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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 μ 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)	Intra tracheal 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 lesions. 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-

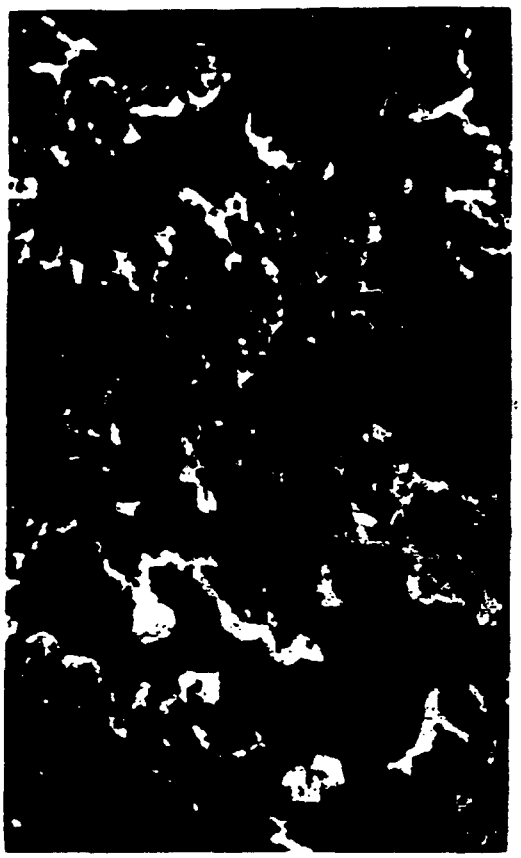

Fig 1.—Ash pattern of acid-insoluble material in lung of rat immediately following intratracheal injection of chrysotile dust. Alveolar ducts are outlined by fairly thick, continuous coating of dust. Some dust, mostly in the form of discontinuous deposits, is also found in some distal alveoli. Section 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[5] 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),

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

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

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 33¼ months later (unstained section, 12.5/0.3 Leitz objective and 0.9 Leitz dark-field 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 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 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 considered to indicate necessarily that no dust was present. It is also of interest that the dark-field examination of the unstained sections with low 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 50 to 100 μ 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 musculatis 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 affected regions. They were considerably smaller than those seen in human lungs but were readily recognized. They were also seen under dark-field illumination with 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-

[illegible] exposed [illegible] [illegible] containing [illegible] the dust [illegible] appearance [illegible] lesions in [illegible] consisted of [illegible] cells [illegible] interconnect [illegible] pigs [illegible] inhaled high concen[illegible] [illegible] of chrysotile dust for [illegible] weeks or longer manifested similar [illegible] asbestotic thickening in the [illegible] portion of the racemus. However, [illegible] number of units [illegible] 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 [illegible] 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 [illegible] either by dissolution or by transport, [illegible] 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,[7] 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,[8] and recently confirmed by Holt et al.[9]

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 siliciosis 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

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 intracellular.

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 in rats, but it has not been adequately studied to permit further characterization.

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PLAINTIFF'S EXHIBIT

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EXPERIMENTAL STUDIES ON ASBESTOS

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Since the establishment of the Pneumoconiosis Research Unit in Johannesburg in 1954, investigations have been undertaken to ascertain whether pulmonary lesions and complications following the inhalation of crocidolite and amosite dusts were similar to those associated with exposure to chrysotile dust.[1] The results indicated that crocidolite and amosite dusts essentially caused an interstitial fibrosis, this was seen to be peribronchiolar and perivascular in distribution during the early stages, and in the later stages the lesions became diffuse, with alveolar wall thickening and peribronchiolar and perivascular fibrosis.

Other clinical investigations suggest that asbestos may be implicated in the development of malignant tumours of the pleurae, and primary neoplasms of this nature have been described by many authors in recent years.[2-9]

The pathological evidence associating these tumours with asbestos appeared to be inconclusive. Wagner,[1] however, reported that 47 cases of mesothelioma had been identified in South Africa up to the end of June 1960. In 45 of these a possible association with exposure to crocidolite from the Northern Cape has been established. Whether the primary factor in the development of these tumours is exposure to asbestos dust, or to a contaminant of it, is as yet not known. According to Wagner, one would expect similar cases from the Lydenburg district, since the nature of the rock, ore and fibre, are almost identical with those of the Northern Cape.

