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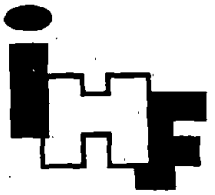

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INDUSTRIAL HEALTH FOUNDATION, INC.

A NONPROFIT ORGANIZATION FOR THE ADVANCEMENT OF HEALTHFUL WORKING CONDITIONS IN INDUSTRY

FINAL

TECHNICAL REPORT

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ASBESTOS DUST:

A STUDY ON THE PATHOGENETIC MECHANISM

by

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INTRODUCTION

The earliest theory attempting to explain the mechanism by which asbestos caused tissue damage, assumed that the inhaled asbestos fibers acted like lances or needles, piercing and otherwise traumatizing cells in the respiratory tract. This theory was somewhat qualified by Gardner and Vorwald [1] who determined that only asbestos fibers longer than 10 μm were capable of causing lung damage. This qualification was, however, denied by King, et al,[2] who found asbestos fibers shorter than 10 μm also causing lung damage.

It is curious that so little effort has been expended to elucidate the mechanism of action of asbestos dust, particularly that by which lung cancer develops. Although pulmonary fibrosis has been produced in animals exposed to asbestos dust, lung cancer has not been observed until relatively recently.[3] We found lung cancer in 30% of surviving rats that had inhaled hammer-milled chrysotile asbestos. Inasmuch as no other laboratory working with asbestos dust had observed the development of lung cancer, it seemed probable that there was something unique about the asbestos dust used by us. We had comminuted chrysotile asbestos to a respirable dust, using the hammer-milling process of Holt, et al.[4] We suspected that some of the metal from the hammer-mill was transferred to the asbestos fibers. This suspicion was strengthened by severe wearing-down of the hammers in the mill, necessitating their replacement. Since many steel alloys contain small amounts of nickel and chromium, these trace metals were suspect. Chemical analysis of the asbestos dust leaving the mill did indeed reveal an increase in the nickel (82%),

cobalt (145%), and chromium content over that originally present. Inasmuch as workers reducing nickel ore and those working with certain chr,me compounds are known to have an increased prevalence of lung cancer, the suspicion seemed justified.

Previous pilot studies in this laboratory attempting to throw light on the mechanism of asbestos dust as a fibrogenic agent have given negative results, i.e., the results have ruled out that certain factors could be operative in the pathogenesis. For instance, chrysotile, being essentially hydrated magnesium silicate, might be considered noxious by virtue of its molecular structure. However, when freshly prepared magnesium silicate was injected intratracheally into rats, no pulmonary fibrosis developed.

When synthetic chrysotile (prepared by reacting sodium silicate and magnesium chloride in a bomb at high temperature and high pressure) was injected intratracheally into rats, no significant disease was produced, and since synthetic chrysotile has an x-ray diffraction pattern as well as electron diffraction pattern identical to the natural product, the crystalline structure of chrysotile cannot be held responsible for the production of biologic damage.

The concept that the pointed ends of asbestos fibers, when inhaled, traumatize lung tissue and cause an inflammatory reaction is still accepted as valid by some even though the mechanical theory for the pathogenesis of silicosis was discredited more than 30 years ago. The continued acceptance of this concept is all the more difficult to understand because a report, published in 1956, clearly indicated that a ceramic fibrous dust composed of a clay-like material (aluminum silicate) when injected intratracheally caused no appreciable pulmonary

damage.[5] That the needle-like character of dust particles is not responsible for lung damage has recently been confirmed in a paper reporting the pulmonary effects of fibrous glass.[6]

The sum-total of what is known regarding the locus of pathogenicity of asbestos dust is, therefore, of a negative nature. The following aspects of asbestos dust have been excluded decisively from playing a significant role in, or even contributing to, the pathogenesis of asbestosis:

1. The needle-shape character of the dust particles.
2. Their crystalline character.
3. Their chemical composition (as a silicate).

