfu/gm, 0–10^7 cfu/gm and 10^1–10^4 EU/gm, respectively) and lowest in the logs of white poplar and red oak (0–10^4 cfu/gm, 0–10^5 cfu/gm and 10^0–10^5 EU/gm, respectively).

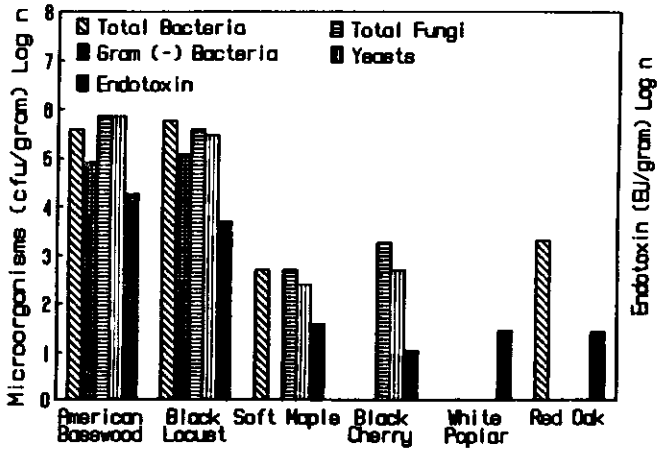

Figure 1. Concentrations of bacteria, fungi and endotoxin in the samples of heartwood collected in August, 1987.

High concentrations of bacteria, fungi and endotoxin were found in all the examined kinds of wood tissue: heartwood (Figures 1–2), sapwood (Figures 3–4) and bark (Figures 5–6). No significant differences were noted between the contamination rates in August and October ($P>0.05$).

In most of the samples of heartwood and sapwood, gram-negative bacteria dominated the total bacteria flora. Except for two cases (Figure 5), this was not observed in the bark samples. In bark samples taken in October, viable gram-negative bacteria were absent completely and the very high level of bacteria found in the bark of the black locust was due to the presence of large numbers of spore-forming bacilli (Figure 6). For each kind of wood tissue (heartwood, sapwood and bark) a significant correlation has been found between the concentrations of gram-negative bacteria and endotoxin ($P<0.05$). No thermophilic actinomycetes were found in the examined wood samples.

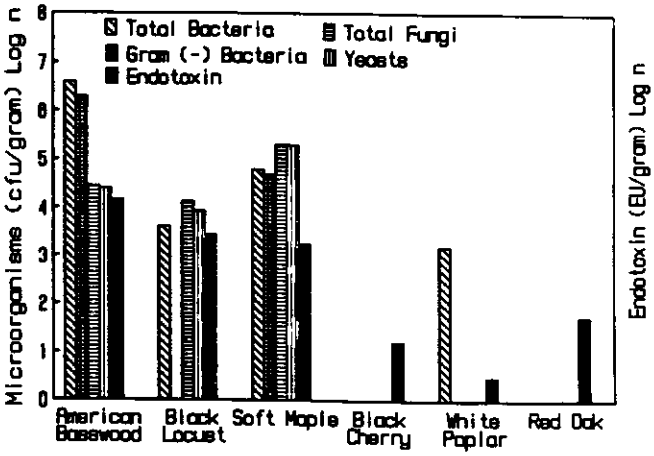

Figure 2. Concentrations of bacteria, fungi and endotoxin in the samples of heartwood collected in October, 1987.

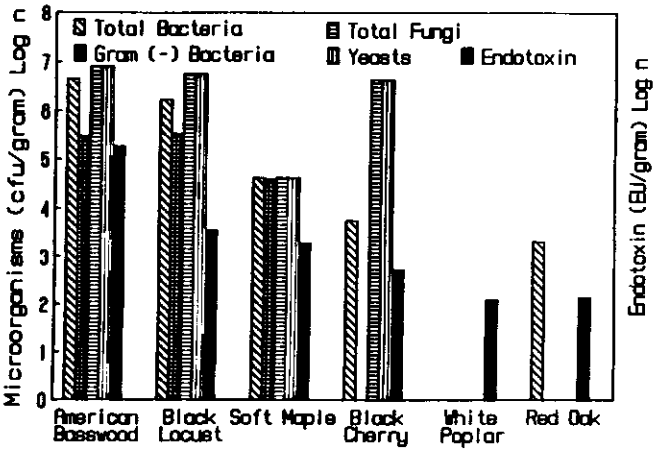

Figure 3. Concentrations of bacteria, fungi and endotoxin in the samples of sapwood collected in August, 1987.

Twelve species and/or genera of gram-negative bacteria and seven genera of gram-positive bacteria were found in the wood samples (Table I). The gram-negative flora comprised five fermentative species (belonging to the *Enterobacteriaceae* family) which, in most cases, were associated with the sapwood and seven non-fermentative species (mostly of the genus *Pseudomonas)* which were mostly associated with the heartwood. Among the gram-positive bacteria, the most common organisms were endospore-forming bacteria of the genus *Bacillus* and coryneform bacteria belonging to the genera *Arthrobacter, Brevibacterium, Corynebacterium* and *Microbacterium.*

In all kinds of wood samples examined, yeasts were the predominant fungi observed (Figures 1–6, Table II) and the

Table I

Species of Bacteria Occurring in Wood Samples

Name of the species	Heartwood	Sapwood	Bark	Maximal concentration (X 10^5 cfu/gram)
GRAM-NEGATIVE BACTERIA				
Fermentative				
Citrobacter freundii	+ (B, M)	++ (B), + (M)		0.10 (Sapwood, B)
Enterobacter agglomerans	++ (B), + (M)	+++ (B), + (M)	++ (B, L)	3.00 (Sapwood, B)
Enterobacter cloacae		++ (M)		0.38 (Sapwood, M)
Klebsiella sp.		+++ (B), + (M)		1.45 (Sapwood, B)
Serratia rubidaea	++ (L)	++ (L)		0.41 (Sapwood, L)
Non-fermentative				
Acinetobacter calcoaceticus	+ (M)		+++ (L)	30.50 (Bark, L)
Agrobacterium radiobacter	+++ (L)	+++ (L)		2.64 (Sapwood, L)
Pseudomonas fluorescens	+ (M)			0.07 (Heartwood, M)
Pseudomonas maltophilia	+ (M)			0.02 (Heartwood, M)
Pseudomonas oryzihabitans	+++ (B)			15.10 (Heartwood, B)
Pseudomonas putida	+++ (B), ++ (M)	+ (B)		3.84 (Heartwood, B)
Pseudomonas stutzeri	++ (B), + (M)	+ (M)		0.45 (Heartwood, B)
GRAM-POSITIVE BACTERIA				
Bacillus spp.	+++ (B), ++ (L), + (M)	+++ (L), + (B, M)	+++ (L, M), ++ (B), + (C, O, P)	154.00 (Bark, L)
Coryneform bacteria*	+++ (B, L), + (P)	+++ (B, L) + (C, M, O)	+++ (L), ++ (B, M), + (C, P)	20.30 (Heartwood, B)
Staphylococcus spp.	+ (M, O, P)	+++ (L), + (O)	+ (P)	5.00 (Sapwood, L)
Streptomyces spp.		+++ (L)	++ (L), + (B)	2.51 (Sapwood, L)

B = American Basswood M = Soft Maple + = occurred in concentration below 1 x 10^4 cfu/gram *Comprise: *Arthrobacter spp.*, *Brevibacterium spp.* *Corynebacterium spp.*.

C = Black Cherry O = Red Oak ++ = occurred in concentration 1 x10^4 - 1 x 10^5 cfu/gram

L = Black Locust P = White Poplar +++ = occurred in concentration over 10^5 cfu/gram

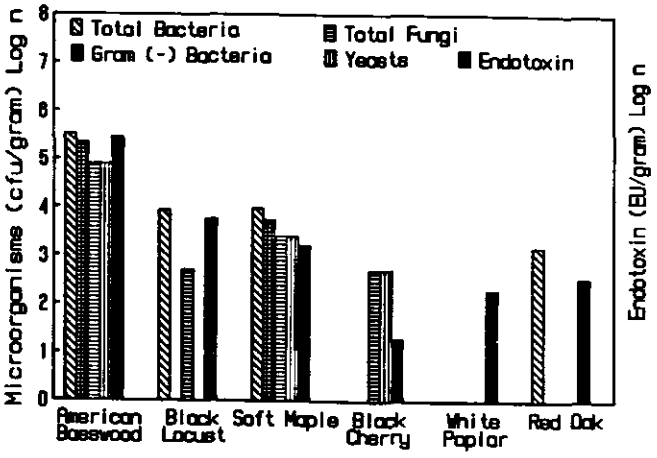

Figure 4. Concentrations of bacteria, fungi and endotoxin in the samples of sapwood collected in October, 1987.

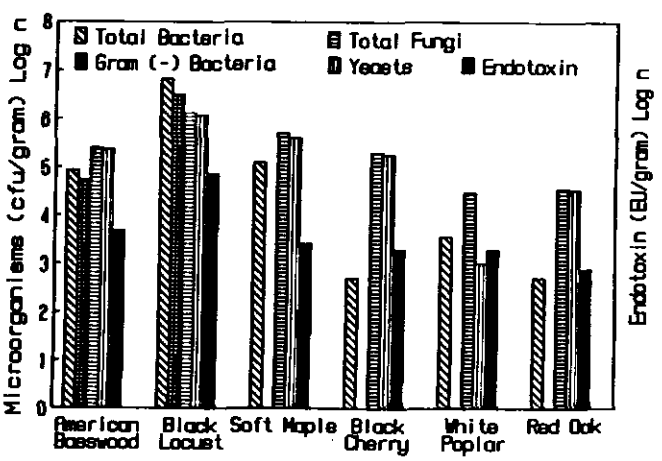

Figure 5. Concentrations of bacteria, fungi and endotoxin in the samples of bark collected in August, 1987.