Attempts to produce tumours in experimental animals by exposing them to an atmosphere of asbestos dust or to intratracheal injection of asbestos have been disappointing.[10] Vorwald *et al.*[11] found that ordinary industrial asbestos dust and long fibres from which the small particles had been separated gave peribronchiolar fibrosis. With dust particles 3μ or less there was little or no reaction in experimental animals. Behrens[12] also reported that pure chrysotile asbestos injected intraperitoneally into mice and intratracheally into rats produced only a non-specific fibrosis that might be due to a foreign-body reaction. At present no evidence is available regarding the pathogenicity of crocidolite in experimental animals, although clinical reports incriminate crocidolite as a possible aetiological factor in the development of malignant tumours in the lung of humans. The present preliminary study was therefore planned to investigate the pathogenicity of crocidolite and a mixture of crocidolite and quartz in the lungs of rats.

TABLE I. SIZE DISTRIBUTION OF PARTICLES OF CROCIDOLITE AND QUARTZ

	Crocidolite %	*Quartz* %
> 5μ	20	10
< 5μ	80	90
< 2μ	40	50
< 1μ	30	30

MATERIALS AND METHODS

Dust. Samples of very pure rock crystal (quartz) and [illegible] obtained from the Department of Geology, University of Stellenbosch. [illegible] asbestos fibres were isolated, cut up as finely as poss[ible] and ground for several days. The rock crystal was crus[hed] and also ground in an agate ball mill. The size distr[ibu]tion of the particles is given in Table I. A mixture [of] quartz and asbestos was prepared in the proportion 25:75. The chemical composition of the crocidolite asbe[stos] is given in Table II.

TABLE II. CHEMICAL ANALYSIS OF CROCIDOLITE (from Vermaas[13])

SiO_2	51·94	CaO	0·1[illegible]
Al_2O_3	0·20	Na_2O	6·0[illegible]
Fe_2O_3	18·64	K_2O	0·0[illegible]
FeO	19·39	H_2O^+	0·0[illegible]
MgO	1·37	H_2O^-	2·5[illegible]
		Total	100·7[illegible]

Animals. Albino rats (*Rattus norvegicus*, Wistar In[sti]tute) weighing 150 - 175 G. were used. Two groups of [...] animals each were injected intratracheally with suspens[ions] of asbestos and a mixture of quartz and asbestos, res[pec]tively.

Dust suspensions. The dusts were suspended in 0[...] saline in the proportion of 50 mg. of dust per 1 ml. [...] samples of each of the dusts were weighed out in sc[rew-]capped bottles, 20 ml. of saline added, and the suspen[sions] sterilized by autoclaving for 20 minutes at 15-lb. press[ure]. The suspensions were shaken before use.

Injection of dusts. The animals were lightly anaesthet[ised] with ether. They were tied on their backs on a slop[ing] dissecting board (± 45°), the tongue retracted with a [...] a speculum introduced through the mouth into the thr[oat] and the head drawn forward and downward until [the vocal] chords were visible. A 16-gauge blunt hollow needl[e ...] inches long, was then inserted into the trachea; a syr[inge] containing 1 ml. of suspension and 1 ml. of air [was] quickly attached and the suspension injected forcibly [into] the lungs.

Duration of experiment. The experiments lasted [...] days and one rat out of each group was killed at 20[-day] intervals. A few animals died and had to be discarded.

Histological technique. The animals were anaesthet[ised] with ether, the trachea exposed, and 10 ml. of [...] formol-saline injected into the lungs by inserting a syr[inge] needle into the trachea. The tracheas were then tied [...] the animals autopsied, and the unopened lungs preserve[d in] 15% formol-saline. After fixation, the lungs were secti[oned] in a sagittal plane near the hilum and whole-lung bl[ocks] were embedded in paraffin wax. Serial sections of [...] thickness were cut from each block. Of these, two [were] stained with haematoxylin and eosin and another imp[reg]nated with silver (Gordon and Sweet[14]). The remai[ning] sections were kept as spares.