The trace-metal theory held that the asbestos mineral per se was not pathogenic but that its dust became pathogenic as it acquired such trace metals as nickel and chromium during contact with the steel alloys of machinery designed to separate the ore from associated rocks, to "open" the fibers, to card, and to spin the fibers. This theory became more attractive when it was recalled that the lung cancer rate of Canadian asbestos miners was reported to have been no higher than that of the surrounding non-mining population.[7] These statistics were in striking contrast to those derived from an English asbestos textile mill where the lung cancer rate of its workers was ten times greater than that of the surrounding population.[8] The relevance of these reports to the trace-metal theory is as follows: The dust to which the asbestos textile workers had been exposed had had maximal contact with machinery and can, therefore, be presumed to have been coated with nickel- and chromium-containing alloys.

In contrast, the dust to which the asbestos miners had been exposed had had minimal contact with machinery and, therefore, can be presumed to have acquired no significant coating by trace-metals.

The purpose of this investigation, therefore, was to test the hypothesis that the locus of pathogenicity of asbestos dust resided not in the asbestos mineral per se but in the trace-metals with which it became coated during the processes that converted asbestos into consumer products.

METHODS AND MATERIALS

Samples of crocidolite, amosite, and Canadian chrysotile were each prepared by five different methods for intratracheal injection. One sample of each was hammer-milled for 12 hours (hammer-mill described by Holt, et al [4]). The purpose of the prolonged hammer-milling was to coat the asbestos with the nickel-containing alloy of which the hammer-mill was composed.

Another sample of each kind of asbestos was ball-milled for two hours and then heated in an electric muffle furnace for two hours at 900° C for chrysotile, at 950° C for crocidolite, and at 1000° C for amosite. The heating was intended to alter the chemical as well as the crystalline structure of the asbestos.

A third sample of each asbestos, after ball-milling, was treated with aqua regia for one hour, washed, treated with 2% EDTA in 1M KOH for another hour, and washed until the pH was 7.0. It was then spex-milled for 3 to 15 minutes so that an aqueous suspension could easily pass through an 18 gauge needle. This chemical treatment was intended to remove trace-metals—particularly nickel—that is present within the mineral crystals.

The fourth sample of each type of asbestos was treated in a manner similar to the third sample, but in addition, it was subsequently also heated in the same manner as the second sample.

For the fifth sample a chunk of the mineral ore of each type of asbestos was denuded of its superficial fibers on one surface, using a piece of broken glass to accomplish this. Additional deeper-lying fibers were then hand-picked with the aid of sharp glass fragments to provide asbestos fibers that had no contact with a metal. These fibers were then Spex-milled for 20 to 30 minutes, using plastic containers and plastic balls.

The different batches of asbestos were dried to constant weight and a standard suspension was made of each. Each of these was then injected intratracheally in 1 ml amounts into a few rats under light ether anesthesia with the aid of an illuminated self-retaining laryngeal speculum that allowed visualization of the vocal chords. The purpose of the preliminary injections was to determine the largest dose that could be administered without a high mortality.

It was found that excessive dosage tended to obstruct the bronchi, causing death of the rats soon after withdrawal of the syringe with its attached long 18-gauge needle used for the injection. The degree to which obstruction resulted depended also on the character of the preparation. Chrysotile had the greatest tendency to become matted and, thereby, to obstruct the bronchi. It was, therefore, necessary to dilute the suspensions of chrysotile so that 1 ml contained no more than 1.75 mg of dust. Other preparations could be injected as concentrated as 7 mg per ml. Only one ml could be injected at one time,

and, generally, one month was allowed to elapse before another intratracheal injection was attempted. Up to four intratracheal injections per rat were administered. The total dosage ranged from 1.75 mg to 21 mg.

RESULTS

There was a fairly high mortality within the first six months after the first intratracheal injection—generally from pneumonia. Added to this injection-related mortality there were two separate epidemics of pneumonia during the four years of this study. One of these occurred in Pittsburgh, and the other, about six months after the animals had been safely transferred to splendid new animal quarters under the supervision of Dr. Garlick, D.V.M., at the Medical University of South Carolina in Charleston. Dr. Garlick stated that the last epidemic had been caused by mycoplasma. At any rate, the records show that a total of 1,537 rats had been injected intratracheally one to four times, but only 727 rats survived six months.

