

THE UNIVERSITY OF MANITOBA

EXPERIMENTAL ASBESTOSIS - THE PATHOLOGIC EFFECTS OF CHRYSOTILE
ASBESTOS ON THE RESPIRATORY TISSUE OF THE RAT

by

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ABSTRACT

The biological effects of chrysotile asbestos dust on the pulmonary tissue of rats were examined both morphologically and cytodynamically. Groups of rats were treated by intratracheal injection with suspensions of natural chrysotile, synthetic chrysotile, fibreglas, and carbon dust. In addition a group of rats were treated likewise with the saline suspending medium alone. The animals were sacrificed at different time intervals after treatment. It was observed that only the natural chrysotile asbestos appeared to exert drastic pathological effects on the pulmonary tissue of the animals. It was therefore postulated that the specific biological effects of chrysotile asbestos did not depend solely on a single factor such as fibrous structure, chemical or physical property, or fibre size of the mineral. Rather a combination of some, if not all of these properties were responsible for the specific biological effects of chrysotile.

The chrysotile whether in a natural or synthetic form exerted pronounced inhibitory effects on the mitotic rate of rat alveolar tissue. It is postulated that the population of alveolar macrophages is most severely affected through the cytotoxic action of the chrysotile.

Although severe weight losses were experienced by the animals treated with the natural mineral, the factor of nutrition was not decisive in determining the decline of the mitotic activity of the cellular populations of the alveolar tissue.

Notwithstanding the decline in the mitotic activity of alveolar tissue, the cellular populations of that tissue were nevertheless capable of thymidine uptake. In fact, the animals treated with the asbestos dusts exhibited a level of thymidine labelling of alveolar tissue significantly greater than the saline controls. This label however, was cleared more rapidly from the asbestos treated groups than from the controls by two weeks after the commencement of treatment. This suggested that the labelled cells were being lost due to the cytotoxicity of the chrysotile upon them.

Unexpectedly, the mesothelium of the visceral pleura of asbestos treated animals demonstrated a significantly greater labelling index than that of saline treated control animals. Furthermore the labelling index of the mesothelium of natural chrysotile treated animals was also significantly greater than that of synthetic chrysotile treated groups. As in the case of the alveolar tissue, the label was also cleared more rapidly from natural asbestos treated animals' mesothelium by two weeks after treatment. These findings suggested that the pleural mesothelial cells are most sensitive to natural chrysotile asbestos even after a short period of exposure.

The utilization of tritium-labelled asbestos of both types enabled a clear localization of these chrysotile dusts within the alveolar tissue of the rats by means of radioautography. It was observed by this means that the synthetic mineral was cleared from the lung with greater efficiency than the natural fibres. In addition, scintillation counting of the radioactivity remaining in the lungs of animals at various times after treatment added support to this view.

Significant pathological lesions were not induced in the respiratory tissues of rats after either short term or long term exposure to dust clouds of natural chrysotile within a specially constructed inhalation exposure chamber. In addition, significant differences cytodynamically could not be detected in these groups although a declining trend in the mitotic activity of either the alveolar tissue or tracheal epithelium of these animals was evident.

Electron microscopic examination of the alveolar tissue of rats exposed to natural chrysotile by either intratracheal injection or inhalation exposure indicated that the basis for cytotoxicity of asbestos upon alveolar macrophages was evident morphologically. Furthermore, there also appeared to be a response of the septal alveolar cells to the mineral fibres. It is speculated that the surface properties of chrysotile in collaboration with the fibrous nature of the mineral fibres may exert a profound influence on the normal surface activity of the alveolar tissue.

IN RETROSPECT

What a man does not work out for himself, he does not have.

Rabbi Ber of Radeshiz

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INTRODUCTION

INTRODUCTION

Man in his introspection is often flung on the horns of a dilemma - from considering the fruits of his mind in all aspects of endeavour, to perpetual self-flagellation on seeing the horrors that he has instigated. Of these horrors, the pollution of his environment by his own industriousness and carelessness, has begun to haunt man for aesthetic, moral, and practical reasons. We are continually informed by our mass communications media of the impending doom of our two basic natural resources on which all others depend - air and water. This dissertation is essentially a study of the effects of one specific type of air pollutant on the mammalian organism. Air pollution is a problem which is causing increasing concern. Whilst our technology has enabled an improvement of the condition of our living standards and a prolongation of our lifespan, it has simultaneously subjected our society to more dangers by producing an evermore deteriorating environment.

