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Experimental Asbestosis

Studies on the Progressiveness of the Pulmonary Fibrosis Caused by Chrysotile Dust

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THE unreliability of symptoms as a criterion for the progression of pulmonary fibrosis was underlined in a medical and psychiatric study of coal miners with respiratory complaints. Here, a very significant association was observed between a recognizable psychoneurotic factor and an apparent worsening of the clinical condition: of 40 patients studied, only 12.5% had disability on "physical" grounds alone; 35% had disability on psychiatric grounds alone; and the remainder on both.[1]

Furthermore, discerning clinicians recognize that opinions concerning the progression of a pulmonary fibrosis based upon the symptomatology (eg, shortness of breath) and a roentgenologic evaluation, even when reenforced by lung function studies may be quite erroneous. In the presence of a superadded coincidental pulmonary illness, such signs and symptoms may be reversible in whole or in part between such episodes, which include covert and overt acute pneumonitides, pulmonary edema, and allergic or chronic bronchitis—with or without emphysema, or emphysema without bronchitis. Such diagnostic difficulties were the subject of a recent symposium on emphysema in industry.

As uncertain as are the clinical criteria of progression of pneumoconiosis, very little help has come from postmortem studies of human lungs or those of experimental animals in defining the criteria by which progression of pneumoconiosis may be determined. This is particularly true of asbestosis.

It is the purpose of this paper to define some of the anatomic stigmata of progression of asbestosis and to determine whether or not asbestosis caused by chrysotile dust is progressive.

Methods and Materials

Lung burdens of chrysotile asbestos dust were imposed upon rats, hamsters, and guinea pigs by exposure in inhalation chambers as well as by intratracheal injection. Most of the animals exposed to dust in an inhalation chamber and reported in this study were part of a larger investigation that will be reported separately.

The inhalation chamber in which all animals (except four guinea pigs) were exposed to dust, measured 8 × 8 × 8 feet. The animals were housed in wire cages that were suspended in racks. Periodically, these cages were rotated so that inequalities in dust exposure caused by position were largely obviated. The exposures were for six hours per day, five days per week. The total exposure varied, as listed in the table.

The chrysotile asbestos was ballmilled and then fed into a hammermill (modified from a design by Holt and Young[2]). This was provided with inlet and outlet tubing so arranged that the comminuted asbestos was fed back continually into the hammermill. At the same time, the ultrafines were allowed to waft upward into the inhalation chamber. Two of these hammermills provided the chamber with sufficient dust to average 86 mg/cu m with a range of 42 mg/cu m to 146 mg/cu m. The asbestos dust cloud was evaluated by sampling with a two-stage size selective device, similar in design to that proposed by Wright[3] and operated at 20 liters/minute. The first stage of the instrument was a horizontal elutriator with selector characteristics recommended by the Johannesburg Pneumoconiosis Conference of 1959, namely acceptance of all particles having terminal settling velocities greater than that of a 7.1μ sphere of density 1 gm/cu cm, 50% acceptance

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of particles, the terminal settling velocity of which is equivalent to 5μ spherical particles of 1 gm/cu cm density, and dropping to zero acceptance of particles having terminal settling velocities equal to a 1μ sphere of 1 gm/cu cm density. All particles penetrating the first stage elutriator were considered "respirable" and were collected on a permeable-membrane filter which acted as the instrument's second stage. The filter was dried and weighed prior to and after collection of the sample. In this manner, it was found that 63% to 79% of the suspended asbestos dust in the exposure chamber was respirable, ie, its terminal settling velocity was less than that of a 7.1μ sphere of 1 gm/cu cm density.

The exhaust air from the inhalation chamber was drawn by suction through a pair of electrostatic precipitators. The chamber air was consequently always under negative pressure as long as the hammermills were operating. When the hammermills were shut down, fresh air was pulled through the chamber before the door was opened.

Periodically, dust from the electrostatic precipitators was collected and weighed. Since a record of the air flow through the chamber was kept, it was possible to calculate the average dust concentration in the chamber air by dividing the weight of the dust collected within a time period by the volume of air flowing through the chamber within that time.

As seen in the table, four guinea pigs were exposed to a concentration of chrysotile dust averaging about 20 mg/cu m. For this exposure, a smaller chamber was used and the dust was prepared by atomizing a suspension of ball-milled chrysotile asbestos at a pressure of 100 lb/sq in and impinging the emergent jet a near-sonic velocity against a tool-steel baffle. The average fiber length of this dust was 0.92μ as determined by measurements from electron photomicrographs. Inasmuch as other data on the size distribution of the dust are not available, it is not possible to state what percentage of the dust suspended in the chamber air was respirable. These animals were exposed for three months to this dust.

For intratracheal injections, the dust collected from the electrostatic precipitators was suspended in water so that 1 ml contained 3.5 mg dust. The rats and hamsters were injected intratracheally, some repeatedly, as listed in the table. The injections were made under light ether anesthesia with the aid of an illuminated speculum that allowed visualization of the vocal chords.