Table II
Fungi Occurring in Wood Samples

Organism	Heartwood	Sapwood	Bark	Maximal concentration (X 10^5 cfu/gram)
DBB- yeasts[a]	+++ (B,L), ++ (M) + (C)	+++ (B,C,L), ++(M)	+++ (B,L,M), ++ (C,O), + (P)	78.35 (Sapwood, B)
DBB+ yeasts[b]	++ (B,L,M)	+++ (B), ++ (C,L)	++ (B,C,L,O)	1.45 (Sapwood, B)
Acremonium sp.	++ (L), + (B)	++ (L)	+++ (M), + (B,P)	14.00 (Bark, M)
Oidiodendron sp.			++ (C,M)	0.79 (Bark, M)
Penicillium sp.	++ (L)	++ (B,L)	++ (L,P), + (B,C,O)	0.72 (Bark, L)
Trichoderma sp.		++ (C)	++ (B,L)	0.49 (Bark, L)
Nonsporulating	+ (B,C,M)		++ (L,M), + (B,O,P)	0.26 (Bark, M)

[a]Negative reaction with Diazonium Blue B (DBB); presumptive Ascomycetes and their anamorphs (includes Candida zeylanoides, other undetermined Candida spp., and Hansenula silvicola.

[b]Positive reaction with DBB; presumptive Basidiomycetes and their anamorphs (includes undetermined Candida spp., Cryptococcus laurentii, and Rhodotorula glutinis).

B = American Basswood M = Soft Maple + = occurred in concentration below 1 x 10^4 cfu/gram

C = Black Cherry O = Red Oak ++ = occurred in concentration 1 x 10^4 - 1 x 10^5 cfu/gram

L = Black Locust P = White Poplar +++ = occurred in concentration over 10^5 cfu/gram

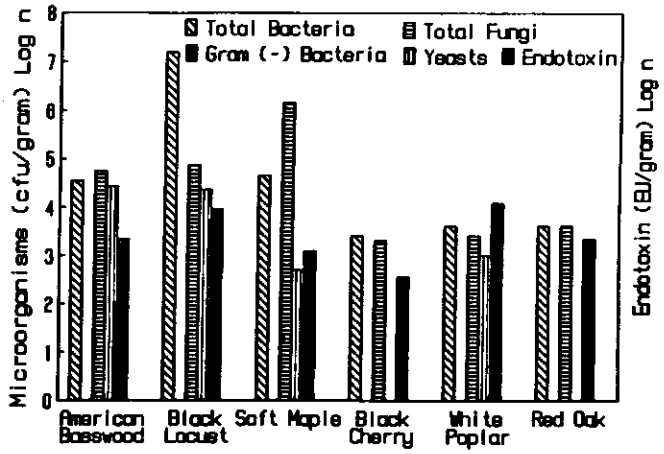

Figure 6. Concentrations of bacteria, fungi and endotoxin in the samples of bark collected in October, 1987.

most numerous among them were presumptive Ascomycetes and their anamorphs, i.e., they gave a negative reaction with Diazonium Blue B (DBB).[13] Yeast fungi tended to be found in the greatest numbers in the sapwood. Species of yeast isolated include undetermined *Candida spp.* (include both DBB+ and DBB− species), *Candida zeylanoides, Cryptococcus laurentii, Hansenula silvicola and Rhodotorula glutinis*.

Molds found in these samples included *Acremonium (Cephalosporium sp., Alternaria sp., Aspergillus fumigatus, Aureobasidium pullulans, Bispora sp., Cladosporium sp., Mortierella sp.,* and *Trichoderma sp.* as well a number of fungi that could not be identified because of their failure to sporulate. The molds that occurred in the greatest numbers were *Acremonium sp., Oidiodendron sp., Penicillium sp.,* and *Trichoderma sp.*. The highest numbers of molds were found in the bark.

DISCUSSION

The levels of microorganisms and endotoxin in timber logs showed notable variation depending on the species of the tree. The concentrations of bacteria and fungi in the most contaminated wood species (basswood, locust) exceeded the level of 10^6 cfu/gm, and were comparable to the values reported for certain organic dusts related to harmful respiratory effects in workers.[3]

The concentration of endotoxin in the wood reached, in many cases, a level of 10^5–10^6 EU/gm which corresponds to the values found in organic materials (grain, silage, mushroom farm pre-flush) associated with the cases of respiratory disorders in exposed workers.[17] This finding is in agreement with the fact that some of the wood samples contained high concentrations of gram-negative bacteria. Among these bacteria were the species *(Enterobacter agglomerans, Klebsiella spp., Pseudomonas putida)* which are known producers of biologically active endotoxin that can cause pulmonary

injury through non-specific stimulation of alveolar macrophages.[19]

The occurrence of high concentrations of fungi in the wood presents another factor of potential respiratory risk for sawmill workers. The *Penicillium* species that were frequently isolated in this study have been reported as a source of the pathogenic respiratory allergens.[6,24] Another potentially pathogenic species are *Aspergillus fumigatus* and *Aureobasidium pullulans*.[8,10,22]

The data conform to some earlier reports on the occurrence of bacteria and fungi in the wood[9,15,16,18] The composition of the microflora of examined logs, characterized by the prevalence of yeasts and gram-negative bacteria indicates that it was in the stage of "pioneer colonization" which precedes the stage of wood decay by brown rot and white rot fungi.[11,20]

The main conclusion from this preliminary study is that some kinds of apparently not decayed timber stored for processing in sawmill contain very high concentrations of "pioneer" microorganisms and their toxins. These organisms may cause respiratory disorders in the woodworkers if inhaled with the sawdust. Although not defined by the current study, the potential problems associated with microbiologically contaminated woods are intriguing and require further research.

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Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

MICROBE EXPOSURE AND THE OCCURRENCE OF ANTIBODIES AGAINST THE EXPOSING MICROBES AMONG WOOD WORKERS IN CELLULOSE INDUSTRY

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ABSTRACT

Exposure to airborne fungal spores and bacteria, and occurrence of antibodies against the most common fungal[14] and bacterial[3] species in sera of the 11 workers were studied in a cellulose factory. Air samples for microbiological analysis were taken by a six-stage Andersen impactor in barking department and on wood chip piles out-of-doors. Barking workers were exposed mainly to bacteria (geometric mean of bacterial concentration 46.3×10^3 cfu/m^3) and to lesser extent to fungal spores (5.9×10^3 cfu/m^3) in contrast to catepillar drivers on wood chip piles (1.5×10^3 cfu/m^3 and 45.5×10^3 cfu/m^3 respectively). Rhodotorula glutinis was the dominating fungal species in the barking department and Aspergillus fumigatus and Penicillium brevicompactum on wood chip piles.

Enzyme-linked immunosorbent assay (ELISA) found differences in IgG-antibody levels between different microbial species as also between different work environments. Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces salmonicolor and Aspergillus niger while lowest levels were found against Rhizopus nigricans, Humicola grisea and Streptomyces albus. Generally, the levels of antibodies against fungal species were 2–5 times higher in the wood chip workers than those in the barking workers. Although the amount of the bacteria in the barking department was about 30 times higher than that on the wood chip piles, no differences in the levels of bacterial antibodies were found between the two groups.

Probably the dry microbial material such as that in wood chip work penetrates into the lower parts of the respiratory tracts and initiates the formation of antibodies more easily than the moist aerosols.

INTRODUCTION

In pulp production wood used as raw material is cutted after barking into chips and stored outdoors in huge piles, where chips are transferred by caterpillars. Chips are stored approximately a couple of months before taking in to the production. During the storage microbiological changes occur in the piles (Bergman and Nilsson 1979, Pellikka and Kotimaa 1983). Some cases of allergic alveolitis have been described among wood workers after exposure to fungal spores (van Assendelft et al. 1985, Lundgren and Rosenhall 1979, Jorgensen and Fjellheim 1982). Wood is barked in big barking drums, where water is used in the process. Water is circulated and becomes contaminated by bacteria and fungi. Process is mostly open and water becomes easily aerosolized. Microbial aerosols from humidifiers may cause so-called humidifier fever (Rylander et al. 1978, Marinkovich and Norey 1983). Because of respiratory symptoms among wood workers in a cellulose factory in central Finland, microbe exposure and the antibodies against 17 most common exposing microbes were investigated.

MATERIALS AND METHODS

Subjects and Serum Samples

Serum samples from six catepillar drivers on wood chip piles and four workers in the barking department were taken within two weeks after air sampling. All the examined workers had work-related symptoms suggesting allergic background with microbial etiology (Table I).

Air Sampling for Estimating the Microbe Exposure

Air samples for microbiological analysis were collected by a six-stage fractionating impactor (model 10-800, Andersen Inc., Georgia, USA) (Andersen 1958). Three sets of media were used in successive samplings on each sampling site: Hagen-medium (incubated at 20°C) was used for mesophilic fungi, the same medium incubated at 40°C was used for thermotolerant fungi and plate count agar was used for total count of mesophilic bacteria. 10 air samples for each microbe group were collected both on wood chip piles and in the barking

Table I

Workers' Age, Type of Work, the Duration of Exposure, Symptoms, and the Clinical Findings

ID.CODE	AGE (YRS)	WORK	DUR.OF EXPOSURE (YRS)	SYMPTOMS R E C F M D	CL. FINDINGS PEF	ESR
VA 1	55	CD	25	- - - F M -	NORMAL	12
SA 2	38	CD	8	R - C - - -	NORMAL	2
LA 3	56	CD	14	- - C F M -	NORMAL	12
LI 4	39	CD	14	- - C F - -	NORMAL	5
KU 5	46	CD	26	R - - F - -	LOWERED	17
TO 6	36	WO/S	7/4	R - - - - D	NORMAL	4
KA 7	47	WO/B	4/3	R E - F M -	NORMAL	7
KA 8	37	B	17	R - C - - -	NORMAL	2
MA 9	37	B	15	- - C - - -	NORMAL	2
HA 10	47	B	17	- - C - - -	NORMAL	5

CD = CATERPILLAR DRIVER
WO = WOOD WORKER OUTDOORS
S = SLASHER
B = BARKING WORKER

R = RHINITIS
E = EYE IRRITATION
C = COUGH
F = FEVER
M = MUSCLE PAIN
D = DYSPNEA

ESR = ERYTHROCYTE SEDIMENTATION RATE

department. After incubation the number of colonies was counted, and the positive hole correction method was performed to calculate the concentrations of viable airborne microbes.