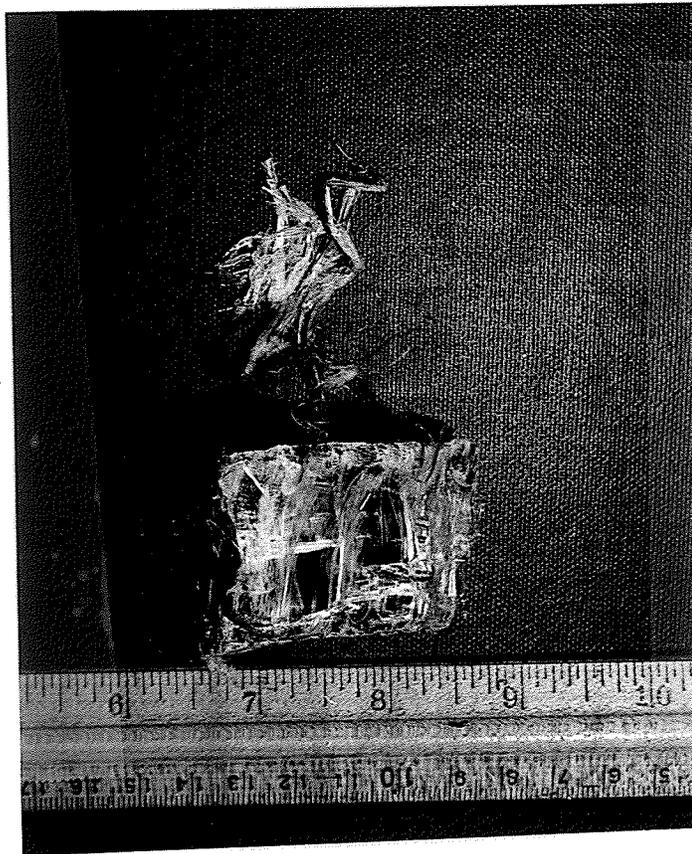
The peculiar minerals collectively named "asbestos" are an excellent example of man's dilemma. These minerals, because of their properties, are employed to great advantage in a myriad of

applications in our society. It is difficult to overestimate their usefulness and importance. Nonetheless, they have an ugly history of causing terrible diseases in man which are often incurable, and whose treatment is usually a delaying action of an inevitable difficult death. The simplest solution to these pathological progressions would be a preventative one, and the most extreme measure, a cessation of employing these minerals. Yet, the latter solution would be highly impractical at present because a substitute for asbestos minerals in their varied applications simply does not exist. Thus, for both academic and practical reasons, an understanding of the disease processes and their initiation by asbestos dusts is the more expedient. Such knowledge would enable the continued use of these minerals, but at the same time would provide information facilitating their proper management to forestall the occurrence of asbestos-related disease. The research described in this thesis was undertaken with these goals in mind.

THE ASBESTOS MINERALS

"Asbestos" is a term that refers collectively to the group of minerals having a fibrous structure. These minerals, all hydrated silicates, have one common property - a crystalline structure that enables their separation into relatively soft, silky fibres. Suitably separated, the asbestos minerals have a fluffy, woolly appearance (Figure 1). These minerals can be classified into two major groups on the basis of their crystalline structure: serpentine and the amphiboles. Chrysotile asbestos, the sole representative of the serpentine group, is the most common of all the asbestiform minerals. More than 95% of the mining and processing of asbestos fibres is of chrysotile. Canada, one of the largest producers, possesses vast mine deposits, the most extensive being in the Province of Quebec. Crocidolite, amosite, anthophyllite, tremolite and actinolite constitute the amphibole group of asbestos minerals. These occur less frequently than chrysotile and are not utilized as extensively; however, certain characteristics of this group render them useful in specific applications.

FIGURE 1: Portion of a vein of natural chrysotile asbestos with some fibres removed and partially separated.