Some animals died at various times following the imposition of the dust burden. Others were killed approximately two years after the imposition of the lung dust burden as shown in the table.

Animals Exposed to, or Injected Intratracheally With, Chrysotile Asbestos Dust

	Species No. Animals	Inhalation Exposure (Months) at Dust Concentration (average) (86 mg/cu m)	(20 mg/cu m)	Intratracheal Injection No. 3.5-mg Injections	Time, End of Exposure to Death (Months)
		Rats			
	2	0.25			11-13
	6	0.5			13-24.5
	6	1			11.25-24
	6	2			11.25-23
	4	4			0.5-21
	4	8			3.5-17
	5		1		21
	5		2		21
	4		4		21
	10		6		21
Total	52				
		Hamsters			
	9		1		13.5-21
	5		2		12.5-16.5
Total	14				
		Guinea Pigs			
	6	0.5-5.5			0
	4		3		0-4
Total	10				

All animals were autopsied, the lungs were removed and expanded with 4% formaldehyde solution under a pressure of 12 cm water. Paraffin sections were cut at 6μ. Cleared unstained sections were examined under dark-field conditions[4] and appropriate fields were photographed on 35 mm film. These sections were then stained with hematoxylin and eosin and the same fields rephotographed. After decolorization and silver impregnation (Gordon and Sweets), the fields were photographed for the third time. A fourth photograph of the same fields was made after the sections had been subjected to microincineration at 600 C for an hour and treated with concentrated hydrochloric acid after cooling.

Representative lesions in the lungs of control animals were also photographed for purposes of comparison with the experimental lessons. There were 16 rats, 29 hamsters, and 13 guinea pigs that had been part of the shipments of the animals later put on test, but had been set aside away from the dust exposure as laboratory controls.

The results of this investigation are based largely upon a comparative study of about 700 fields selected from the sections of 76 animals.

Results

Rats (Intratracheal Injection).—Immedi-

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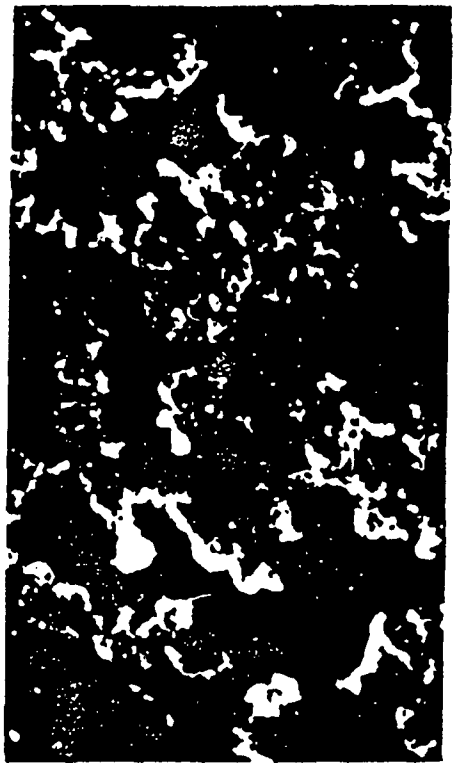

Fig 1.—Ash pattern of acid-insoluble material in lung of rat immediately following intratracheal injection of chrysotile dust. Alveolar ducts are outlined by fairly thick, continuous coating of dust. Some dust, mostly in the form of discontinuous deposits, is also found in some distal alveoli. Section was subjected to microincineration followed by treatment with concentrated hydrochloric acid (× 150).

Fig 2.—Ash pattern of acid-insoluble material, three days after intratracheal injection of chrysotile dust in rat. Dust, now in the form of flocculent masses of spiculated aggregates, seems to form casts of alveolar ducts and fills their lumens. Very little dust is noted in the peripheral alveoli (× 150).

ately after the intratracheal injection of chrysotile, the dust was seen applied as a uniform and continuous, dense coating upon the respiratory surfaces of the alveolar ducts and their evaginating alveoli (Fig 1). The lumens were everywhere widely patent. Surface coating by the dust of some of the distal alveoli was also observed, but not regularly.

After 72 hours, the proximal portion of the racemus[6] was a nearly solid, cellular structure in which the lumens of the respiratory bronchiole and alveolar ducts were obliterated by a polypoid mass of ovoid and plump spindle-shaped cells which also obliterated the lumens of the evaginating alveoli. A highly significant change in the distribution of the asbestos dust was now observed. The dust permeated the occlusive inflammatory tissue and thereby formed a cast of what was once the lumen (Fig 2). It was, however, no longer compactly disposed; rather, it was distributed in a flocculent manner. Nevertheless, the total amount of dust in the lumens of the alveolar ducts appeared to be considerably greater three days after the intratracheal injection than that present immediately after the injection. At the same time, less asbestos dust was observed in the more peripherally situated alveoli. It is probable that the increase in the amount of dust in the alveolar ducts was derived from the more peripheral alveoli. Although some of the finer dust may have been intracellular, the greater bulk, by far, was extracellular, being imprisoned in the inter-

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Fig 3.—Minimal, moderately healed asbestotic lesion in rat exposed to chrysotile dust for one month, then pastured and killed 24 months later. Lesion consists of a partially thickened alveolar duct. Mural thickening is caused by hypocellular connective tissue that has obliterated alveoli near bottom of field (hematoxylin and eosin, (× 150).