Antigens

For antigen preparation 14 fungal end 3 bacterial strains were subcultured from original cultivation plates in nutrient broth containing 5 g/l peptone (Difco Laboratories, Detroit, Mich., USA) and 3 g/l beef extract (Difco) at optimal temperature for each species. Bacterial growth was harvested by centrifugation and fungal growth by filtering, and washed three times by distilled water. Microbial pellets were disrupted mechanically (Ultra Turrax, Janke and Kunkel, Staufen i Breisgan, FRG) and then by ultrasonic disintegrator (Soniprep 150, MSE, Crawley, U.K.). The supernatants after a centrifugation at 40,000 g for 30 min were used as ELISA antigens.

Antibody Determination

IgG-antibodies were determinated by enzyme-linked immunosorbent assay (ELISA) carried out on disposable polystyrene microtiter plates (Immumoplate I, Nunc, Denmark). Microbial sonicates were used as antigen and alkaline phosphatase-labelled swine anti-human IgG (Orion Diagnostica, Espoo, Finland) was used as conjugate. Antibody levels were given as ELISA absorbance at a serum dilution of 1:100, read at 405 nm by a Titertek Multiskan (Eflab, Helsinki, Finland).

RESULTS

Microbe Exposure

Marked qualitative differences were found in the microbial exposure of caterpillar drivers and barking workers. Barking workers were exposed mainly to bacteria (geometric mean of bacterial concentration 46.3×10^3 cfu/m^3) and to lesser extent to fungal spores (5.9×10^3 cfu/m^3) in contrast to caterpillar drivers on wood chip piles and 1.5×10^3 cfu/m^3 and 4.5×10^3 cfu/m^3 respectively) (Table II). Rhodotorula glutinis was the dominating fungal species in the barking department, and Aspergillus fumigatus and Penicillium brevicompactum on wood chip piles (Table III).

Antibodies

Differences in antibody levels between different microbial species as also between different work environments were found by ELISA (Table IV). Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces

Table II

Total Concentrations of Airborne Bacteria and Fungi (cfu/m^3) in the Barking Department and on Wood Chip Piles Outdoors ($\bar{x}$ = geometric mean)

	BARKING DEPARTMENT (n=10)		ON WOOD CHIP PILES (n=10)	
MICROBE GROUP	$\bar{x}$	RANGE	$\bar{x}$	RANGE
BACTERIA	46000	9200-230000	1500	770-35000
FUNGI	5900	1400-70000	45000	1000-200000

Table III

Concentrations of Airborne Microbes (cfu/m^3) in the Barking Department and on the Wood Chip Piles Outdoors ($\bar{x}$ = geometric mean)

	BARKING DEPARTMENT (n=10)		ON WOOD CHIP PILES (n=10)	
	$\bar{x}$	RANGE	$\bar{x}$	RANGE
Aspergillus fumigatus	28	0-740	40000	880-200000
Aspergillus niger	2	0-62	9	0-190
Humicola grisea	0	-	9	0-48
Paecilomyces variotii	2	0-41	6	0-110
Penicillium brevicompactum	500	41-3400	6000	12-88000
Rhizopus nigricans	2	0-33	3	0-71
Streptomyces albus	2	0-80	7	0-170
Trichoderma viride	110	17-490	5	0-24
Aureobasidium pullulans	5	0-44	2	0-24
Cephalosporium curtipes	0	-	0	-
Cladosporium cladosporioides	52	0-180	9	0-570
Geotrichum candidum	2	0-21	7	0-150
Phialophora bubakii	0	-	0	-
Rhodotorula glutinis	3900	510-65000	54	0-560
Sporobolomyces salmonicolor	1	0-10	8	0-150
Bacterium 1	930	180-4700	5	0-120
Bacterium 2	33000	8300-210000	9	0-800

Table IV

Antibody Levels ($\bar{x}$ + S.E.) Against the Microbes Found in the Working Environment in Barking Workers and in Wood Chip Workers

MICROBE	BARKING WOS (N=4) $\bar{X}$ + S.E.		WOOD CHIP WOS (N=6) $\bar{X}$ + S.E.	
ASP. FUMIGATUS	0.248	0.057	1.080	0.210
ASP. NIGER	0.477	0.120	1.362	0.242
HUM. GRISEA	0.074	0.011	0.136	0.015
PAEC. VARIOTII	0.408	0.082	1.528	0.103
PENIC. BREVICOMPACTUM	0.157	0.070	1.145	0.201
RHIZ. NIGRICANS	0.115	0.039	0.128	0.033
STR. ALBUS	0.087	0.014	0.172	0.029
TRICH. VIRIDE	0.443	0.107	0.810	0.194
AUR. PULLULANS	0.297	0.074	0.594	0.065
CEPH. CURTIPES	0.683	0.162	1.085	0.094
CLAD. CLADOSPORIOIDES	0.262	0.092	0.853	0.137
GEOTR. CANDIDUM	0.302	0.066	0.469	0.095
PHIL. BUBAKII	0.107	0.029	0.420	0.112
RHODOT. GLUTINIS	0.408	0.040	0.967	0.128
SPOROB. SALMONICOLOR	0.392	0.059	1.377	0.230
BACTERIUM 1	0.425	0.075	0.498	0.066
BACTERIUM 2	0.810	0.254	0.812	0.122

salmonicolor and Aspergillus niger while lowest were found against Rhizopus nigricans, Humicola grisea and Streptomyces albus. Generally, the levels of antibodies against fungal species were 2–5 times higher in the wood chip workers than those in the barking workers. No differences in the levels of bacterial antibodies were found between the two groups.

DISCUSSION

In the cellulose factory, the concentrations of airborne microbes except for bacteria were significantly higher on dusty wood chip piles than in the barking department, and correspondingly, the levels of antibodies against fungal species were higher in the wood chip workers than in the barking workers. Highest antibody levels were found against Paecilomyces variotii, Sporobolomyces salmonicolor, and Aspergillus niger and lowest antibody levels were found against Streptomyces albus, Humicola grisea and Rhizopus nigricans reflecting differences in capability of the species to stimulate a formation of antibodies. Although the amount of the bacteria in the barking department was about 30 times higher because of aerosolized processing water than that on the wood chip piles, no differences in the levels of antibodies against bacteria were found between the caterpillar drivers and the barking workers. Probably the dry microbial material such as that in wood chip penetrates into the lower parts of the respiratory tracts and initiates the formation of antibodies more easily than the moist aerosols.

Fever and muscle pain as work-related symptoms in wood chip workers suggest the diagnosis of allergic alveolitis, which is supported also by high antibody levels in this group (Terho 1982), whereas rhinitis and cough found mostly in barking workers with low antibody levels seem not to be IgG-mediated reactions.

The comparison of antibody findings in the cellulose factory with those in office workers gave a surprising result. The barking workers' antibody levels were not at all higher than those in bank clerks with minimal exposure to airborne microbes (unpubl. data). The dry microbial material occurring in wood chip work and in office work penetrates probably easily into the alveoli and initiates the formation of antibodies more effectively than the moist aerosols irrespective of the amount of antigen.

These results suggest that in addition to microbial concentration the physical nature of aerosols should be considered for evaluating the health risks caused by airborne microbes. On the other hand, the immunization against occupationally exposing microbes could be diminished by controlled air-humidifying to prevent allergic respiratory diseases.

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ETIOLOGICAL INVESTIGATION OF FARMER'S LUNG —SEROLOGICAL STUDY

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SUMMARY

The reactions of precipitins in serum against the antigens from two strains of T. vulgaris were shown in 46.7 and 66.7% in 30 patients with farmer's lung, significantly higher than those in the control groups, while the reactions against M. faeni and A. fumigatus were low in the patients' group and not significantly higher than those in two control groups. The results indicated that the main etiological agents of farmer's lung were some strains of T. vulgaris in the patients.

INTRODUCTION

From 1980 through 1981, an epidemiological survey was conducted among 1054 hay grinders in Dafeng County, Jiangsu Province. 120 of them had history of farmer's lung disease. During follow-up study of these 120 grinders, acute episodes of farmer's lung after exposure to mouldy hay dust were seen in 67 of them.[1] Meanwhile, a microbiological study of sputum of these patients and mouldy hay samples from their workplaces was performed. 80 strains of thermophilic actinomycetes were isolated from these samples, 61.2% of them being T. vulgaris.[2] In order to confirm whether T. vulgaris was the main etiological agent of farmer's lung in that county, we studied the precipitins in serum from the patients using serological method.

MATERIALS AND METHODS

Antigens

Six strains of Thermoactinomycetes, including 4 strains of T. vulgaris called 801, 806, 816, 832 and 2 strains of Thermophilic nocadia called 835 and 836, which were isolated from mouldy hay collected from the workplaces of the patients with farmer's lung, were selected and then, antigens were prepared by using a modified Salvaggio's method.[3] Meanwhile, the strains of M. faeni 1., T. vulgaris 2. and T. candidus, one of each, provided by Dr. V. P. Kurup (Medical College of Wisconsin) were also selected to prepare antigens. The antigens were diluted to 30 or 40 mg per ml of normal saline, when they were used. In addition, other antigens including those form M. faeni 2., T. vulgaris 1. provided by Dr. J. H. Edwards (MRC Pneumoconiosis Unit) and A. fumigatus provided by Dr. J. Marx, JR (Marshfield Medical Foundation) were also used for detecting precipitins in serological test. Besides, the extracts from mouldy hay was prepared by using a modified Williams' method and diluted to 12 mg per ml of normal saline, also employed in the serological test.