①

Man's fascination with these peculiar minerals dates back at least 2,500 years (Collins, 1967). Their high resistance to destruction by heat and, as it follows, the resistance to heat of cloth woven from asbestos fibres, aroused man's inquisitive curiosity. The etymology of these minerals in various languages illustrates this circumstance. The word "asbestos" is derived from the Greek verb "sbennumi," meaning to quench, die down, or extinguish. Although this meaning contradicts the heat resistant properties of asbestos, it was probably derived from the glowing appearance of asbestos under conditions of extreme heat which were not sufficient to bring about its destruction by dehydration. Thus, the mineral was described as a fabulous stone which was reputed to burn with an everlasting (unextinguishable) flame. The descriptive noun for asbestos is "amiante" in French, and "amiantes" in Spanish and Italian, meaning a material that is indefilable. "Steinflachs," meaning flax-like stone describes the minerals in German. French-Canadian miners have described asbestos as "pierre-a-cotton" ("stone of cotton"). Despite the great interest previously expressed in general, asbestos minerals did not attain industrial significance until the 1860's, at which time the Canadian asbestos deposits were discovered. Since that time, world production became augmented by staggering degrees (Hendry, 1965). This increase is directly correlated to the multiplicity of applications in which asbestos is employed. These "mineral(s) of a thousand uses" as named by Hendry (1965), are utilized especially in the building industry where their

binding properties and tensile strength render them highly suitable for incorporation into the manufacture of various cement products, as well as floor tile. The heat resistant nature of the asbestos minerals serves an important function in the production of insulating material. The shipbuilding industry is a large consumer as is the construction industry. Wagner (1968) related the interesting fact that at the battle of Jutland (1916), the world's two largest man-made deposits of asbestos (in the form of dreadnoughts) met in mortal conflict. Countless other applications for asbestos minerals exist which, although employing lesser quantities of asbestos, nonetheless represent a major consumption in toto. Of these, the manufacture of brake-shoe linings for automobiles, thereby utilizing the excellent frictional resistance of asbestos, represents a large annual consumption.

Although some of the extreme biological effects of asbestos have been known for many years, it has only been recently that the vast production and myriad of uses of this mineral have been considered in relation to the welfare of human populations. As it will be apparent from following sections, much evidence exists concerning the health problem asbestos presents to humans. Nevertheless, owing to the latency of its effects, full knowledge of the extent of the present hazard of asbestos will not be available for some years. The necessity for the understanding and clarification of the biological effects of asbestos minerals is both profound and

pressing. Inasmuch as chrysotile is by far the most prevalent asbestos mineral in use, and because Canada is one of the largest producers and suppliers, the present investigation emphasizes the biological effects of that mineral. Wherever applicable, however, discussions on the amphibole group of minerals are included for comparison.

CHRYSOTILE ASBESTOS

Formation

The formation of chrysotile has been the subject of considerable geologic and petrologic research. Chrysotile is invariably found in serpentine rock. It is believed that the formation of chrysotile occurred as the result of two separate metamorphic reactions. The first reaction was the formation of serpentine by hydrothermal alteration in ultrabasic rocks of volcanic origin. Occurring at some later time, the second reaction yielded chrysotile in the cracks and fissures of the native serpentine rock by the recrystallization of the serpentine. This recrystallization occurred in an aqueous solution which reprecipitated the new crystalline form of the rock as chrysotile under appropriate hydrothermal conditions (Speil and

Leinweber, 1969). Although the physical properties differ, the chemical compositions of chrysotile and serpentine are identical, both being hydrated magnesium silicates and having the empirical formula $Mg_3(Si_2O_5)(OH)_4$. Chrysotile has also been synthesized in the laboratory from magnesium oxide, silica and water under controlled hydrothermal conditions. For instance, Bowen and Tuttle (1949) successfully synthesized chrysotile by this means. It is quite probable that the first successful attempts at synthesis occurred as early as 1927 (Speil and Leinweber, 1969) but the lack of sophisticated characterization by electron microscopy and X-ray diffraction techniques did then not facilitate its veritable identity. In contrast to the natural mineral, synthetic chrysotile is composed mainly of single fibres. The synthesis of large bundles of parallel fibres has not been successfully achieved so far despite the addition of trace impurities, mineralizers or the variations of experimental conditions. Nevertheless, at least small fibre bundles of ultramicroscopic dimensions were successfully produced by a number of authors (Yang, 1960; Gross et al., 1970; Turnock et al., 1970).