Grading. The grading of the maturity and severit[y of] the lesions was done according to the new system of [...] *et al.*[15] as follows:

Grade of fibrosis	Amount of fibrosis
Loose reticulin, no collagen	Very few nodules
Compact reticulin with or without collagen	Few nodules
Somewhat cellular but made up mostly of collagen	Moderate number of nodules
Wholly composed of collagen fibres and virtually acellular	Many silicotic nodules
Acellular, collagenous, confluent	Large areas involved

RESULTS

Macroscopic Appearances

Macroscopic examination of the lungs of the animals the asbestos and quartz-asbestos groups showed no outstanding differences over the first 100 days. The dust distribution between the two lungs tended to be irregular, nd more dust apparently entered the left lung than the ght lung. Foci of dust were seen on the surface of the ings, but no fibrotic changes could be observed.

After 100 days the lungs of the quartz-asbestos animals became firmer and also larger in size compared to the ungs of the asbestos group. Areas of fibrosis, particularly n the dorsal aspects of the left lungs, were seen while in he asbestos group the lungs remained soft with only a ew minute lesions visible on the pleural surfaces.

Microscopic Appearances

The grade of fibrosis and the amount of fibrosis produced by asbestos and an asbestos-quartz admixture in the lungs of rats over a period of 220 days are given in Table III.

TABLE III. GRADE AND AMOUNT OF FIBROSIS PRODUCED BY ASBESTOS AND AN ASBESTOS-QUARTZ ADMIXTURE IN THE LUNGS OF RATS

Days of survival	Asbestos			Asbestos and Quartz		
	Grade of fibrosis	Amount of fibrosis	Total fibrosis	Grade of fibrosis	Amount of fibrosis	Total fibrosis
20	1	2	2*	1	3	3
40	1	2	2	1	3	3
60	1	3	3	1	4	4
80	2	4	8*	1	3	3
100	1	1	1	1	2	2
120	1	3	3	2	3	6
140	1	3	3	2	3	6
160	2	1	2*	2	4	8
180	1	3	3	1	1	9
200	3	2	6*	3	3	9
220	1	1	1	1	3	9*

*Indicates cases with infection.

The pathological changes caused by these two dust samples followed more or less the same pattern over the first 100 days after the intratracheal administration of the dusts. Some differences were observed concerning the cell types involved, the rate of phagocytosis of the dusts, and the sites of tissue reaction, as well as the incidence of infection. On the introduction of both dusts a typical foreign-body reaction was found. The reaction in the quartz-asbestos group was more severe and numerous mononuclear cells and leukocytes were mobilized, much more so than in the lungs of the asbestos group, where most of the asbestos particles were phagocytosed within 80 days and relatively limited cell destruction occurred compared to the asbestos-quartz group. In the latter group some asbestos and quartz particles were found lying free

Asbestos dust alone was readily ingested by the macro phages and transported to the vicinity of the small bron chioli, where big aggregates of dust eventually accumu

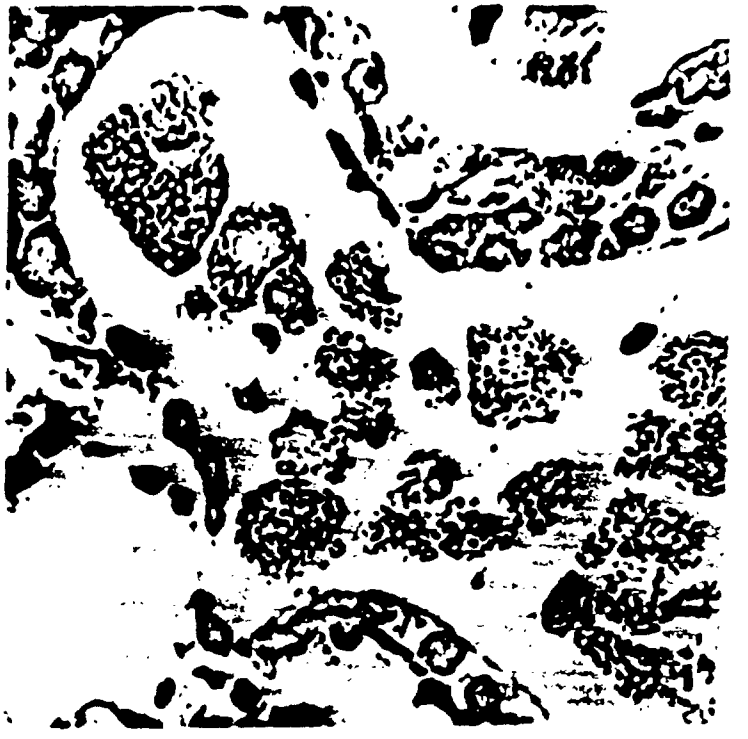

Fig. 1. Lung section showing numerous macrophages loaded with asbestos particles in the vicinity of a bronchiole, with some alveolar wall thickening, 60 days after the intratracheal injection of 50 mg. of pure asbestos dust. (H. & E. × 450.)