Serum Samples

Serum samples from 30 of these 67 patients were collected just one month after they ground mouldy hay. 30 serum samples from healthy people with no history of exposure to mouldy hay in the same area matched with the patients in sex and age were selected as control group A. Another 29 serum samples were collected from the healthy students in Shanghai Medical University as the control group B.

Serological Test

The presence of precipitins against the antigens was tested by using modified Ouchterlony's agar-gel double-diffusion assay.[5]

RESULTS

It was shown that the reactions against two strains of T. vulgaris 1. and 2. were 46.7 and 66.7% in the patients' group, significantly higher than those in the control groups, whereas the reaction against T. candidus was 80% higher than that in group B, and it had not much difference with the control group A. Besides, the reactions against M. faeni 1. and 2. and A. fumigatus were rather low (16.7, 3.3 and 9.1%) in the patients' group and not significantly higher than those in the two control groups.

The reactions against six strains of thermophilic actinomycetes named T. vulgaris 801, 806, 816 and 832 and Thermophilic nocardia 835 and 836 ranged from 13.3 to 80.0% in the patients' group. The reactions against T. vulgaris 816 was 36.7% in the patients' group, significantly higher than that in the two control groups, and those against T. vulgaris 806 and 832 were 80.0 and 33.3% in the patients' group, significantly higher than those in the group B, but not in the group A. Besides, reactions against T. vulgaris 801, Thermophilic nocardia 835 and 836 and the extracts of mouldy hay in the patients' group were not significantly higher than those in the control group.

DISCUSSION

The precipitin test against farmer's lung antigens has been widely used in clinical diagnosis and epidemiological survey of the farmer's lung disease. The positive reactions against these antigens always indicate that the people have the history of exposure to them. Based on these reactions, the etiological agents of farmer's lung could be determined.[6] In our study, the precipitins against a variety of farmer's lung antigens in sera from the patients with farmer's lung in Dafeng County, Jiangsu Province were tested and it was found that the percentages of positive reaction against three strains of T. vulgaris 1., 2. and 816 were 46.7, 66.7 and 36.7% in the patients with farmer's lung, respectively, which were significantly higher than those in the two control groups. The results might indicate that the main causative agents were some strains of T. vulgaris. The microbiological study of the mouldy hay from that county and sputum from the patients had also indicated that T. vulgaris was the dominant thermophilic actinomyces in the samples, while M. faeni was not found in them[2] So, the findings of our serological study and the microbiological study were consistent with each other.

Pepys had reported that the percentage of positive reaction against M. faeni in the patients with farmer's lung was as high as 85% in Britain.[7] So, the main causative agent of the disease was M. faeni in Britain. But in Finland, Terho found that the main etiological antigen of farmer's lung was from T. vulgaris.[8] Perhaps the difference might be referred to the different way of preparing and storing hay, perhaps also climatic differences and differences in crop types. In Dafeng County as well as other area in east part of China, hay before stocking would be sun-dried as much as it could be and then stocked outdoors. In this instance, the weather is rather humid and warm in these regions, but the time is not long enough for M. Faeni to grow in the stacks, which might be the reason why the percentage of positive serological reaction against M. faeni was very low in the patients in that county.

It was reported that the reactions against different strains of T. vulgaris in the same group of patients with farmer's lung might be significantly different from each other, and similar results could be found from different strains of T. candidus.[8,9,10] In our study, it was also found that the reactions against six strains of T. vulgaris in the patients with farmer's lung ranged widely from 13.3 to 80.0%. These findings may indicate that different strains of T. vulgaris could have different antigens. Therefore, a variety of strains of T. vulgaris should be used to test precipitins in serum from the patients with farmer's lung.

In addition, the reactions against the extracts from Thermophilic nocardia and mouldy hay in the patients was found not significant in this study.

In conclusion, it may be said that the etiological agents of farmer's lung in Dafeng County were mainly from some strains of T. vulgaris, but not M. faeni, and different strains of T. vulgaris should be applied to detect precipitins in serum diagnosis of farmer's lung.

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IN SITU QUANTITATION OF NON-FIBROUS INORGANIC PARTICLE BURDEN IN LUNG TISSUE USING SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY ANALYSIS

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ABSTRACT

To investigate the interaction of inhaled inorganic particulates with the lung one needs quantitative information on the particulate burden of lung tissue. The vast majority of tissue samples (biopsies and autopsies) are fixed with formaldehyde and embedded in paraffin wax. Over the past 16 years, I have attempted to obtain maximal analytical use of such tissue. Standard paraffin sections of tissue are analyzed in the scanning electron microscope (SEM) using secondary electron and backscatter electron imaging (BEI). Inorganic material is detected in the BEI and is analyzed using Energy Dispersive X-ray Analysis (EDXA). Quantitation of the tissue particulate burden is readily accomplished using a point-counting, morphometric approach.

I have collected quantitative data from analyses of particulates in over 400 lung samples. The data includes numerous comparative digestion analyses. The results from this *in situ* method correlate well with other analytic methods, and with comparison of results from other laboratories using similar or other techniques—with the exception of submicrometer metal particles which are better represented in the *in situ* analyses, as they may be lost during digestion and filtration. Currently, of over 30,000 particles in the data base, major non-fibrous particles are comprised of 13.8% silica, 47.7% silicates and 38.6% metals. These data, plus data on medical and occupational histories, smoking, age, sex, pathologic diagnoses, etc. are being queried regarding normal and diseased lungs.

No Paper provided.

PULMONARY FIBROSIS ASSOCIATED WITH SMOKING IN MEN RESIDING IN A CLEAN-AIR ENVIRONMENT

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ABSTRACT

The role of cigarette smoking in the pathogenesis of pulmonary fibrosis has not been defined. This question is important to pathologists concerned with pneumoconiosis since many inhalant-related lesions result in fibrosis in the smaller airways. Using Gough-Wentworth slices and microscope tissue sections, we analyzed lungs of Vermont males over a range of ages who died suddenly and unexpectedly and were autopsied. Gough sections were analyzed by the method of Thurlbeck and by planimetry. Microscopical tissue was evaluated by the method of Hogg, et al. (Med. J. Aust. 142:605, 1985). Postmortem interviews with next-of-kin were conducted by trained nurse epidemiologists to determine cigarette use and possible occupational exposures.

Overall, enphysematous changes were not striking, but there was a gradual increase in scores with advancing age in both smokers and nonsmokers using both techniques of analysis. In microscopic sections, inflammation reflected by cellular infiltration in the walls of bronchioles and the presence of intraluminal macrophages was most prominent in younger smokers, whereas fibrosis of the walls of the bronchioles increased with age among smokers. Inflammation and the lung fibrosis indices in smokers and nonsmokers differed significantly. This study provided an opportunity to evaluate pulmonary changes associated with smoking among men living in a clean air environment, and not employed in dusty trades. In addition, it excludes possible terminal effects on the tissue morphology. A significant association of smoking with fibrosis of the membranous and respiratory bronchioles was found. The data suggest that respiratory bronchiolitis may be a contributory pathogenetic factor.

No Paper provided.

ACCUMULATION AND COMPOSITION OF INHALED PARTICULATES IN HUMAN LUNGS

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SUMMARY

The black particulate matters deposited and accumulated in the autopsied human lung of deceased residents of the Tokyo Metropolitan area with no history of lung disease were separated, and their composing elements and substances were identified using several analytical techniques. The origin of lung contamination was examined. The carbon free radicals detected from human lungs were an original finding. Alpha-quartz was identified, carbon element, minerals and trace elements were determined, asbestos fibers were found and a result of the mutagenicity test on deposits was positive. The results observed in most cases were almost identical to the composition of an urban atmosphere. As for the exogenous factors related to the formation of pulmonary lesions, the effect of tobacco smoking cannot be ignored. In our pathohistological study, the observations of pulmonary lesions were found to be related to smoking. In view of the high concentration of element observed therein, it is considered that multiple factors participate in the development of exogenous pulmonary disease due to substances in the environment. These findings may be important in elucidating factors involved in the development of the lung disease due to particle deposition.

INTRODUCTION

The pulmonary anthracosis which has generally been assumed to have little pathological significance has been used as a simple indicator for estimating exogenous lung contamination. In studing the effects of suspended particulate matters in the atmospheric environment, it should be noted that the amount of black dusts deposited in a life time in human lungs depend upon various factors such as age, place of residence, smoking habits, and occupation. Published research reports pertain to anthracosis in the lung of such animals as dog, monkey, pigeon and autopsied human tissues.[1,2,3] These studies were conducted from the pathological standpoint. However, there has been a need to chemically analyze the composition of black deposits only, because it has been thought that black deposits are mainly composed of inhaled suspended particulate matters in the atmosphere. In a report published[4] in which Ohta was a co-author, multiple elements analysis was first conducted on anthracosis using spark source mass spectrometry. The ongoing studies[5,6,7] have concentrated on the establishment of a relatively large base of data obtained by analyzing particulate matters isolated from autopsied lungs not only for element but also for accumulated toxic materials using several analytical techniques.

MATERIALS AND METHODS

Lung Specimens and Pathohistological Findings

The autopsied lung samples used for this study were taken exclusively from people living in the Tokyo Metropolitan area with no history of lung disease. The age of 108 cases ranged from the second decade to the ninth decade. A defined site of the left upper autopsied lung lobes of these cases was employed, and pathohistological observations were examined.