Structure

The crystalline structure of chrysotile has been the subject of study for well over 40 years. Warren and Bragge (1930) were the

first to determine that its structure was crystalline. The crystal structure of chrysotile consists essentially of an infinite silica sheet $(\text{Si}_2\text{O}_5)_n$ where all the silica tetrahedra are pointing in the same direction. On one side of the sheet is attached a brucite layer $\text{Mg}(\text{OH})_2$ in which two out of every three hydroxyls are replaced by the apical hydrogens of the silica tetrahedra. The resulting structure is a double sheet as graphically demonstrated in Figure 2. A strain is produced in the structure by a mismatch in the dimensions of the silica and brucite sheets. Various postulates were presented concerning the structural means of relieving this strain. The proposal gaining the greatest support was that the structural alteration of strain relief was a curvature of the sheet with the brucite layer on the outside. Whittaker (1953, 55, 56, 57) demonstrated by means of X-ray diffraction studies that the crystal lattice was definitely curved. Later, Maser et al. (1960) demonstrated the tubular structure of chrysotile by electron microscopy. Additional electron microscopic evidence was provided by Yada (1967). The high resolution electron micrographs produced by Yada supported the work of Whittaker, and suggested that the basic structural unit was a single magnesia silica sheet rather than the double sheet as previously suggested. The wall of a single fibre of chrysotile was hypothesized to consist of twice the basic spiral element of 5 silica magnesia units with a thickness of 70 \AA . The diameter of a single fibre is