Table 1 lists the distribution of the six-month survivors and of the total number of injected rats per group. As previously indicated, chrysotile had the lowest percentage of survivors (32%), presumably because of its tendency for the fibers to form mats and obstruct bronchi. An exception was seen in the "treated and heated" chrysotile group (82%) in which the dust had apparently been so severely altered that it did not cause significant bronchial or bronchiolar obstruction. In the sections this dust showed microscopically no discernible fibers and it had an amorphous black appearance. Whereas the conversion of the semitransparent fibers into black amorphous particles or masses was fairly

complete in the "treated and heated" chrysotile group, this was incomplete in the crocidolite "treated and heated" and in the chrysotile "treated" group. It is tempting to explain the extremely low survival rates of the "treated" chrysotile and amosite groups (15% and 19%, respectively) on a severe softening of the fibers by the aqua regia treatment, thereby enhancing their tendency to form mats and to obstruct bronchi.

The intratracheal injection of asbestos dust caused lesions in the air-conducting tubes as well as in the lung depths—the alveolar ducts and associated alveoli. The intrabronchial lesions consisted of sessile polypi that often occupied a major portion of the bronchial cross section (Fig. 1). The polyp consisted of a mass of matted asbestos dust permeated and surrounded by inflammatory tissue that initially was highly cellular but rapidly lost most of its exudative cells while retaining its histiocytes. The surface of these inflammatory polypi became rapidly covered by columnar bronchial epithelium—usually within one week. In many bronchi the polypoid inflammatory tissue emanating from one mucosal ulcer was joined by similar tissue originating from several other ulcers. The result was that the bronchial lumen, as seen in cross-section, appeared to be converted into circumferentially arranged narrow channels lined by columnar bronchial epithelium—all within the confines of the bronchial wall (Fig. 2). Inspite of the manifest bronchial obstruction, atelectasis from this cause was usually absent. When atelectasis was present, it was usually found in older animals where it was associated with endemic chronic bronchitis. In the atelectatic regions more of the injected dust and more lesions were often seen than in expanded regions of the lungs. The impression was gained that the

excessive amounts of dust and lesions in the atelectatic regions were greater than could be accounted for by compression of the tissue.

The more peripheral lesions involving the alveolar ducts and alveoli also began as intraluminal granulation tissue that sequestrated the injected dust. However, it did not often exhibit a polypoid structure, but the alveolar duct and associated alveoli were usually destroyed. As the exudative cells disappeared and the collagen content of the inflammatory tissue increased, the resulting scars underwent shrinkage so that the original alveolar structures became unrecognizable even in preparations impregnated with silver to outline the reticulin fibers (Fig. 3). Discrete collagenous thickening of septal walls was also noted in relation to some of these coarse scars (Fig. 4).

The dust was often associated with multinucleated giant cells and clusters of macrophages. The appearance of the dust varied, depending upon whether it had been heated,or treated with acid and then heated. The treated-and-then-heated variety of dust characteristically appeared as black,opaque, and structureless particles or masses. The otherwise-modified asbestos dusts, inclusive of the aqua-regia-treated dust, were recognizable in the sections as semi-transparent fibers.

Since all but a few of the animals died of intercurrent disease, acute pneumonia was present in one or more of the lobes. This pneumonic consolidation tended to obscure lesions produced by the asbestos dust. In most animals it became necessary to concentrate attention to those regions of the lung in which pneumonia was minimal or absent. Nevertheless, it was generally possible to recognize lesions or stigmata (macrophages and giant cells) caused by injected

asbestos dust even in the presence of pneumonic consolidation. However, the impression was gained that the amount of demonstrable dust and the number of lesions were greatly reduced in pneumonic regions.

Attempts have been made to quantify the changes induced in the lungs of rats by the various modifications of asbestos dusts. The results of this quantification are listed in Table 2.