lated. The dust lesions in the asbestos lungs were therefo primarily encountered around the bronchioli and to lesser extent in lung areas with a relatively high conce tration of dust. These lung areas were well marked alveolar wall thickening and the accumulation of numero macrophages and giant cells, loaded with asbestos partic (Fig. 1). The quartz-asbestos mixture, on the other han provoked a general and widespread tissue reaction which the bronchioli, blood vessels and alveolar walls w involved. It appeared as if the presence of a small conce tration of quartz interfered with the phagocytosis of dust to such an extent that a diffuse fibrosis resulted.

Hyperplasia of the lymph nodes of the lung was common in the asbestos group except in cases w infection. In the quartz-asbestos lungs the nodes appea to be hyperplastic and infiltrated with fibrous tissue.

The incidence of lung infection in animals injected w pure asbestos tended to be higher than in those wh received the asbestos-quartz mixture. From the histolog sections it was observed that the bronchioli of seve lungs injected with asbestos dust were comple blocked with mucus and infiltrated by leukocytes, mac phages, and mononuclear cells. Dust lesions in these l areas, isolated from the respiratory tree, showed a mar increase in collagen content and progressed to grad fibrosis (Fig. 2). In animals without infection the prog sion of the lesions was relatively slow and they reac only grade-1 fibrosis after 220 days (Fig. 3).

In the quartz-asbestos group an acute infection

...only one animal and the fibrotic changes in this ...could therefore not be attributed to infection. Although the progression of the lesions in this group was produced in this period (Fig. 4) compared to a grade ... animals without infection, in the asbestos group.

No malignant tumours or any neoplasms of the p...

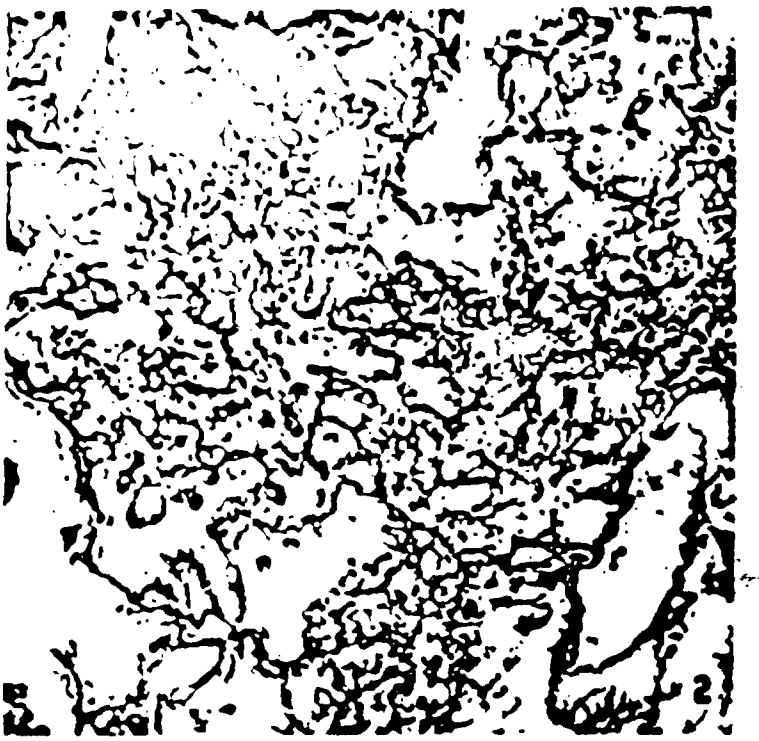

Fig. 3. Rat lung with infection, 200 days after the injection of 50 mg. of asbestos dust, showing grade-2 fibrosis. (Silver impregnation × 150.)