Separation of Black Deposits from the Lung Tissue

The lung tissue was dissolved in alkaline solution. First, a test was made to determine whether 0.5N NaOH or 0.5N KOH would be satisfactory. Results by these solutions were not so different in regard to dissolve the lung tissue. After weighing the lung tissue which was kept in room temperature after removal from storage at −80°C, they were cut into small pieces and placed in polyethylene bottles with demineralized water to eliminate blood. After repeating this procedure for a few times using high speed centrifugation, 0.5N NaOH which was used for many samples were poured into the bottles. By repeated ultrahigh speed centrifugations at 12,000 rpm and 30,000 rpm, the solid residue was retained. The final residue was then washed using water, ethanol, acetone and finally dried. These black powders were used as samples for analysis.

Deposition Rate and Observation of Particulates Using Scanning Electron Microanalyzer (SEM-EDAX)

Elementary Analysis

1. Determination of elementary content using SEM-EDAX for obtaining general survey of the particulate components
2. Quantitative analysis by neutron activation

The analysis of Mn, V, Al and Ti in 92 samples was completed, and other selected 13 samples were analyzed into trace elements. The samples were irradiated for 30 sec. for short halflife nuclides and for 5 hours for long half life nuclides at 1.5×10^{12} n/cm^2 • sec.

3. Determination of carbon content using CHO Elemental Analyzer

   In the analysis of total carbon content, CHO Elemental Analyzer was used. A sample measured precisely to 0.3 mg or 1.2 mg was placed in a sample container. Elemental carbon content was measured by combustion at 300°C for 30 min. The volatilized carbon was calculated by subtracting the weight of residue carbon from the total carbon.

Detection of Free Radicals in the Black Particulate Deposited in Lungs Using Electron Spin Resonance (ESR)

Soot, tobacco, other kinds of smoke and products of combustion are serious sources of harmful particulates. Samples of black deposits from the lung which were removed with tweezers without any chemical treatment were lyophilized and approximately 20 mg of each sample was subjected to ESR analysis at room temperature. Solid DPPH was used as standard for the g factor and its benezene solutions was used for the estimation of radical concentration.

Mutagenicity Test for Black Deposits in the Lung

A mutagenicity test for black deposits isolated directly from the lung tissue was examined by the Ames Test. The strain used for this test was Salmonella typhimurium TA98 and TA100.

Identification of Crystallized Materials in Deposited Dust

The crystallized material in the black dust which was treated with alkaline solution was identified using X-ray diffraction for 50 cases.

Detection of Asbestos

The asbestos fibers were detected and identified using a transmission electron microscope (TEM) coupled with X-ray microanalyzer for selected samples.

RESULTS AND DISCUSSION

Pathohistological Findings

Some of 108 cases were detected to have pathohistological findings. The main observed findings were chronic bronchiolitis, emphysema and pavement epithelium metaplasia. These cases were found in relating to smoking considerable amount of cigarettes. This is especially true of 9 cases found in this study.

Deposition Rate and Observation of Deposited Particulates

The deposition rate of inhaled dust was positively correlated with age. Correlation factor (r) was 0.65 ($n=95$, $p<0.001$). The particle size and shape of collected dust particles from human lungs were observed that an individual particulate was approximately 0.1 micron in diameter, and many particles had aggregated into clumps.

Elementary Composition of Deposited Particulates and their Accumulation in the Lung

Usually Mg, Al, Si, P, S, K, Ca, Ti and Fe were detected in almost all samples, while Cl and Zn were detected in many samples, those contents were represented in weight percent (wt%). The concentration of Hg, Cr, Fe, Zn, Co, Ag, Sb, Cd and As in 13 specimen's samples were determined by neutron activation analysis. In case of chromium worker, Cr concentration was very high because of exposure to hexavalent chromium. V and Mn in the particulate are considered to originate from artificial sources, such as fuel or combustion, Al and Ti are assumed coming from soil or sand in the natural environmental sources. The concentration of these elements were determined. We attempted to correlate the concentration of element to age. The correlation factor (r) of Al was $r=0.48$ ($n=92$, ***), that of V, $r=0.40$ ($n=91$, ***), that of Si, $r=0.46$ ($n=95$, ***), that of Fe, $r=0.34$ ($n=95$, ***). These elements showed a positive correlation to age, that is, they were accumulated in the lung according to increase in age. However, Mn and Ti were not correlated to age. And furthermore, Ca concentration showed a negative correlation to age ($r=-0.56$, $n=72$,***). The average total carbon content was 55 wt% ($n=77$). The data comparing the total carbon content between smokers and nonsmokers were not discriminating. The average content of elemental carbon was 39 wt% ($n=39$). The volatilized carbon was considered to be organic carbon.

Determination of Free Radicals in Black Deposits

Carbon-centered free radicals were detected in all 21 specimens. Figure 1-a shows an ESR spectrum from specimen A who was 81 year-old woman, where a narrow singlet is seen with a width of 2.7G and a factor of 2.0025. This spectral component is designated R_1. This is more evident in the spectrum obtained with a wider field sweep in Figure 1-b. Such a broad signal apparently arises from inorganic magnetic species in the black deposits. Carbon-centered free radicals are not among those substances commonly expected to be contained in the air dust. Thermolysis or combustion of hydrocarbons is essentially a free radical process accompanying bond cleavage. As a simple comparison, tar and ash from Japanese cigarettes were collected and measured. The observed spectra were the same as that shown in Figure 1. The intensity of R_1 component in each specimen measured by the height of the derivative peaks was obtained.

Mutagenicity Test for Black Deposits

A mutagenicity test which is called the Ames Test was used to determine the black deposits, which were isolated directly from lung tissues, and a small amount of them was used in this test. They were set in the center of an agar plate, the so-called spot test. The strains used for mutagenesis testing were salmonella typhimurium TA98 and TA100, S-9(−) and S-9(+). some inhibition was observed, however, His^+ revertants were not more than the numbers of spontaneous revertants both in S-9(−) and in S-9(+). The result was that one sample was positive in both of TA98 and TA100 to

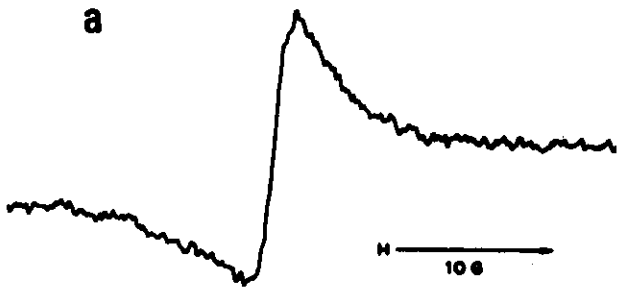

Figure 1-a. ESR spectrum of carbon free radical signal of specimen A who lived in the center of Tokyo for 60 years. Age: 81, Tobacco (−). The signal consists primary of R_1 type radical case. Gain = 4×10^4. Modulation width = 1G at g = 2.00.

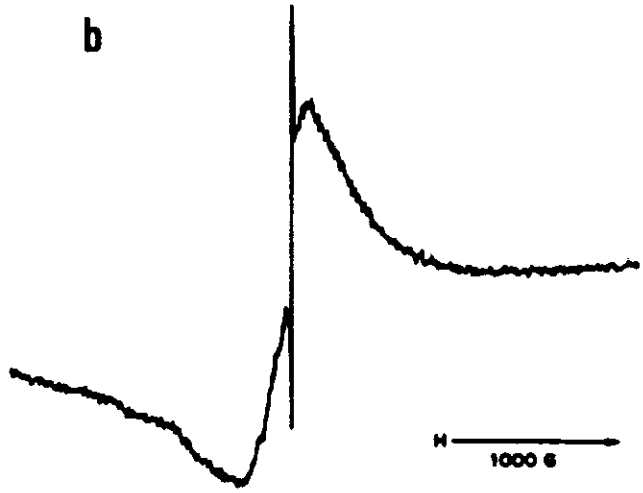

Figure 1-b. The whole ESR spectrum of the same specimen taken with a wide magnetic field sweep. Besides R_1 radical (the sharp signal in the center), a very broad absorption due to inorganic magnetic species is observed. Gain = 1×10^4. Modulation width = 10G.

S-9(−) and S-9(+), 3 samples were probably positive in TA98 only.

Identification of Crystal Structure in the Deposited Particulates

Alpha quartz was detected in 55 samples and crystallized stearate calcium was also detected. In a few samples, talc and ferric hydroxide were detected. Alpha quartz ($\alpha-SiO_2$) is a natural mineral, originating from soil and rock. They were blown up in the atmosphere. The stearate calcium detected in the lung deposit was produced by chemical procedures with alkaline solution at 40°C for several days.

Detection of Asbestos Fibers in the Black Deposits

Asbestos fibers were detected in three cases among the 10 samples. We studied them using a TEM-XMA to identify asbestos fibers in the lung deposits. Chrysotile fibers were found in specimen B who was 78 year-old medical doctor, tremolite fibers and crocidolite fibers were detected in specimen C who was 65 year-old man. These fibers were qualitatively examined. However, other samples were not detected asbestos fibers.

CONCLUSION

The black dust deposited and accumulated in the human lung were separated and through the identification of their composed elements, crystallized materials, carbon free radicals, asbestos fibers and the mutagenicity test, the origin of lung contamination was examined. The results observed in most cases were identical to the composition of an urban atmosphere except for several cases, which were depended on their profession. The one case was a hexavalent chromium worker, and others were laborers worked at a industrial factory, public engineering works, construction industry etc. As for the exogenous factors related to the formation of pulmonary lesions, the effect of smoking cannot be ignored. We have detected free radicals in the human lung deposits as an original finding related to smoking and soot. A mutagenicity test for black deposits also was examined and a few cases were positive. Some of these data provide a large information base for future work and will be useful for making a risk evaluation for lung contamination by low exposure to toxic substances.