It appears from the tabulation that the heated dusts (whether heated and treated or only heated) caused bronchial lesions in only about one-half of the percentage of the animals found with such lesions when they had been injected with the various kinds of unheated asbestos dusts, 35% versus 80%. The greatest difference was noted in the chrysotile groups where the figures were 19% versus 86%. In the crocidolite groups the ratio was 22%:78%. No significant difference was observed between the amosite groups, 67%:77%.

Using the presence of giant cells and macrophages (in significant numbers) as a criterion for comparing the effect of different dusts, no significant difference was encountered between the incidence of affected animals injected with heated or unheated asbestos dusts, although in the chrysotile groups the incidence was 42%:5%, heated versus unheated dusts.

It is important to note that neither the addition of trace-metals to the asbestos, as was accomplished by prolonged hammer-milling, nor the removal of trace-metals from the asbestos with aqua regia and EDTA, nor the asbestos that had no contact with metal resulted in significant differences in the incidence or character of the lesions produced in rat lungs.

Equally important is the fact that no asbestos-related lung tumor occurred in these intratracheally injected rats, although there were a small number of reticulum cell sarcomas. These are common in this strain of rats, occurring spontaneously in laboratory control animals.

DISCUSSION

It is difficult to explain the failure of lung cancers to develop in the rats at the dosages of asbestos employed. Identical dosages (14 mg and 21 mg) of intratracheally administered chrysotile dust have previously resulted in lung cancer.[3]

The development of bronchial polypoid lesions or the conversion of a bronchial cross section into concentrically arranged narrow channels should not be used as a criterion of fibrogenicity. Such lesions are known to occur when filamentous dusts known to be nonfibrogenic, such as ceramic aluminum silicate and fiber glass, are injected intratracheally.[9] Inasmuch as these bronchial lesions do not develop in rats inhaling fiber glass dust in high concentrations,[6] the development of these bronchial lesions must be considered artefactual.

The paucity of dust and lesions in pneumonic regions deserves comment. Aside from the obfuscating effect of the pneumonic exudate, there is very probably an actual reduction in the amount of dust and in those lesions that are still composed of reticulin fibers. This probability is based on clinical and experimental evidence pointing to the ability of edema fluid to mobilize dust sequestered within the lung and to disrupt and cause lysis of inflammatory stroma composed of reticulin fibers.[10, 11]

In view of the difficulty of attaining a sufficiently high dust burden in the lungs by intratracheal injections without encountering an unacceptably high mortality, and because of the artefactual bronchial lesions inherent in this procedure, it would appear that this technique is less than satisfactory and that the inhalation technique is to be preferred. However, the inhalation technique, as generally used, would not be feasible because of the extremely large amounts of each variety of dust required—many kilograms instead of milligrams. There could, however, be a solution to this quandary. The use of a properly designed inhalation chamber that exposes only the heads of animals to the dust and recirculates the dust would require only a few hundred grams of each dust. The disadvantage, of course, would be that only a small number of animals per chamber can be exposed. This disadvantage could be mitigated somewhat by the possibility of sterilizing the chamber air with continuous ultraviolet irradiation and thereby minimizing respiratory infections in the exposed animals.

It appears from this investigation that, although the pathogenicity of chrysotile asbestos dust is not attributable to:

1. its fibrous-shaped particles,
2. its magnesium silicate molecule, or
3. its crystalline character,

nevertheless, heating to a high temperature destroys or weakens its fibrogenicity and carcinogenicity as it does also the other types of asbestos. Presumably, heating the asbestos affects its pathogenicity by modifying the crystalline structure and chemical composition; yet, neither of these apparently determines pathogenicity!

We are faced with an enigmatic situation for which there is as yet no answer!

CONCLUSIONS

The salient facts established by this study are the following:

1. The intratracheal injection of the three most important asbestos dusts into rats caused no cancer production, whether these dusts were natural or had been modified by the addition or subtraction of trace metals, or whether these dusts had been modified by heating.

2. Heating of the asbestos dusts caused a significant diminution in the fibrogenicity of the asbestos dust similar to a reduction in their carcinogenicity when injected intrapleurally.