Fig. 4. Rat lung section, 200 days after the injection dust consisting of 12·5 mg. quartz + 37·5 mg. asbest... showing massive grade-3 fibrosis. (Silver impregnation 150.)

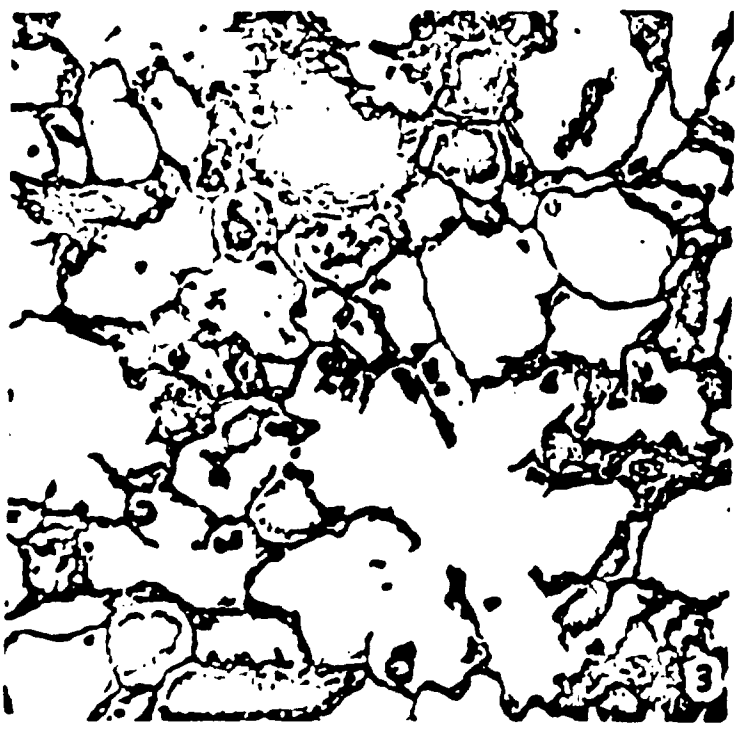

Fig. 4. Rat lung without infection, 220 days after the injection of 50 mg. of asbestos dust, showing grade-1 fibrosis, dust aggregates, and alveolar wall thickening. (Silver impregnation × 150.)

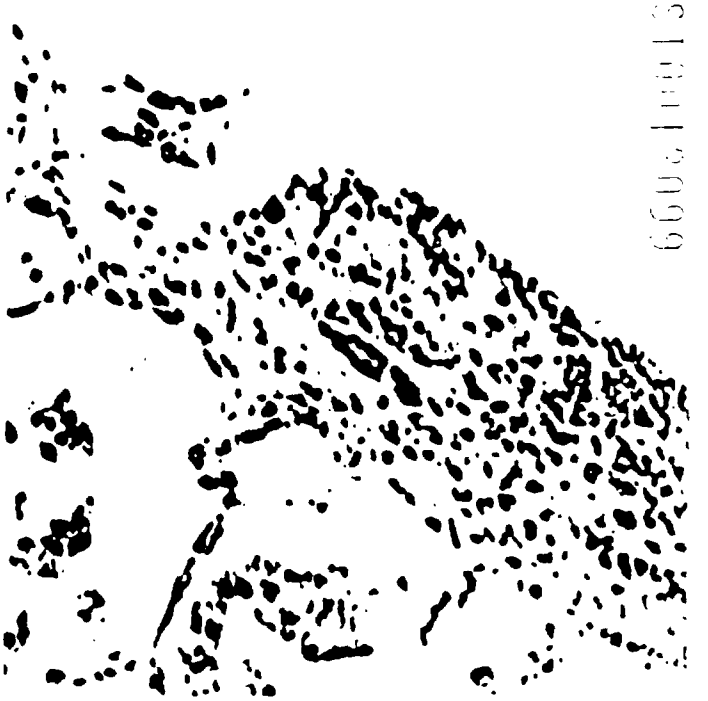

Fig. 5. Part of lung section, 180 days after injection of quartz-asbestos mixture, showing pleural thickening w... mononuclear-cell infiltration. (H. & E. × 150.)

exceptionally slow up to 100 days, a marked increase in ...and thereafter till the termination of the

were observed, although pleural thickening was found ... a few cases with infection and in most animals inje...