Fig 4.—Field same as in Fig 3 after silver impregnation shows that scar is composed on nonbranching, largely parallel, thick collagen fibers that are fairly densely arranged (Gordon and Sweets, (× 150).

stices of the precollagenous stroma of the inflammatory tissue.

When examined one year or longer after the intratracheal dust injection, asbestotic fibrosis was found in all rats so injected. The characteristic lesion was sharply limited to the alveolar duct as well as to the short respiratory bronchiole and consisted of moderate to severe collagenous thickening of the wall, often also associated with striking hyperplasia of smooth muscle (in more than one third of the injected animals). The collagenous mural thickening involved much of the wall and resulted in the obliteration of many of the evaginating alveoli. Generally speaking, the involvement of the alveolar ducts, even in animals injected with but a single dose of 3.5 mg of chrysotile dust, was greater than the minimal lesion observed in rats that had inhaled high concentrations of the dust for one month. As judged from the examination of a single section of both lungs of the rats, greater dust dosage was often, but not always, associated with an increased incidence of thickened alveolar ducts. The occurrence of larger collagenous scars containing small remnants of air spaces seemed also to be related to the multiplicity of the asbestos dust injections, as was a metaplasia to the columnar variety of the epithelium lining the surviving evaginating alveoli.

The sharply delimited mural thickening of the alveolar ducts (Fig 3) and respiratory bronchioles, with the collagenized, nonarborescent, thick stromal fibers (Fig 4), and the associated reduced cellularity (Fig 3),

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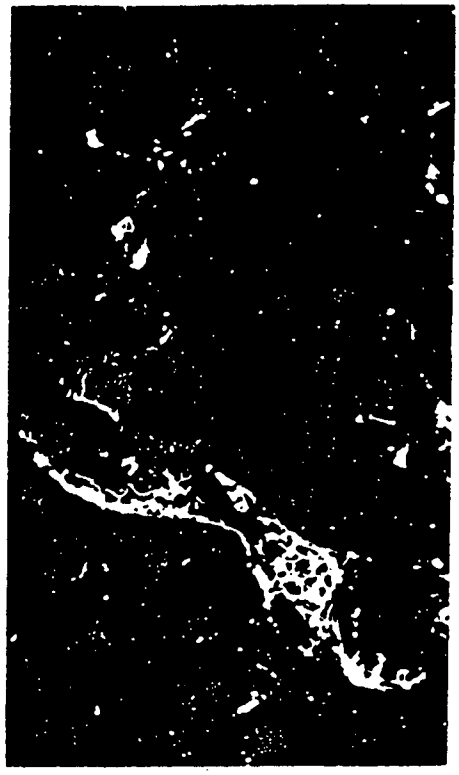

Fig 5.—Ash pattern of acid-insoluble material of same field as in Fig 3 after microincineration and treatment with concentrated hydrochloric acid, showing the presence of tangled masses of asbestos fibers in the regions of the scar tissue (× 150).

Fig 6.—Hyperplasia of smooth muscle in rat injected twice with 3.5 mg chrysotile dust and killed 21 months later. Well-defined bundles of smooth muscle are found in relation to alveolar ducts where the only normally present muscle is situated around mouths of evaginating alveoli (hematoxylin and eosin, × 150).

represented a healed or healing inflammation. Within this scar tissue, apparently sequestered compact masses of asbestos fibers were often strikingly prominent after microincineration (Fig 5.) On the other hand, many scars were seen that contained little or no demonstrable dust. The sharp delimitation of the scars was accentuated by the adjacent normal delicate alveolar walls. These were characterized by single, nonbranching argyrophilic fibers. Nevertheless, in some of the rats, there were also foci of a more active inflammation. Such foci were marked by cellular thickening of alveolar walls and by considerable desquamation. Here, the stroma was arborescent, the side branches tending to give structural support to the increased number of alveolar cells. Since similar inflammatory foci of cellular alveolar mural thickening were also found in the lungs of control rats not exposed to, or injected with, dust, it is believed that they were caused by unrelated spontaneous disease.

No asbestos bodies were found in rats exposed to or injected with chrysotile asbestos dust.