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CARCINOMA OF THE LUNG AND SILICOSIS: PATHOLOGICAL STUDY

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INTRODUCTION

The relationship between silicosis and lung carcinoma can be approached from both epidemiologic and morphologic viewpoints. The majority of epidemiologic studies indicate that lung carcinoma occurs less frequently in coal miners than in comparable populations. However, excess lung carcinoma has been reported among metal miners, pottery workers, foundry workers and silicotic patients.

To our knowledge, detailed morphologic studies of lung carcinoma in silicotic patients have not been reported.

This is a report on pathologic evaluation of lung carcinoma associated with silicosis which was reviewed between 1960 and 1986 at our Laboratory.

MATERIALS AND METHODS

Between 1960 and 1986, the authors evaluated about 450 autopsies of silicosis. Of these, 140 were our own consecutive autopsies in our Laboratory and remaining 310 were kindly provided to us from other hospitals in Japan. Carcinoma of the lung was seen in 48 of the autopsies.

Pathological studies, including cell types, cancer sites, severity of silicosis, were performed in the 48 cases of lung carcinoma associated with silicosis.

Severity of silicosis was determined by extent of progressive massive fibrosis as follows; (1) mild silicosis; silicosis without PMF (simple silicosis); (2) medium silicosis: silicosis with small PMF that were formed within lung segment; (3) severe silicosis: silicosis with large PMF including some segments.

RESULTS

Carcinoma of the lung was seen in 25 of our own consecutive autopsies, an incidence of 19.9%. The incidence of lung carcinoma was definitely elevated among mild silicosis and lowered among severe silicosis (Table I).

Table II shows the distribution of lung carcinomas by histologic cell type in all 48 lung carcinomas.

Over all, the predominant cancer was squamous cell carcinoma (54.2%) followed by small-cell carcinoma (22.9%) and adenocarcinoma (14.6%). There was a clear trend of squamous cell carcinomas arising in the larger airways, whereas the adenocarcinoma was found only in peripheral lung tissue.

More tumors were observed in the right lung, but the difference was not observed between upper and lower lobes (Table III). In case of mild silicosis, the majority of tumors arose in the right, upper and larger airways. On the other hand, in case of medium and severe silicosis, more tumors arose in the left, lower and peripheral lung tissues.

The distribution of primary focus in the large bronchi are illustrated in Figure 1. In case of mild silicosis, many tumors arose in stem and lobar bronchi, whereas many tumors arose in segmental bronchi in case of medium and severe silicosis.

In case of silicosis with PMF, the majority of tumors arose in the segmental bronchi leading to PMF. (Figure 2 shows the typical case of such cases.)

The primary foci of tumors arising in peripheral lung tissues were illustrated in Figure 3. Almost all the tumors in periph-

Table I

Incidence of Lung Carcinomoma Among Our Autopsy Cases

Severity of Pn.	Number of Cases	Lung Carcinoma	%
Mild	40	13	32.5
Medium	51	10	19.6
Severe	49	2	4.1
Total	140	25	17.9

Table II

Distribution of Lung Carcinoma Associated with Pneumoconiosis Cases by Histologic Type

	Central Type	Peripheral Type	Unknown	Total	%
Squamous Cell Ca.	15	10	1	26	54.2
Adenocarcinoma	0	6	1	7	14.6
Small-Cell Ca.	6	4	1	11	22.9
Large-Cell Ca.	2	1	1	4	8.3
Total	23	21	4	48	100.0

Table III

Location in Lung of Tumors by Site of Origin

	Mild Pn.	Medium Pn.	Severe Pn.	Medium+Sever	Total
Right:Left	16: 7	7: 7	4: 3	11: 10	27: 17
Upper:Middle:Lower	14: 0: 9	5: 1: 8	4: 0: 3	9: 1: 11	23: 1: 20
Central:Peripheral	14: 9	7: 7	2: 5	9: 12	23: 21
Total	23	14	7	21	44

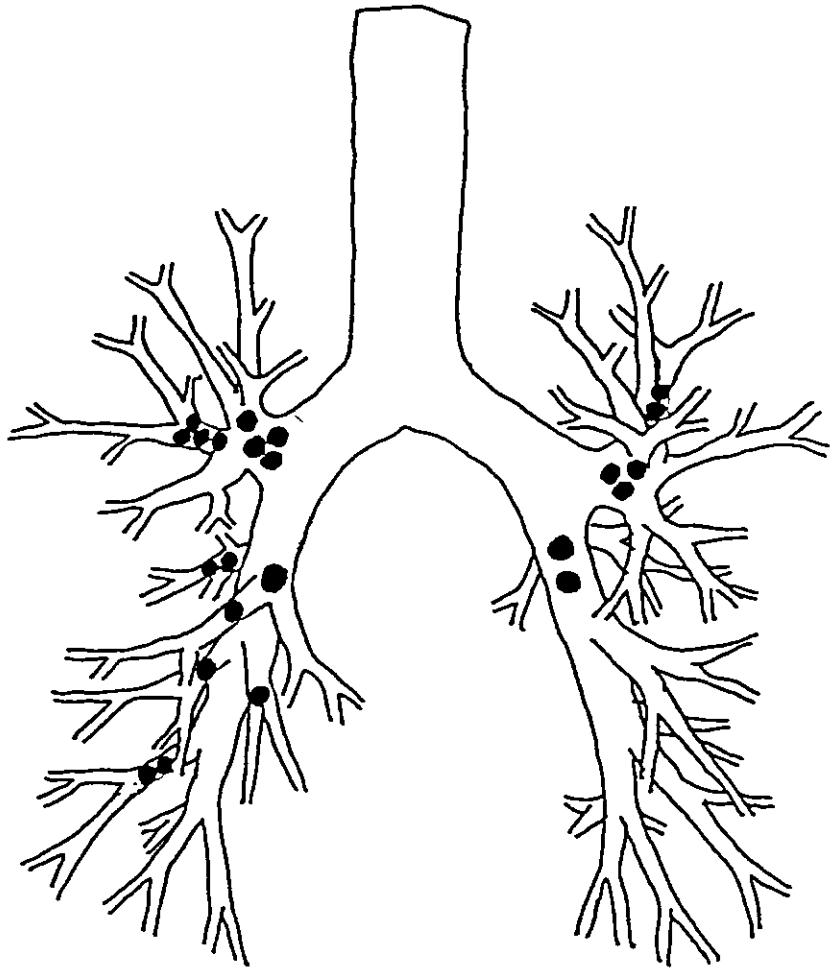
eral lung tissues arose in S_2, S_3, S_6, S_9 where PMF were usually formed.

The majority of the tumors in peripheral lung tissues were centered on closely adjacent to PMF or originated on the basis of pathologic course to PMF. In these cases, there were three cases of scar cancer arising in scar tissues of PMF (Figure 4 and Figure 5).

Diffuse interstitial fibrosis of the lung was often associated with silicosis. Five cases of carcinoma of lung were found in these cases (Figure 6).

SUMMARY

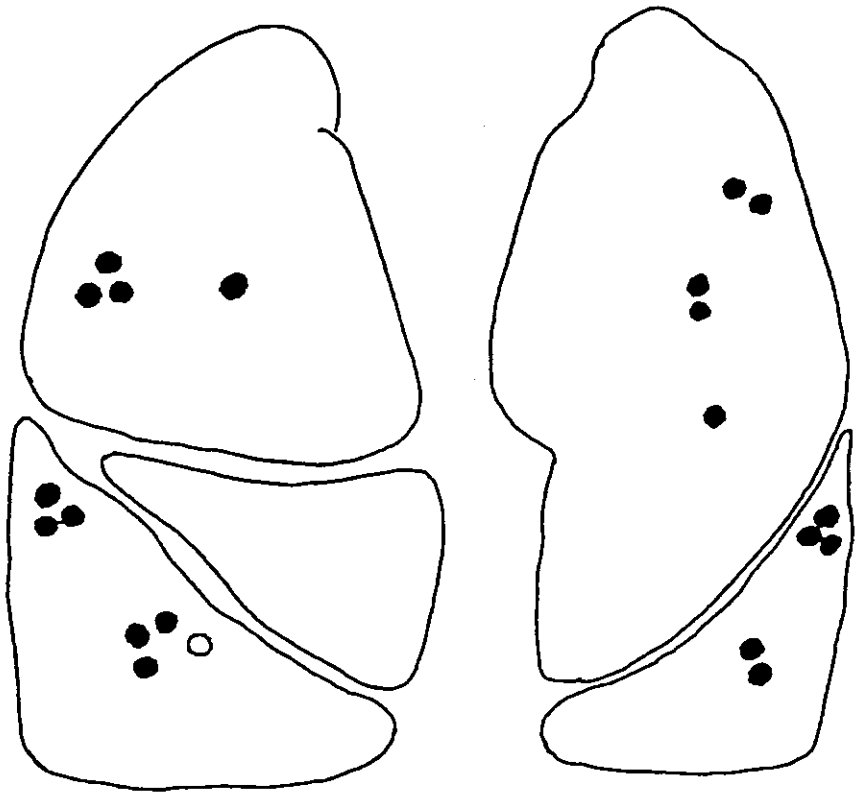
The data indicate the close relationship between pathological, changes of lung tissues by dust exposure and carcinoma of the lung.



	Right Lung	Left Lung
Upper Lobe Bronchus	4	3
Lower Lobe Bronchus	1	2
B_2 (B_{1+2})	4	2
B_4	1	0
B_6	2	0
B_7	1	0
B_8	1	0
B_9	2	0
Total	16	7

Figure 1. Location in bronchial trees of central type of tumors by site of origin.

Figure 2. Squamous cell carcinoma originated from bronchus leading to progressive massive fibrosis.



	Right Lung	Left Lung
S_2 (S_{1+2})	3	2
S_3	1	2
S_4	0	1
S_6	3	3
S_7	1	—
S_8	3	2
Total	11	10

Figure 3. Location in lung of peripheral type of tumors by site of origin.

Figure 4. Scar cancer originated from anterior portion of progressive massive fibrosis.

