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16. Abstract (Limit: 200 words) The pulmonary concentration of asbestos (1332214) dust was determined in rats which had inhaled asbestos dust or had been injected with this dust. Particular attention was given to the pulmonary concentration of asbestos dust in rats with cancer. This was compared to the amount of asbestos dust concentrated in the lungs of rats without cancer. A comparison was also made of the dust concentration in the lungs of rats where lung clearance was impaired and in rats with presumably normal clearance. The silica (14808607) content of the lungs of rats that had inhaled high concentrations of asbestos over a 16 month period was extremely low. The differences in the silica content between groups that had lung cancer and those that had no lung cancer was small. No group showed a decisive difference in pulmonary silica content. The lungs used in this study had been stored in formaldehyde solution for 5 to 6 years prior to analysis. Results indicated that lung tissue containing deposits of finely divided chrysotile dust may lose this dust by dissolution if kept submerged in aqueous formaldehyde solution for a prolonged period. The author recommends that asbestos determinations should be made on fresh lung tissue or on lung tissue that has been fume fixed after removal from the body and dried.

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INTRODUCTION

It is generally agreed that there is a dose-effect relationship between the inhalation of asbestos dust and the development of lung cancer. An early epidemiologic study of workers in a large asbestos textile factoryin England pointed out that the lung cancer incidence among the workers was ten times that of the general population.[1] Following publication of this report, good "housekeeping" measures were instituted,and a few years ago the publication of the results of a new survey of the same factory's employees indicated that the incidence and risk of developing lung cancer in those workers was now no higher than it was in the general population.[2] This suggests that, at least so far as the development of lung cancer is concerned, there may be a level of asbestos dust exposure at which the risk of developing lung cancer is remote.

In contrast to this apparent dose-effect relationship stand, the so-called neighborhood cases of mesothelioma in which only indirect, but no occupational, exposure to asbestos dust could be documented.[3, 4] These cases would seem to suggest that only a few asbestos fibers stored in lungs would suffice to cause the development of a mesothelioma in infants[3]as well as in adults. The implication in case reports of this kind is that a dose-effect relationship of asbestos dust is nonexistent.

Another way of expressing this is that any exposure level of asbestos dust above zero may cause the development of lung cancer. This, voiced as a "concern" or fear, seems to be contradicted by the finding that the lungs of virtually all adult city dwellers contain asbestos fibers,[5, 6, 7] which is probably related to the discovery that the ambient air of cities and some rural areas contains asbestos fibers.

Data from Mt. Sinai Medical School in New York City and from the University of California at Berkeley indicate that the ambient air of these two cities contains chrysotile asbestos fibers. My laboratory, with the collaboration of Dr. John M. G. Davis, Institute of Occupational Medicine, Edinburgh, Scotland, has made similar findings in the dust isolated from the lungs of Pittsburghers. We have developed methods for quantitating the fibrous dust particles visible in the light microscope (L-M) which had been isolated from the lungs of city dwellers [8, 9] and have more recently adapted the quantitation for electron-microscope (E-M) enumeration.[10] Using the latter, it was found in one case (a six-year-old child) that 8% of the fibers visible with the L-M were chrysotile but that 35% of all fibers (L-M plus E-M) were chrysotile. Thus, it seems that there may be an appreciable accumulation of asbestos dust in the lungs of city dwellers who are not occupationally exposed to asbestos dust.

Governmental agencies are concerned with the health effects of community-type pollution with asbestos dust which occurs in large urban centers, particularly where asbestos-containing materials are sprayed on the steel skeletons of buildings during their construction. Unfortunately, there are uncertainties and even complete lack of data in regard to how much exposure to asbestos dust, if any, over the total number of years of employment in an occupational situation, and over an entire lifetime in other people, would result neither in pulmonary fibrosis nor in cancer. If no dose-effect relationship can be established for asbestos dust, then the only real safeguard against the health effects of this dust is zero-exposure.

The fact that the institution of good housekeeping measures in a large asbestos textile factory has been instrumental in lowering the incidence of lung cancer from ten times that of the general population to one no larger than the latter is suggestive of a dose-effect relationship of asbestos.