Rats (Inhalation).—The characteristic minimal lesion, as found a year or more after the pulmonary deposition of chrysotile dust, consisted of focal collagenous thickening affecting patches of alveolar duct wall between some of the evaginating alveoli (Fig 3 and 4). With more extensive deposition of the chrysotile fibers, the mural fibrosis tended first to narrow the mouths of the evaginating alveoli and then to close them

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Fig 7.—One of smaller cellular foci in lungs of hamster injected twice intratracheally with 3.5 mg chrysotile dust. Animal died one year later. The alveolar structure of cellular tissue is obliterated by cellular proliferations. There is a suggestion of a polypoid mass slightly to the right and above the center of the field (hematoxylin and eosin, × 150).

Fig 8.—Silver impregnation of same field as in Fig 7 shows a plethora of branching and interconnecting argyrophilic fibers forming thick alveolar walls and projecting into air spaces as polypoid masses of various sizes. Largest polypoid mass is one referred to in Fig 7. It fills the lumen of alveolar duct (Gordon and Sweets, × 150).

off entirely. The fibrosis also dipped into some of the evaginating alveoli, partially or completely obliterating them. In this manner, the respiratory bronchiole and adjoining alveolar ducts may become converted into relatively smooth-walled tubes with few or no evaginations. Most of the wall thickness of these tubes was attributable to the fibrotically obliterated alveoli that originally evaginated from the former.

The cellularity of the thickened structures varied considerably depending upon the time interval between the deposition of the dust upon the parenchymal surfaces and the death of the animal. In the florid stage of the inflammation, as seen in a rat exposed to the chrysotile dust for four months and killed two weeks later, the cells in the affected regions were large and numerous while the stroma consisted of an arborescent network of argyrophilic fibers in which the cells were enmeshed. In time, the argyrophilic fibers became thickened, condensed, and lost their arborescence as well as their argyrophilia; the latter coincident with their collagenous conversion (Fig 4). During this process of stromal maturation, the cellularity of the tissue diminished and the cells elongated to resemble fibrocytes.

In some of the rats examined a year or longer after the dust exposure, there were, in addition to the collagenous foci, cellular foci of more active inflammation that had no apparent relation to the proximal portion of the

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Fig 9.—Ash pattern of acid-insoluble material shows that region of polypoid mass in alveolar duct is densely permeated by tangled masses of asbestos fibers. Smaller masses and aggregates of dust are found in peripheral alveoli and other air spaces (× 150).

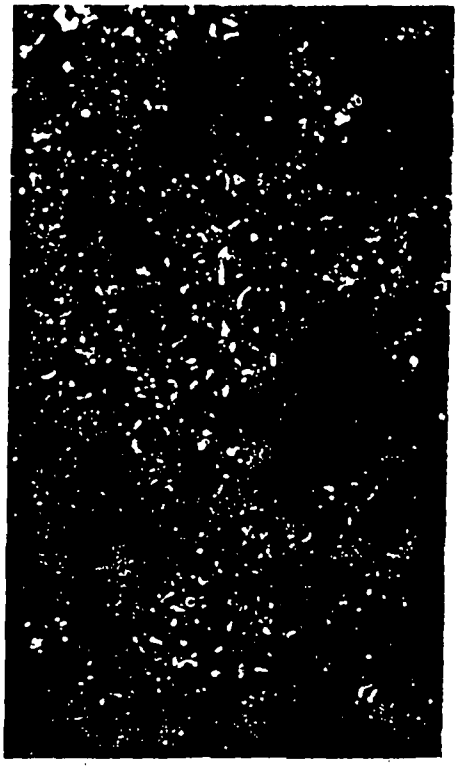

Fig 10.—Numerous luminescent asbestos bodies in region of asbestotic inflammation, from the lung of a hamster that received intratracheal injection of 3.5 mg chrysotile dust and died 13¾ months later (unstained section; 12.5/0.3 Leitz objective and 0.9 Leitz darkfield condenser × 150).

racemus. Some of these foci may also contain variable numbers of polymorphonuclear leukocytes. Similar cellular foci of active inflammation were also found in some of the unexposed laboratory control rats of comparable age.

Lung sections of all 18 rats exposed one month or longer to heavy concentrations of chrysotile dust exhibited minimal fibrotic pulmonary lesions one year or longer after the exposure. In contrast, in but one of six rats exposed to the same dust for only two weeks were similar changes found. The other five exposed animals had lungs that did not differ from those of unexposed rats. In neither of two rats exposed to the same dust for only one week, was there any evidence of asbestotic fibrosis 11 and 13 months, respectively, after exposure.

Compared with the amount of acid-insoluble ash seen in the lung sections shortly after the dust exposure, it appeared that only a small fraction of this amount of dust could be seen in the lungs of rats one or more years after the exposure. Nevertheless, in view of the ultramicroscopic size of much of the chrysotile dust, the failure of this method to demonstrate mineral dust in the tissue must not be construed to indicate necessarily that no dust was present. It is also of interest that the darkfield examination of the unstained sections with dry objectives was negative for luminescent material, even in regions later shown to contain abundant acid-insoluble mineral ash.