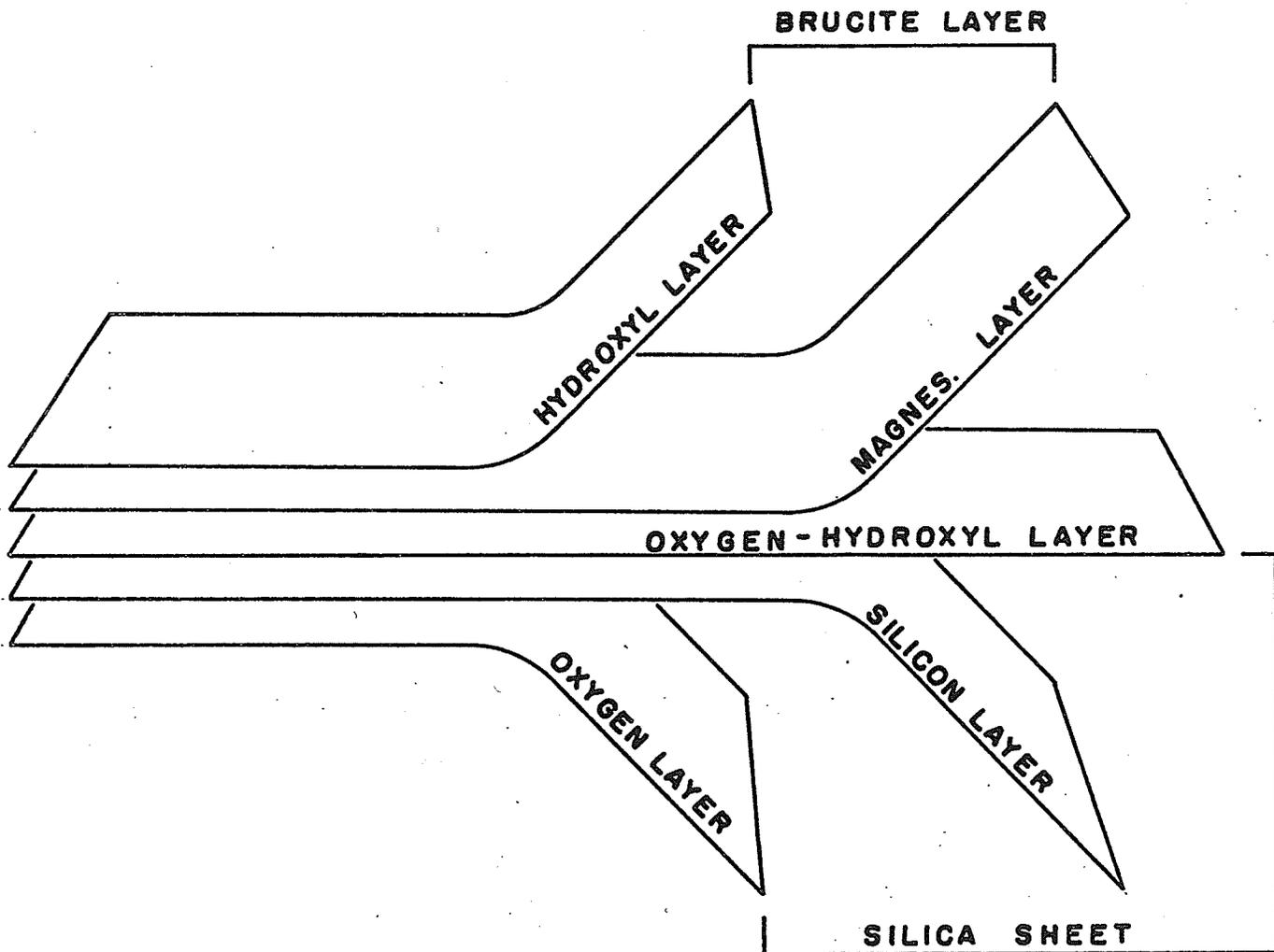


FIGURE 2: Diagrammatic representation of the unit cell composition of chrysotile (after Speil and Leinweber (1969)).

approximately 240 \AA . The fibres of chrysotile are therefore tubular, as well as hollow and often containing amorphous material. The other varieties of serpentine - lizardite and antigorite exhibit the similar basic chemical composition and sheet structure as does chrysotile but differ in their crystalline structures. These differences are believed to be indicative of different means of relieving the strain in the crystal. Neither lizardite nor antigorite are fibrous minerals. They are not employed industrially to any significant extent.

Behaviour - a brief survey of the physical and chemical properties of chrysotile

It is the chemical and physical properties of chrysotile that determine its usefulness industrially. Furthermore, some if not all of the selfsame properties of chrysotile play a significant role in determining the pathological effects of this mineral fibre when introduced into biological matter. It is of importance, therefore, to understand basically the chemical and physical properties of chrysotile insofar as they may exert important influences on its biological reactivity.

Tensile Strength

Chrysotile exhibits an extremely high tensile strength. This property renders this variety of asbestos very useful as a reinforcing fibre material. The tensile strength of chrysotile has been shown by Zukowski and Gaze (1959) to depend on inter-fibrillar connections rather than on the true fibre bundles. Surprisingly, the tensile strength of this mineral approaches that of steel and yet chrysotile can be readily separated into a mass of soft, cotton-like material.

Harshness

Chrysotile may be found in a variety of forms which exhibit differing degrees of harshness. Harshness refers to the flexural modulus of the fibres or simply, the resistance of fibres to being bent. Commercially, harsh chrysotile fibres are more easily processed. Woodroffe (1956) postulated that the harshness of chrysotile was ascribable to the natural water content of the mineral. In contrast, Whittaker and Zussman (1967) proposed that harshness was dependent on the relative occurrence of two similar crystalline forms - clino- and ortho-chrysotile.

Thermal Decomposition

The decomposition of chrysotile by heat is of little significance in relation to its biological effects in mammalian tissues. However, the destruction of chrysotile by this means is quite important in the assessment of the contributions of chrysotile dust to the atmosphere from various industrial applications where the mineral is employed. In this regard it was observed by Sinclair (1970) as also by Lynch (1970), that more than 98% of the chrysotile in brake-shoe linings of automobiles becomes destroyed by extreme temperatures in association with mechanical stress disintegration.

Destruction by Acid

Of all the serpentine minerals, chrysotile is the most susceptible to attack by acids. Inasmuch as the amphibole varieties of asbestos are much more resistant to acid, they are utilized in certain applications where that property is desirable. Strong acids rapidly destroy chrysotile by removing the brucite layer, leaving behind a residue of amorphous silica exhibiting a fragile fibrous morphology (Badollet, 1963). Chrysotile is

attacked similarly but to a lesser extent by weak acids. Water likewise brings about the gradual dissolution of chrysotile. Holt and Clark (1960) extracting chrysotile with boiling water, reported that the resultant solution contained magnesium and orthosilic acid. They proposed that the dissolution of chrysotile occurred as the result of the loss of magnesium ions. The colloidal silica remaining was then hydrolyzed to orthosilic acid. It is apparent that chrysotile is slowly soluble in water under the conditions of extraction. Yet, the extent of its solubility in physiological fluids at body temperature is still unknown. It is highly likely that such dissolution is minimal if not infinitesimal.

Surface Characteristics

Perhaps the most significant properties of chrysotile in relation to its pathogenic effects on pulmonary and other tissues are its surface characteristics. Because of the high specific surface area that varies with the degree of fibre bundle separation, and is augmented considerably by its tubular structure, chrysotile is able to adsorb large quantities of water and other substances. Furthermore, the positive surface charge of chrysotile within physiological fluids enhances the adsorption of water molecules which are themselves extremely polar (Speil and Leinweber, 1969). As the result of these surface properties, chrysotile swells

considerably, forming a colloid-like suspension in both water and tissue fluids.