There are, in addition, certain data derived from our previous experimental study [11] which tend to support the existence of a dose-effect relationship for asbestos. Of 132 rats which were subjected to inhalation of asbestos dust, 71 had had intratracheal applications of 5% sodium hydroxide for the purpose of damaging the escalator mechanism and thereby impairing the rate of clearance of asbestos dust from the lungs. The cancer rate was 48% in the rats treated with sodium hydroxide (15 out of 31 rats which survived 16 months or longer), whereas the rats not treated with sodium hydroxide had a cancer rate of only 24% (10 out of 41 survivors). Presumably, the rats treated with sodium hydroxide had a larger lung burden of asbestos dust than did the untreated animals. It cannot be argued that painting the tracheal mucosa with lye predisposed the tissue to cancer because no cancer arose from the trachea or from the larger bronchi. All tumors were situated in the peripheral portions of the lungs or were associated with bronchioles.

Less meaningful data are noted in the group of 19 rats injected intratracheally with asbestos and surviving 16 months or longer. Whereas only three of these animals developed lung cancer, all three had received multiple doses of asbestos dust. None of the rats receiving but a single intratracheal dose of asbestos developed cancer.

The purpose of this study was to determine the pulmonary concentration of asbestos dust in rats which had inhaled asbestos dust or had been injected with this dust. In particular, the pulmonary concentration of asbestos dust in rats with cancer was to be compared with the asbestos dust concentration in the lungs of rats without cancer. In addition, a comparison of the dust concentrations was to be made between rats in which the lung clearance was impaired and rats with presumably normal clearance.

The determination that a dose-effect relationship of chrysotile asbestos does exist insofar as the development of lung cancer—if this be the conclusion—should allay somewhat the alarm reaction evoked by the recently acquired knowledge of the presence of chrysotile asbestos fibers in ambient city air and in the lungs of city dwellers. Although the results regarding the chrysotile dust burdens in the lungs of rats cannot be extrapolated to human lungs, in terms of mg. dust per gm. of dry lung, they may provide a "ball park" level of dust which, with a proper safety factor, may be useful in setting human tolerance levels.

METHOD AND MATERIALS

The lungs of the rats of the previous investigation in which pulmonary cancers developed in association with the pulmonary deposition of chrysotile dust had been stored in formaldehyde solution over the past seven years. Each of these had been tied within a gauze bag with its identification number. A variable number of such bags had been placed in large jars without regard to the diagnosis. That is, lungs without cancer were to be found in the same jar as lungs with cancer, as were lungs of rats that had had no exposure to asbestos.

The lungs were dissected free of the trachea, bronchi, and other tissues. They were then sliced and dried to constant weight. The dried lungs were then ground to a coarse powder in an agate mortar and silica determinations were made on the ground material according to the method of King, et al.[12] This method involves heating a mixture of the ground lung with anhydrous sodium carbonate in platinum crucibles, first causing the organic material to ignite, and later, the silicious material to combine with the sodium carbonate to form soluble sodium silicate. This is a clear melt and generally required nearly two hours of heating. The color reaction produced by the addition of ammonium molybdate followed by sulfuric acid and amino-naphthol-sulfonic acid reducing agent was compared in a colorimeter against the color similarly produced with a known standard silica solution.

All determinations were made in duplicate and the results agreed with each other within 1%. When the duplicate results did not agree within this limit, the determinations were repeated. The methodology was also controlled by adding known amounts of silica to normal lungs and determining the recovery. It was found that the recovery was in the neighborhood of 99%.

The lungs from 50 rats that had inhaled asbestos but had not been treated with lye (to impair the tracheal mucosa) were analyzed. Seven of these lungs had cancer. There were fifteen lungs from rats that had inhaled asbestos but had also received intratracheal treatments with 5% sodium hydroxide to impair clearance. All of these had lung cancer. Three lungs were from rats that had developed lung cancer after the intratracheal injection of asbestos. There were also lungs from 14 control animals. Ten of these had been treated with sodium hydroxide and had not been exposed to dust, and the other four were laboratory controls.

RESULTS

The results of the silica determinations are listed in Table 1.

Table 1

Silica Content of Lungs of Rats with and without Asbestos Exposure

Animal Group	No. of Rats	Average Time of Exposure to Death (months)	Average Silica Content of Lungs µg/gm dry Lung
Asbestos Inhalation No Tumors	43	21	550
Asbestos Inhalation with Tumors	7	25	568
Asbestos Inhalation plus Intratracheal NaOH	15	26	612
Intratracheal Asbestos Injection	3	21	458
Intratracheal NaOH No Asbestos	10	23	557

The statistical treatment of these results is given in Table 2.