In the lungs of a number of rats exposed to chrysotile dust two months or more, there

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were bundles of smooth muscle that often constituted masses 50μ to 100μ in thickness (Fig 6). These muscle bundles were sharply delimited and related to the wall of thickened alveolar ducts. They had no connection with blood or lymph vessels nor could a relationship to the muscularis of a terminal bronchiole be demonstrated. In the newly formed connective tissue about some of the alveolar ducts and respiratory bronchioles, there were scattered gland-like structures. These pseudo-glands represent surviving units of the evaginating alveoli that have become lined by tall columnar epithelium, occasionally ciliated. No acid-insoluble mineral ash was demonstrable in the area occupied by the proliferated muscle fibers. The regions, pervaded by the more cellular and active inflammation, some containing leukocytes, were also devoid of demonstrable mineral ash.

Hamsters (Intratracheal Injection).—Of 19 hamsters, 16 died as a result of the asbestotic inflammation that followed the intratracheal injection of chrysotile dust. The three survivors had received only one injection and were killed 21 months after the imposition of the lung dust burden. The average survival of the hamsters that had one intratracheal injection was 18⅔ months, with a range of 13¾ to 20½ months. The animals that had received two intratracheal injections survived, on the average, 14¼ months from the time of the first injection, with a range of 12½ to 16½ months. The three survivors that were killed after 21 months were emaciated and also had extensive asbestotic disease.

Many of the lung sections showed extensive consolidation with relatively few patent air spaces. In other lung sections, the consolidation was patchy, seemingly concentrated about larger air spaces with relatively little aerated tissue between the consolidated portions. The consolidated portions were composed of masses of cells with vesicular round or oval nuclei—apparently alveolar cells (Fig 7). Leukocytes were not seen. Alveoli or larger air spaces usually could not be identified in these regions except after silver impregnation (Fig 8). The argyrophilic mural stroma of all air spaces in the solidified regions was greatly thickened by arborescent fibers that formed a loose network encroaching upon the lumens. Occasionally, the argyrophilic stroma extended into the lumen of an alveolar duct or a bronchiole in a polypoid manner, occluding it. The condensation of thin argyrophilic fibers to form parallel thick fibers devoid of arborescence and the transformation into collagen, characteristic of the lesions in rats, was not seen at all or to a very minor extent in these hamsters. For the most part, the occlusion of the air spaces was caused mainly by massed alveolar cells, but the contribution of the argyrophilic stroma should not be dismissed. It should also be emphasized that in contrast to the situation in rat lungs where the involvement was sharply limited to the alveolar duct and its evaginating alveoli, there was no such delimitation in the hamster lung. Here, the cellular as well as the stromal proliferation extended without abatement to the peripheral alveoli, thus accounting for the diffuseness of the consolidation.

Following incineration, abundant acid-insoluble mineral dust was observed in the involved racemi. Many individual fibers could be identified; some straight or wavy, others coiled and forming tangled masses. Compared with the amount of dust found in rat lungs, the chrysotile dust in the hamster lungs was much more abundant and more diffusely distributed (Fig 9). Asbestos bodies were found in abundance throughout the affected regions. They were considerably smaller than those seen in human lungs but were readily recognized. They were best seen under dark-field illumination with dry objectives in unstained sections (Fig 10). However, there was no parallelism between the number of luminescent asbestos bodies seen in the unstained sections and the amount of dust found in the same field after microincineration. The amount of dust present was much greater than might be suspected from the number of asbestos bodies observed. Most of the dust and the asbestos bodies were situated in the air spaces. When observed within tissue, the asbestos bodies and the dust were found trapped within the network of inflammatory argyrophilic fibers and associated cells that tended to occlude the air spaces.

Guinea Pigs (Inhalation).—Barely recognizable, minimal mural thickening of an occasional alveolar duct and respiratory bron-

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chiole was found in the lungs of guinea pigs that had been exposed for three months to an atmosphere containing 20 mg/cu m of chrysotile dust. Similar to the appearance of such lesions in hamsters, the mural thickening consisted of proliferated alveolar cells and their supporting stroma of interconnecting argyrophilic fibers. The lung sections of guinea pigs that had inhaled high concentrations (86 mg/cu m) of chrysotile dust for two weeks or longer, manifested similar minimal mural asbestotic thickening in the proximal portion of the racemus. However, the number of units (racemi) involved was much greater than that in the animals exposed to the lower dust concentration for three months. No evidence of healing (significant reduction in cellularity of the lesion and collagenization of the stroma) was seen in animals examined up to seven months after the beginning of the exposure. Following microincineration and treatment with acid, the amount of ash seen in the guinea pig lung sections was comparable to that noted in rats that had inhaled chrysotile dust. Asbestos bodies found in the lung sections were few and very small.