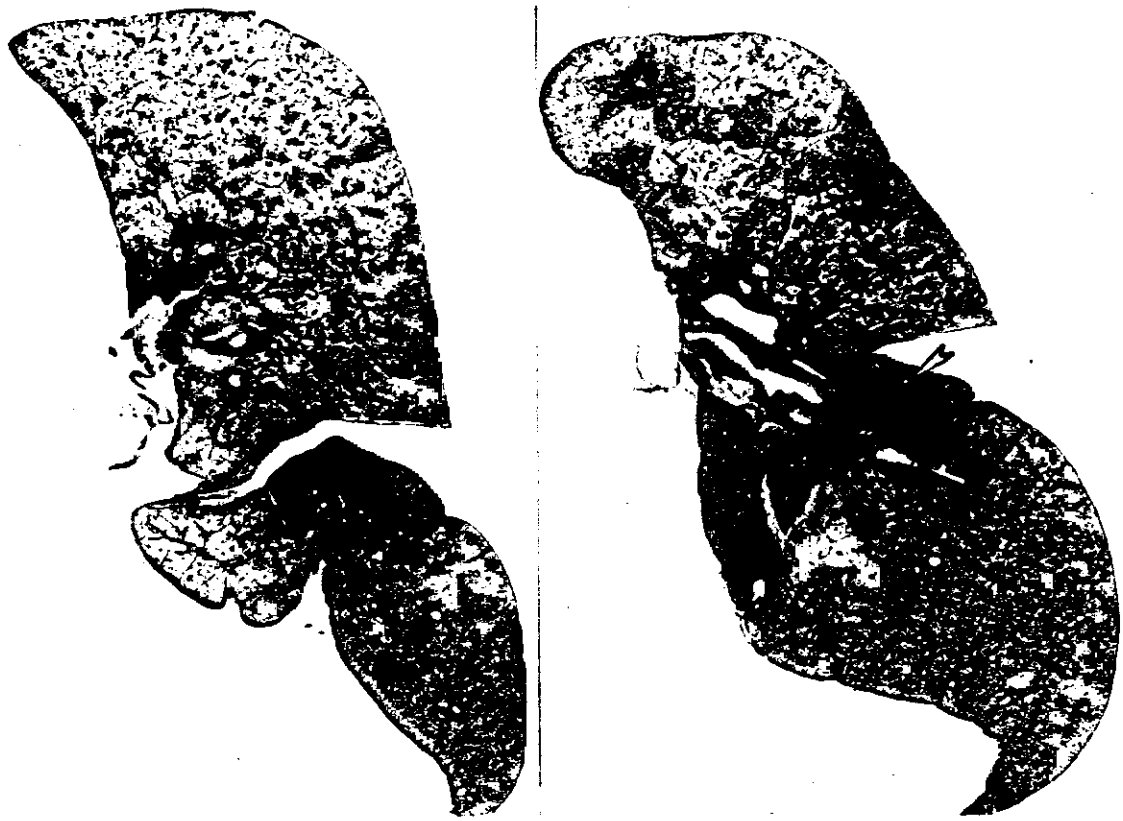


Figure 5. Scar cancer originated on the basis of pathologic course to progressive massive fibrosis.

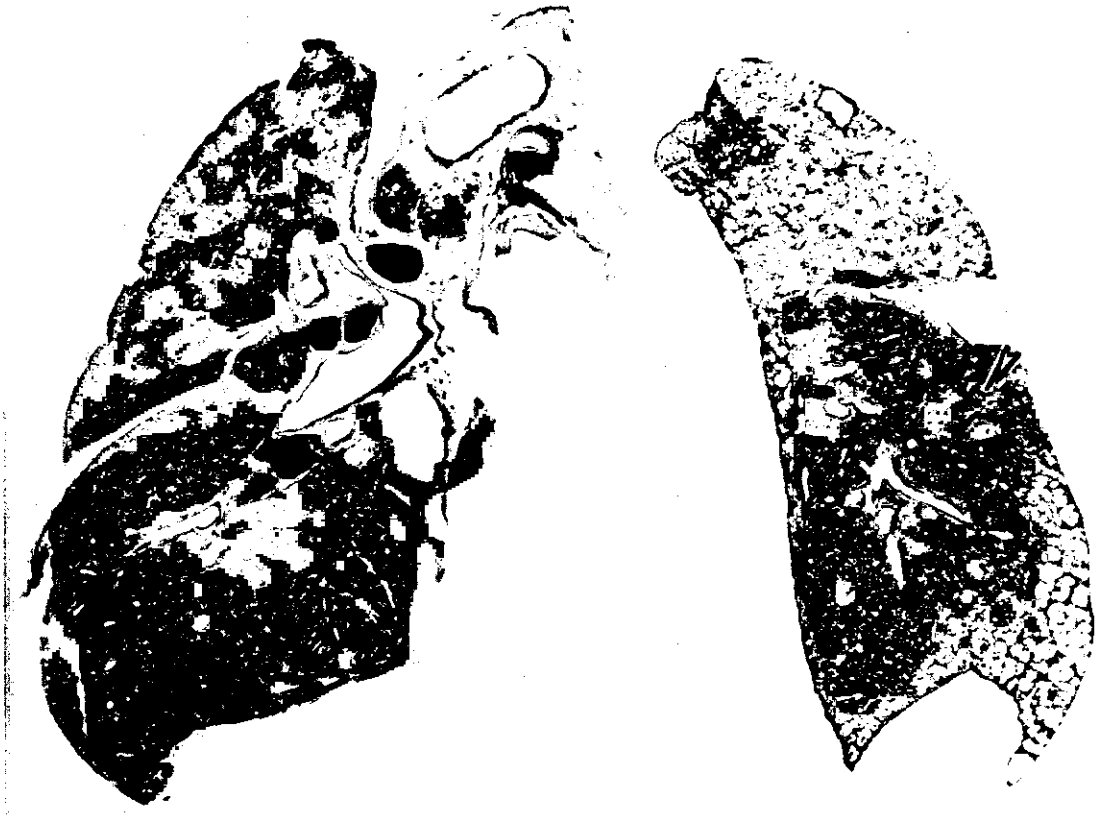


Figure 6. Adenocarcinoma associated with mild pneumoconiosis and diffuse interstitial fibrosis.

STUDY ON DUST PARTICLE SIZE IN AUTOPSIED LUNGS OF UNDERGROUND COALMINERS

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INTRODUCTION

Some scholars suggested that particle less than 5 μ m was most harmful. Others thought that particle less than 5–7 μm had the selective effect on the lungs. Some countries adopted the respirable dust concentration as dust standard.[1] But both the measurement of particle size distribution of dust after death from the lungs of coalminers and the experimental study on dust retained in the respirable organ of animals failed to reach a consensus about hygienic evaluation of different fractions of particle sizes. Dust 40–60 μm in diameter were found at autopsy.[2] Professor Chen Hongquan[3] observed particle size distribution of dust at autopsy, using biological microscope and scanning electron microscope and suggested that the particles with dia. below 2, 5 and 10 μm made up 65.2, 88.4 and 95% of total number. Author[4] thought that dust more than 5–7 μm must be considered when working out limit standard of dust and monitoring dust in production environment.

In this paper, our results of study on particle size distribution of dust in underground coalminers' lungs were reported.

MATERIALS AND METHODS

Subjects

120 histological sections were at random sampled from the lung sections of 60 autopsies (2 sections per case), who had been exposed to coal dust or with Coal Workers' Pneumoconiosis for this study, most of them had been coal mining workers and the few had been rock drifting workers. In view of the difference of physical and chemical properties of coal mineral dusts, 120 sections were divided into two dust groups which were treated with digestion and microincineration, respectively.

Hydrogen Peroxide Digestion

The specimen were digested in 70 ± 5 centigrade temperature H_2O_2 for 3 hrs and treated with concentrated HCl and observed by polarizing microscope.

Microincineration

Thin sections with their waxembedding material were moved by washing in xylene, dried and microincinerated in a muffle furnace at 540 centigrade temperature for 4 hrs, treated with concentrated HCl.

Size-Groups by Particle Size

Dusts were divided into down to 2, 2–5, 5–7, 7–10 and over 10 μm size-groups by geometric projection diameter. Fractions of particle numbers and masses of dust were calculated.

RESULTS

Number Distribution of Particle Sizes of Dust in lungs

The observations of the treated specimens which were divided into many parts equally were performed using the X 400 light microscope and Polarizing microscope. 500 particles were measured per section. Results were in Table I. The % of particle numbers was similar in the small particles of two dust-groups, significantly different in two dust-groups of particle 7–10 μm and over 10 μm in dia.($t > t_{0.01}$ $P < 0.01$). In mineral dust-group, numbers of particle 7–10 μm in dia. made up 7.7 % of all mineral particle numbers. In coal dust-group, number of particle in 7–10 μm dia. only constituted 4.4 % of all coal particle numbers, number of dust >10 μm covered 0.4% and 0.2% respectively in mineral dust-group and in coal dust-group. It was clear that large particle mineral dust predominated over that of coal dust.

To account of different definitions of the respirable dust in the inspirable dust curve recommended by some countries and organisms[5] Table I was changed into Table II. Grain size distribution >7 μm fraction had significant difference in mineral dust-group and coal dust group ($t > t_{0.01}$, $P < 0.01$).