Table 2

Statistical Grouping of Results

	Group	Mean Silica Content	S.D.	No. of Rats	t	d.f.
a	Animals with cancer	568	270	7	0.188	48
	Animals without cancer	547	276	43		
b	Animals with lye treatment	612	282	15	0.768	63
	Animals without lye treatment	550	273	50		
c	Animals with asbestos dust	564	274	65	0.022	
	Animals without asbestos dust	566	150	14		

It was assumed that the results on the lungs were normally distributed, and F-tests were made to verify the assumption of equal variances. A t-test was then run on the data from a and b. A weighted t-test was used on c.

It is apparent that the silica content of the lungs of rats that had inhaled high concentrations of asbestos over a period of 16 months was extremely low and that differences in the silica content between groups that had lung cancer and that had no lung cancer was small. As a matter of fact, no group showed a decisive difference in the pulmonary silica content regardless of treatment or even if it had received no dust exposure. This was borne out in the statistical evaluation.

COMMENTS

These results proved extremely disappointing and led to the suspicion that errors in technique were responsible, particularly when the pulmonary silica concentration of some rats that had had no dust exposure was found to be higher than that of the dust-exposed animals. However, the duplicate values checked, and when the determinations were repeated, the newly-found values were very close to the original recorded values.

Once the analytical values were accepted as correct, the explanation was not difficult to find. Chrysotile is the most soluble of the three most commonly used forms of asbestos. Since approximately five to six years had elapsed between the time the lungs were first placed in formaldehyde solution and the time they were removed from the solution for analysis, a considerable amount of the stored chrysotile could have gone into solution within this time period. There are two reasons why the chrysotile dust stored in the lungs should have undergone dissolution readily. One reason is that the dust to which the rats had been exposed was ultrafine—the vast majority of the fibers could be seen only with an electron microscope. The fineness of the fibers greatly facilitated their dissolution. The second reason could explain why chrysotile tends to undergo dissolution more readily than the other types of asbestos. This reason is related to the tubular structure of the basic chrysotile unit which has an internal as well as an external surface. If the internal surface were also accessible to a leaching fluid, accelerated dissolution would be explained. A recent study in this laboratory has clearly demonstrated that

the central canal of chrysotile fibrils is indeed accessible to aqueous fluids. The fibrils illustrated in Fig. 1 show the presence of lead within the tubular lumen of fibrils that had been soaked in lead acetate solution.

If the lungs of the rats had been stored, each in its own container, and if a silica determination had been made of the fluid in the container with a suitable correction for the silica derived from the glass of the container, then the silica content of the lung tissue plus the corrected silica content of the fluid would have approximated the silica content of the lungs at the time of their submersion in the preserving fluid. Because a variable number of lungs from different groups of rats had been stored in jars containing aqueous formaldehyde solution, silica determinations would not have given any indication how much of the silica was derived from any specific lung.

CONCLUSIONS

The results of this investigation suggest that lung tissue containing deposits of finely divided chrysotile dust may lose this dust by dissolution if kept submerged in aqueous formaldehyde solution over a period of five to six years. Consequently, chemical determinations of chrysotile in lungs as silica will give misleading results if such lung tissue has been stored in aqueous fluid for appreciable periods—unless such lungs have been stored individually, and the corrected silica content of the fluid is added to that of the lungs.

Inasmuch as cancerous lungs and noncancerous lungs from asbestos-exposed rats, as well as lungs from unexposed rats, were stored in variable numbers in common jars, it was not possible to determine how much chrysotile

dissolved out of which lung. The unexpectedly high rate of dissolution of chrysotile dust in conjunction with the manner of storing a number of lungs in one jar of aqueous formaldehyde solution, made it impossible to estimate the level of asbestos dust burdens in the lungs of the experimental animals.

Ideally, asbestos determinations—particularly of chrysotile—should be made on fresh lung tissue or on lung tissue that has been fume-fixed after removal from the body and dried.

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LEGEND OF ILLUSTRATION

Fig. 1 Electron photomicrographs of chrysotile fibers that had been soaked in lead acetate solution. The presence of electron-dense material within the tubular lumens of the crystals suggests that the lumen of the fibrils was accessible to the solution. X 70, 000