Comment

A chronic pulmonary inflammation may be termed progressive if it extends from its original site into adjoining, previously normal air spaces, and if it remains active, retaining its argyrophilic precollagenous stroma and high cellularity. Such a pulmonary inflammation may be considered healed if its argyrophilic stroma has been completely collagenized while its cellularity has become considerably reduced.

In the rat that has inhaled high concentrations of chrysotile asbestos fibers for only a few months or has been injected intratracheally with this dust, the asbestotic inflammation remains sharply limited to the proximal portion of the racemus and heals by becoming transformed into a hypocellular collagenous scar. Thus, it would appear proper to classify asbestosis caused by chrysotile dust as nonprogressive in the rat.

It is of basic interest that appreciable amounts of asbestos fibers are demonstrable within the scars of healed or healing inflammation. This would suggest that the disappearance of chrysotile dust from the lesion, either by dissolution or by transport, is not a sine qua non of healing; but that healing, at least in the rat, does take place—even in the presence of this dust. One would like to think of the formation of the asbestos body as a protective mechanism by which the asbestos fiber becomes sequestrated and the tissues safeguarded from further irritant action by the fiber. To what extent this mechanism may apply is not known; but it does seem that in rats, in which asbestos bodies are not demonstrable (with the optical microscope),[6] healing occurs in the presence of apparently naked fibers.

The diminution, in time, of the amount of dust demonstrable in the tissue and its apparent disappearance in some of the scars, poses an interesting question in regard to the mechanism by which the asbestos fibers disappear. It is commonly believed that chrysotile fibers have a relatively high solubility in tissue fluid. This would seem to account for the inability to demonstrate asbestos fibers in some of the asbestos bodies found in human asbestotic lungs. However, dissolution of the fibers, particularly of the coarser ones, would require a long time—so that it would be difficult to explain the failure of peripheral alveoli to become involved by inflammation. Furthermore, high solubility would not be consistent with the sharp inflammatory localization of the asbestotic lesion in the proximal portion of the racemus in rats. It is much more reasonable to explain this sharp localization of the inflammation on a fairly prompt removal of the inhaled or injected irritant dust from the peripheral alveoli and the subsequent stagnation of the dust in the proximal portions of the racemus.[7] The transport of the chrysotile dust from the peripheral alveoli is effected by the alveolar clearance mechanism consisting of a proximally moving film of alveolar fluid.[8] This transport is dramatically illustrated by the increase in, and concentration of dust in the lumen of the alveolar duct within 72 hours after the intratracheal injection of chrysotile dust (cf Fig 1 and 2). It may be of interest at this point to note that the localization of the early asbestotic lesion to the proximal portion of the racemus was first described by Vorwald et al,[6] and recently confirmed by Holt et al.[9]

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The reaction of the hamster lung to chrysotile dust is the antithesis of that observed in rats. The lesion extends from the proximal portion of the racemus to the peripheral alveoli, thereby producing extensive consolidations in the hamster lungs. The consolidations are composed of obliterated air spaces, the lumens of which are filled with masses of alveolar cells, mostly desquamated (Fig 6). Although the inflammation involves the entire racemus in this animal, the proximal portion shows more severe involvement than the peripheral portion. It is this extensiveness of asbestotic involvement with consequent pulmonary inadequacy that accounts for the high mortality of the hamsters.

With the previously given definition of progression in mind, it is seen that the diffuse involvement of the racemus in the hamster would fit the first requirement for progressiveness of the disease, if it can be shown that the inflammation originally was confined to the proximal portion of the racemus and then extended into the peripheral air spaces. This information, unfortunately, is not available from the present investigation. However, the finding of a more severe involvement of the proximal portion of the racemus favors the probability that such an extension took place. The second requirement of progression, ie, that the inflammation remain active and nonhealing, is a conspicuous feature of the asbestotic hamster lungs. The failure of the argyrophilic fibers to lose their arborescence and to collagenize and the persistance of the extreme cellularity of the involved tissues nearly two years after the intrapulmonary dust deposition is incontrovertible evidence of nonhealing.

Two possible explanations come to mind for the difference in the disease produced in the rat and the hamster by the same chrysotile dust. One explanation is that the reactivity of hamster tissue to the dust is greater than that of rat tissue. The other is that the hamster's alveolar clearance mechanism is not as capable as that of rats in transporting the dust from the peripheral air spaces proximally. As will be seen below, both explanations apply.

There is some evidence that the hamster's pulmonary clearance mechanism is not as effective as that of the rat. This is seen in the considerably larger amount of acid-insoluble mineral ash demonstrable in the hamster lung sections than in those of rats. Further evidence is seen in the diffuseness of the dust distribution in the hamster (Fig 9) in contrast to its restriction to the proximal portion of the racemus as in the rat (Fig 5).