Mass Distribution of Dust Particle Size in Lungs

Accumulative distribution derived from number distribution of particle size was plotted on logarithmic normal log-probability graph paper so as to attain number distribution N(D) which was necessary to account and more minute than the measuring of groups by means of geometric projection using microscope. Total mass of particles with dia. ranging from D_1 to D_2 is given by

$$m_{1,2} = \int_{D_1}^{D_2} \rho \, \alpha_v \, D^3 \, N(D) \, dD$$

Table I
Number Distribution of Particle Sizes of Two Dust-Groups

Types of Dusts	Number of Samples	% of Number Distribution of Particle Sizes (μm)				
		<2	2-5	>5	7-10	>10
Mineral	53	55.6	25.8	10.5	7.7	0.4
Coal	57	57.6	26.5	11.2	4.4	0.2

Table II
Numbers of Particle Sizes of Two Dust-Groups

Types of Dusts	Number of Samples	% of Particle Sizes (μm)			
		<5	5-7	7-10	>10
Mineral	53	72.9	18.6	8.1	0.4
Coal	57	79.1	15.9	4.7	0.3
Mean		76.0	17.2	6.4	0.4

Here: ρ: Particle Density
α_v: Volume Shape Factor of Particle
D: Geometrical Projective Diameter of Particle.

If composition of particles and mechanism of producing particle are same, ρ and α_v are not related to particle size, so the formula above is changed into:

$$m_{1,2} = \rho \alpha_v \int_{D_1}^{D_2} D^3 N(D)\, dD$$

But relation between N(D) and D measured really showed that distributions of N(D) was different within the range of particle sizes considered. For the sake of convenience, integral method of numerical value was used. So:

$$m_{1,2} = \rho \alpha_v \sum_{D_i=D_1}^{D_2} D_i^3 N(D_i)$$

Within the given range of Particle size ($D_1 - D_2$), the per cent of particle mass in total particle mass is:

$$F_{1,2} = \frac{m_{1,2}}{m_t} = \frac{\rho \alpha_v \sum_{D_i=D_1}^{D_2} D_i^3 N(D_i)}{\rho \alpha_v \sum_{D_i=D_{min}}^{D_{max}} N(D_i) D_i^3} = \frac{\sum_{D_i=D_1}^{D_2} D_i^3 N(D_i)}{\sum_{D_i=D_{min}}^{D_{max}} D_i^3 N(D_i)}$$

Where D_{min} and D_{max} are the smallest and largest particle diameters. M_t is total mass. The calculated results were shown in Table III and Figure 1. It was seen in Table III that masses of Particle greater than 5, 7 or 10 μm in size made up respectively 83.7 ± 3.3, 66.3 ± 6.1 and 14.2 ± 7.7

Table III
Mass Percent of Particle Sizes in Two Dust-Groups

Types of Coal	Number of Samples	% Mass of Particle Sizes (μm) <5	5-7	7-10	>10
Mineral	53	16.3	17.4	52.1	14.2
Coal	57	22.5	11.3	55.5	12.5
Mean		19.4	14.3	53.8	12.5

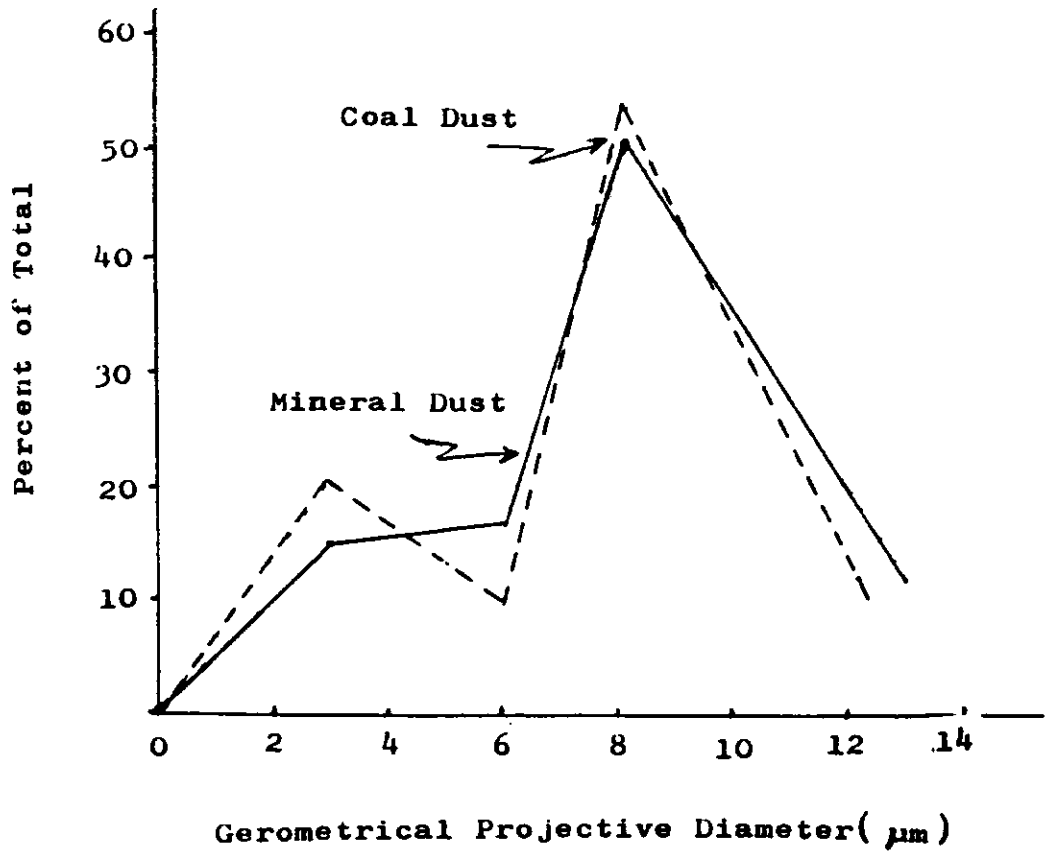

Figure 1. Mass percent of particle sizes of two types of dusts.

percent of the total mass in mineral dust in autopsic lung tissue and in coal dust in lung, masses of particle with more than 5, 7 or 10 μm in dia. accounted for 77.6 ± 7.2, 45.3 ± 10.4 and 10.8 ± 8.4 % of total mass, respectively. Significance tests showed that particle fractions over 5 μm and 7 μm of two types of dusts were significantly different ($t > t_{0.01}$, $P < 0.05$)and that fractions >10 μm of two types of dust had statistical significance($t > t\ 0.05$, $P < 0.05$).

Relationship between the Mass and the Number Distribution of Dust Particle Sizes in Autopsic Lungs

The mass and the number distributions of dust particle sizes in the lung tissue were studied. (Figure 2). Figure 2 illustrated that number of dust < 5 μm amounted to 76.0% of total number, but its mass was only 19.4%, of total mass; that number of dust >5 μm made up only 24.0% , but its mass accounted for 80.6% of total mass and that number of dust † 7 μm was 6.8% total number, its mass was 66.3% total mass and that number of dust >10 μm was 0.4% of total number, its mass constituted 12.5%, total mass.

Some scholars had observed 37297 airborne particles of samples from the gold mine and come to the conclusion that number of particle <1 μm made up 92/% of total number and its mass only 10.5% weight of sample, which was correspondence with our results.

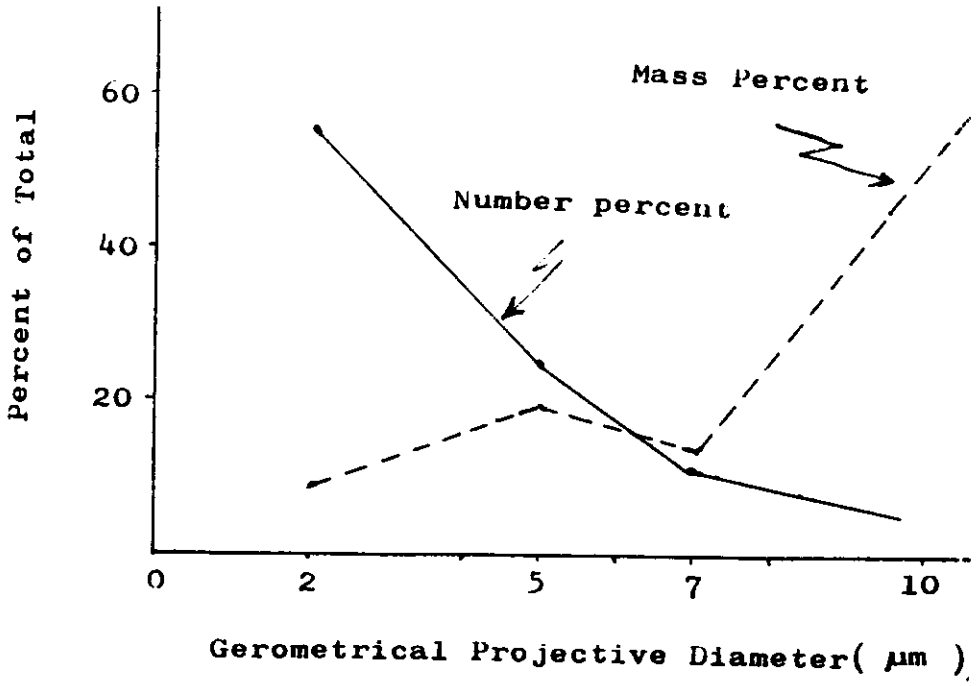

Figure 2. Comparison of percent content of particle sizes of dusts in autopsic lungs.

DISCUSSION

Some scholars[2] had injected the same weight of quartz 0.8–2.0μm and 5–10 μm in dia. into two groups of rats (A group and B group), respectively and observed 19 small dust focuses and 3 large dust focuses in A group and 47 small dust focuses and 15 large dust focuses in B group. It may be seen that quartz 5–7 μm.in dia. caused the more and the larger dust focus than quartz 0.8–2.0 μm in dia.. Hence, the respirable dust concentration was only part of dust concentration.

Other articles[6,7,8] and our study confirmed that the level of pathological change and categories by X-ray were closely relative to mass and content of dust retained in lungs. Such research has shown that CWP is related to exposure to respirable dust, partially dust 2 μm or larger in size, is the most important factor associated with CWP. So we think that when drawing up the dust hygiene standard and monitoring dust concentration, we considered not only the respirable dust concentration but also the total dust concentration.

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