3. In view of the failure of either the addition of trace metals to, or their removal from, asbestos dust to modify the fibrogenic reaction engendered, the trace-metal hypothesis for the pathogenic potential of asbestos dust must be abandoned. Unfortunately, no other theory for the pathogenic potential of asbestos exists or has been suggested!

TABLE 1

Tabulation of the Six-Month Survivors and the Total Number of Rats Injected

Mode of Treatment	Amosite		Crocidolite		Chrysotile		Summation	
	No.	%	No.	%	No.	%	No.	%
Treated [a] and Heated [b]	58/73	79	49/116	42	32/39	82	139/228	61
Heated [b]	75/115	65	94/131	72	42/115	37	211/361	58
Hammer-milled	71/85	84	72/152	47	33/110	30	176/347	51
Treated [a]	18/96	19	48/98	50	24/161	15	90/355	25
Metal-free	23/95	35	56/74	76	32/77	42	111/246	45
Summation	245/464	53	319/571	56	163/502	32	727/1537	47

(a) "Treated" involved treatment with aqua regia for one hour followed by EDTA to remove trace metals.

(b) Heating was at 900° C, 950° C, or 1000° C for one hour.

The numerator represents the number of rats surviving 6 months or longer.

The denominator represents the total number of rats injected.

TABLE 2

Distribution of Rats with Dust-Related Lesions

Mode of Treatment	Incidence of Animals with Lesions							
	Amosite		Crocidolite		Chrysotile		Summation	
	No.	%	No.	%	No.	%	No.	%
	Bronchial Lesions							
Heated	18/22	82	12/34	35	6/17	35	36/73	49
Treated and Heated	8/17	47	0/21	0	1/19	5	9/57	16
Summation	26/39	67	12/55	22	7/36	19	45/130	35
Metal-free	9/12	75	15/16	94	13/13	100	37/41	90
Treated	12/13	92	15/17	88	9/12	75	36/42	86
Hammer-milled	12/18	67	16/26	61	16/19	84	44/63	70
Summation	33/43	77	46/59	78	38/44	86	117/146	80
	Peripheral Scars							
Heated	6/22	27	12/34	35	7/17	41	25/73	34
Treated and Heated	0/17	0	0/21	0	8/19	42	8/57	14
Summation	6/39	15	12/55	22	15/36	42	33/130	25
Metal-free	12/12	100	16/16	100	13/13	100	41/41	100
Treated	13/13	100	17/17	100	12/12	100	42/42	100
Hammer-milled	17/18	94	26/26	100	19/19	100	62/63	98
Summation	42/43	98	59/59	100	44/44	100	145/146	99

TABLE 2 (continued)

Mode of Treatment	Incidence of Animals with Lesions							
	Amosite		Crocidolite		Chrysotile		Summation	
	No.	%	No.	%	No.	%	No.	%
	Giant Cells and Macrophages							
Heated	19/22	86	29/34	85	9/17	53	57/73	78
Treated and Heated	17/17	100	21/21	100	6/19	32	44/57	77
Summation	36/39	92	50/55	91	15/36	42	101/130	78
Metal-free	12/12	100	6/17	35	0/13	0	18/42	42
Treated	12/13	92	13/17	76	2/12	16	27/42	64
Hammer-milled	17/18	94	13/26	50	0/19	0	30/63	48
Summation	41/43	95	32/60	53	2/44	5	75/147	51

See Table 1 for explanations of fractions and "Heated" and "Treated."

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LEGENDS OF ILLUSTRATIONS

Fig. 1 A polyp, composed of inflammatory tissue containing much asbestos, projects into the lumen of a bronchus. Its surface is covered by normal bronchial epithelium. From a rat injected intratracheally with 19 mg. hammer-milled chrysotile and dead 11 1/2 months later. Hematoxylin and eosin. X 100.

Fig. 2 There is conversion of a bronchiolar lumen into multiple narrow channels by synechiae that developed between the polyp and the bronchiolar wall. From a rat injected intratracheally with 19 mg hammer-milled chrysotile and dead 10 1/4 months later. Hematoxylin and eosin. X 250.