The reactivity of hamster tissue to chrysotile fibers is significantly different from that of rat tissue, the former being much more florid. This is seen not only in the plentiful production of asbestos bodies in hamster lungs but also in the failure of argyrophilic precollagenous fibers to mature into collagen as well as in the undiminishing cellularity of the inflammatory tissue even after a lapse of nearly two years. This difference in the reactivity of lung tissue in the rats on the one hand and in hamsters on the other is not unique to chrysotile dust inasmuch as similar and quite comparable differences in these two species have been noted in their reactivity to quartz dust.[10]

It is highly probable that interspecies differences in the efficiency of the pulmonary clearance mechanism play an important role in the susceptibility of the different species to certain pulmonary diseases. It is recognized, for example, that horses and mules working during their lifetime in mines exposed to quartz dust will have accumulated a negligible amount of silica in the lungs and will demonstrate no silicosis;[11] whereas miners in the same workings will have varying degrees of silicosis as well as a significantly elevated pulmonary silica content. We have recently exposed rats and mice simultaneously to quartz dust in the same inhalation chamber for the same length of time. All animals were killed immediately after the end of the dust exposure. Well-defined silicotic nodules were present in the rat lungs; whereas, the mouse lungs were absolutely normal, and no acid-insoluble dust could be demonstrated in the mouse-lung sections after microincineration (unpublished data).

The above observations are relevant to the present investigation insofar as such interspecies differences tend to make extrapolation from animal data to human disease unreliable. The questions to be resolved are whether the efficiency of the clearance mechanism of man approximates that of the

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rat or of the hamster and whether the tissue reactivity of the rat or of the hamster more closely resembles that of man.

A study of the ash-pattern (microincineration) of a number of lungs from asbestos workers (exclusive of cases of silicoasbestosis) has disclosed no instance in which the amount of mineral dust in the lung sections has been more than scanty.[12,13] Other investigators have found upon chemical analysis that the silicate content of manifestly asbestotic lungs was surprisingly low.[14,15] These observations approximate the findings in the rat. Another similarity of human chrysotile asbestosis to findings in asbestotic rat lungs is the complete collagenization with associated acellularity that may be found in "burned-out" cases of human asbestosis.[13] Nevertheless, in spite of the similarity of the collagenization in man and rat, there is an important and significant difference. The early disease in rats is multifocal, affecting the proximal portion of the racemus; whereas in man such multifocal distribution has not been described—only diffuse involvement. A possible explanation of the diffuseness of asbestotic involvement in human lungs could be that the inflammation, initially confined to the proximal portion of the racemus because of continued exposure, spreads peripherally, thereby becoming confluent with that of neighboring racemi. The fact that an asbestotic inflammation may "burn out" in man, does not rule out the possibility that this inflammation remained active and progressive for some years after exposure had ceased before finally attaining the terminal healed stage. There is, unfortunately, no information on the activity of the asbestotic inflammatory process in human lungs at various intervals following removal from further exposure to the specific dust.

In guinea pigs, the results of the inhalation of chrysotile dust are very similar to those observed in rats with respect to the site and extent of the lesion, but conversion of argyrophilic stroma to collagen is not encountered in the sections of guinea pigs that had been killed up to seven months after the initiation of the dust exposure. The amount of dust found in the lung sections is scanty and is limited to the proximal portion of the racemus. However, the number of animals exposed to the different dust concentrations is too small, and the time allowed for maturation of the lesion too short to permit definitive conclusions.

The results of this investigation also have given some insight into the relative pathogenicity of chrysotile asbestos dust for rats insofar as a single intratracheal injection of 3.5 mg of this dust has produced minimal asbestotic lesions in rats. This result may be compared with that of King, et al[16] who found that a quantity of more than 2 mg and less than 5 mg of quartz dust injected intratracheally into rats was the smallest amount of silica capable of producing demonstrable silicosis in this animal. Thus, it seems that for the rat, chrysotile has about the same order of pathogenicity as quartz dust.

For the hamster, the smallest amount of intratracheally injected chrysotile dust capable of producing minimal lesions has not been determined. This dose will apparently be smaller than that found for rats.

By the inhalation technique, using a very high concentration of respirable chrysotile dust (86 mg/cu m), the exposure time required to produce minimal asbestosis in rats and guinea pigs seems to be somewhere between 60 and 120 hours.

The hyperplasia of smooth muscle observed in the asbestotic rats is very similar to such hyperplasia observed in human asbestosis cases studied in this laboratory and is also similar to that found in bronchiolar emphysema of the lung.[17] Although the exact origin of the muscle was not determined, its location made it appear most likely that it was derived from the circular muscle in the alveolar ducts that regulate the openings of the evaginating alveoli.

Summary and Conclusions

Rats, hamsters, and guinea pigs were given various lung dust burdens of chrysotile asbestos; some, by inhalation and others, by intratracheal injection. An attempt was made to determine the smallest amount of chrysotile dust that would produce a recognizable minimal asbestotic lesion. The time lapse between the imposition of the lung dust burden and the death of the animal varied from zero to 24 months.