[illegible] the presence of reticulin in the lungs of rats injected with pure asbestos particles and an asbestos-quartz admixture was found to differ significantly in the later stages of the experiment. In the asbestos-quartz group a progressive increase of reticulin and collagen in the dust lesions was observed, resulting terminally in the production of compact, partially acellular, collagenous nodules. In the asbestos group progression of the initial reticulin was limited and it ended with only slight increase in compactness with or without a few fine collagen fibres in cases with acute infection.

The present results demonstrate that fine asbestos particles are of a very low toxicity to lung tissue and produce only peribronchiolar, and to a lesser degree perivascular, reticulin networks. Even in cases with acute infections, fibrogenesis did not progress beyond grade-2 fibrosis. The typical asbestosis of humans could not be induced with pure fine asbestos dust in the lungs of rats. However, the addition of silica dust (12.5 mg.) to the same asbestos dust (12.5 mg.) led to massive fibrosis (advanced grade-3). Both the maturity and the amount of fibrosis seemed excessive when compared to the lesions caused by equivalent amounts of the individual dusts. The microscopic pattern of the pathological changes appeared to be very similar to those described by Gloyne[16] and Lynch and Cannon[17] in humans. Bouser *et al.*[18] concluded that the common factor in the lungs of miners of asbestos and iron-ore is silica and it is suggested that this may be the carcinogenic agent, causing pulmonary fibrosis, which precedes the initiation of the malignant process. Doll[5] also pointed out that the risk of lung cancer in asbestos workers was of the order of 10 times that experienced by other men. Probably this risk was greater before 1933 and has become progressively less during recent years as the duration of employment under the old dusty conditions has decreased.

The fact that no malignant tumours have been produced by either crocidolite asbestos or a quartz-asbestos admixture might be due to the very high resistance of the rat species to neoplasms, as well as to the limited duration of the present experiments. However, it appeared that free silica enhanced the pathogenicity of asbestos dust and that in the presence of silica some pleural changes have been produced, although not of a malignant nature.

Recently it was pointed out by Harrington[19] that oils containing 3,4 benzpyrene and related substances occur in crocidolite and amosite. The significance of the association between asbestos fibres and hydrocarbons on the one hand and the production of malignant neoplasms on the other hand could not be assessed from the present experiments, because weathered crocidolite has been used in which hydrocarbon content might have been extremely low. However, it is doubtful whether the concentration of benzpyrene in virgin crocidolite would be high enough to produce neoplasms, seeing that coal dust with a relatively higher concentration appeared to be inert.

The present results support the conclusions of Bouser *et al.*[18] that silica in combination with asbestos may be extremely dangerous.

SUMMARY

The pathogenicity of pure, very fine crocidolite particles and of a mixture of crocidolite and quartz was investigated after intratracheal injection of 50 mg. of these dusts into the lungs of rats. The proliferation of reticulin due to these two dusts respectively was found to differ significantly.

The crocidolite particles elicited an initial reticulin that became slightly more compact, with or without collagen fibres, especially in cases with acute infection. It appeared that exposure only to asbestos dust predisposed to acute respiratory infections.

The asbestos-quartz admixture gave a progressive increase in reticulin and collagen, resulting in partially acellular collagenous nodules (grade-3 fibrosis). The histological picture of the pathological changes appeared to be very similar to those observed in human asbestosis. Asbestos dust in combination with a small amount of free silica should be regarded as extremely dangerous.

No malignant tumours have been produced in the experimental period by either asbestos or the asbestos-quartz admixture. Some non-malignant pleural changes were observed in animals of the asbestos group with inflammation, and in animals of the asbestos-quartz group.

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A CASE OF PERSISTENT THYMUS DIVERTICULA IN THE NECK

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The persistence of stalks of thymic tissue in the neck, demonstrating the embryological origin of the thymus gland from the entoderm of the third pharyngeal pouch, is undoubtedly a rare condition. In the course of 30 years' experience of teaching anatomy this is the first dissecting room. Descriptions of such a congenital abnormality are absent in many standard anatomical and embryological textbooks, and only a few references could be found. In Braus' *Anatomie des Menschen* (19[illegible]) there is a schematic and hypothetical drawing of the n