Fig. 3 This is a characteristic example of scars encountered after intratracheal injections of asbestos in rats. The scar, composed of cellular, partially collagenized inflammatory tissue, contains asbestos fibers and an occasional vestigeal alveolus lined by metaplastic epithelium. The elongated shape and size of the scar suggests that it represents an obliterated alveolar duct with its evaginating alveoli. From a rat dead 9 months after intratracheal injections of 21 mg metal-free crocidolite. Hematoxylin and eosin. X250.

Fig. 4 Peripheral collagenous septal thickening resulting from the intratracheal injection of 21 mg metal-free crocidolite in a rat that died 9 months later. Hematoxylin and eosin. X 250.

Fig. 1

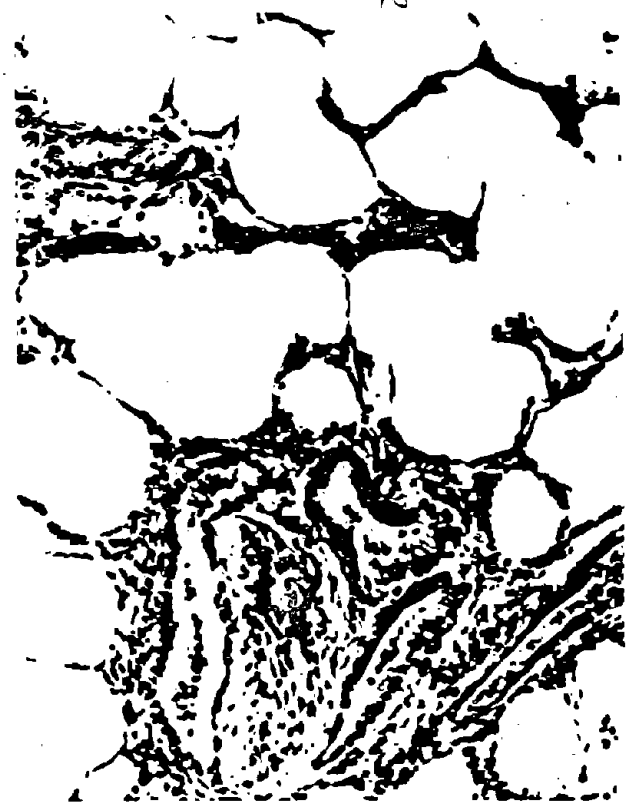

Fig. 2

Fig. 3

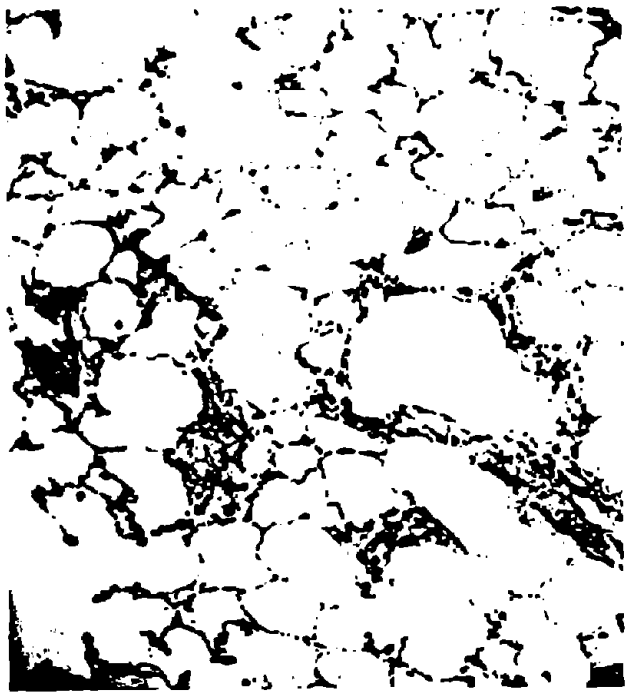

Fig. 4