The following conclusions have been reached:

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1. The characteristic early asbestotic lesion is situated in, and sharply localized to, the proximal portion of the pulmonary racemus which, in the rat, consists of a very short respiratory bronchiole and adjoining alveolar ducts.

2. Asbestosis in rats, caused by chrysotile dust, is nonprogressive.

3. The evidence for the nonprogressiveness of chrysotile asbestosis in rats consists of the observations that the minimal asbestotic lesion is limited to the wall of the respiratory bronchiole and of the adjoining alveolar ducts and does not extend into adjoining normal alveoli and that the minimal asbestotic lesions heal by becoming collagenized and hypocellular.

4. The localization of the minimal asbestotic lesions is attributed to the proximal transport of dust from peripheral air spaces by the alveolar clearance mechanism and subsequent stagnation of the transported dust in the proximal portion of the racemus.

5. In rats chrysotile asbestotic lesions heal in the absence of demonstrable (optical microscope) asbestos bodies and in the presence of chrysotile fibers, the latter becoming trapped in the scar tissue.

6. There is a considerable reduction in the amount of chrysotile dust found in the lung sections of rats one year or more after the imposition of the lung dust burden as compared with the amount of dust present shortly after the dust burden was imposed.

7. The reaction of hamster lungs to chrysotile dust is the antithesis of that observed in rats. The lesion is progressive, extending into peripheral air spaces so as to involve the entire racemus and thereby producing extensive consolidations leading to the death of the animals. The lesions do not heal, since the precollagenous stroma and the high cellularity persists through the two-year period of observation. At the same time, the amount of asbestos dust demonstrable in the sections remains large and diffusely distributed.

8. The progressiveness of the chrysotile asbestotic lesion in hamsters is ascribed to a less effective pulmonary clearance mechanism and to a greater reactivity of the pulmonary tissue than exist in rats.

9. The early chrysotile asbestotic lesion in guinea pigs resembles that of rats, but it has not been adequately studied to permit further characterization.

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References

1. Ross, W.D., et al: Emotional Aspects of Respiratory Disorders Among Coal Miners, *JAMA*, 156:484 (Oct 2) 1954.

2. Holt, P.F., and Young, D.K.: A Dust-Feed Mechanism Suitable for Fibrous Dust, *Ann Occup Hyg* 2:249, 1960.

3. Wright, B.M.: A Size-Selecting Sampler for Air-Borne Dust, *Brit J Industr Med* 11:284, 1954.

4. Gross, P., and Tolker, E.B.: Dust Particles in Lung Sections: Some Notes on Methods of Their Visualization, *Arch Environ Health* 12:213, 1966.

5. von Hayek, H.: *The Human Lung*, V.E. Krahl (trans), New York: Hafner Publishing Company, Inc., p 172.

6. Vorwald, A.J.; Durkan, T.M.; and Pratt, P.C.: Experimental Studies of Asbestosis, *Arch Industr Hyg* 3:1, 1951.

7. Gross, P.; Pfitzer, E.A.; and Hatch, T.F.: Alveolar Clearance: *Its Relation to Lesions of the Respiratory Bronchiole*, *Amer Rev Resp Dis*, 94:10, 1966.

8. Hatch, T.F., and Gross, P.: Pulmonary Deposition and Retention of Inhaled Aerosols, New York: Academic Press, Inc., 1964, p 69.

9. Holt, P.F.; Mills, J.; and Young, D.K.: Early Effects of Chrysotile Asbestos Dust on Rat Lung, *J Path Bact* 87:15, 1964.

10. Gross, P.; deVilliers, A.J.; and deTreville, R.T.P.: Experimental Silicosis, *Arch Path* 84:87-94, 1967.

11. Einbrodt, H.J.; Klosterkötter, W.; and Metze, H.: Vergleichende Untersuchungen über die Korngrössen retinierter Stäube in den Lungen von Mensch und Tier, *Beitr Silikoseforsch* 6:491, 1963.

12. Gross, P., and Smith, K.W.: The Topographic Distribution of Mineral Dusts in Some Pneumoconiotic Lungs, *Dis Chest* 35:140, 1959.

13. Gross, P.: "Pathology of the Pneumoconioses," in Lanza, A.J.L. (ed.): *The Pneumoconioses*, New York: Grune and Stratton, Inc., 1963, p 51.

14. Knox, J.F., and Beattie, J.: Mineral Content of the Lungs After Exposure to Asbestos Dust, *Arch Industr Hyg* 10:23, 1950.

15. Nagelschmidt, G.: Some Observations of the Dust Content and Composition in Lungs With Asbestosis, *Ann NY Acad Sci* 132:64-76, 1955.

16. Ray, S.C.; King, E.J.; and Harrison, C.V.: The Action of Variable Amounts of Quartz on the Lungs of Rats, *Brit J Industr Med* 8:62, 1951.

17. Siebert, F.T., and Fisher, E.R.: Bronchiolar Emphysema, *Amer J Path* 33:1137, 1957.

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