The following section deals with the biological effects of this fibrous mineral. Inasmuch as the initial examinations of the problem of asbestos-related disease were largely epidemiological, that section is essentially a historical review of the epidemiological findings concerning the problem. Subsequently, prior to entering upon a review of the experimental work pertaining to asbestos-related disease of the pulmonary tissue, the normal structure and also the histophysiological activity of this tissue shall be considered. This order of presentation, teleologically sound, will present the platform from which the present study largely originated.

THE HAZARD OF ASBESTOS

With the increased production and utilization of asbestos under existing industrial environmental conditions in the late nineteenth and early twentieth centuries, the exposure of man to these minerals was inevitable. Although many persons may have become affected and succumbed as the result of such exposure before the turn of the century, such cases were not recognized and thus not reported in the literature. The first pathology report related to asbestos exposure was published by Murray (1907). Murray had examined a man who was completely debilitated as the result of pulmonary disease. Autopsy findings signified extensively fibrosed lungs. Microscopic examination of the lung tissue revealed the presence of asbestos fibres ("spicules of asbestos"). This man had been the sole survivor of a group of ten men who were hired ten years previously by an asbestos processing factory. This was the first documented case of the industrial disease that was simply and appropriately designated - "asbestosis". Fourteen years later, Cooke (1924) reported the second case of asbestosis in the literature, later commenting with awe that, "we have never seen anything parallel to this in pneumoconiosis due to other dusts, nor have we been able to find such occurrence in the literature" (Cooke,

1927). Fibres of asbestos ranging in length from three to over 300 micra were observed in the lung tissue of that patient. The "curious bodies" of asbestosis first observed by Fahr (1914) were also in that case encountered within lung tissue. These curious bodies, also referred to as "peculiar," "asbestosis," and "asbestos" bodies, were nodular, dumbbell-shaped structures and contained asbestos fibres as a central core (Gloyne, 1929); they were frequent in the lung tissue of victims of asbestosis. The bodies were first postulated to be the result of a laying down of blood pigment onto asbestos fibres (Gloyne, 1929).

Oliver (1927) emphasized the importance of tuberculosis as a complicating secondary factor in asbestosis, stating that "since fibrotic changes are developing . . . there is almost sure to develop, if such has not already taken place, pulmonary tuberculosis". It is apparent that prior to the discovery and use of antibiotics, the later profound effects of exposure to asbestos were obscured or prevented from expressing themselves, because most persons heavily exposed to asbestos dust probably succumbed earlier to other pulmonary disorders. McDonald (1927) re-examined histologically the case reported by Cooke and observed extensive concurrent tuberculosis as well as a diffuse interstitial pneumonia with chronic bronchitis. Characteristically, many small bronchi were obliterated, the fibrosis of these often

extending into adjacent alveolar tissue. Wood (1929) examined 16 cases of asbestosis radiologically and observed in most instances, a massive fibrosis of the lung as well as a thickened pleura. Wood et al. (1929) reported particularly on a severe case of asbestosis. A 34-year old woman, who had worked for nine years in an asbestos factory where precautions were not taken against dust exposure, died as the result of massive fibrosis of the lungs. Even at the time of Murray's first report it was assumed that asbestosis was of little significance as an industrial disease and that the disease would occur rarely once preventative measures were employed. Murray (1907) himself, stated innocently: "One hears generally speaking that considerable trouble is now taken to prevent the inhalation of dust and so the disease is not so likely to occur as heretofore".

Not until the report by Lynch (1935) of a single case of carcinoma of the lung in association with asbestosis was there any suspicion of a carcinogenic property of asbestos. The relationship between asbestos exposure and pulmonary carcinogenesis became further substantiated only subsequently with the report of Doll (1955). That author observed that in a cohort of 113 men exposed to asbestos dust for at least 20 years, of the 39 that died, 19 were afflicted with both asbestosis and lung cancer. In that same year Bonser (1955) reported 14 cases of lung cancer among 72 post-mortem diagnoses of asbestosis. Shortly thereafter, Braun

and Traun (1958) asserted on the basis of their epidemiological investigations that an unusual risk for the development of lung cancer did not exist among asbestos workers in Quebec. This study met later with severe criticism on the basis of its methodology (Mancuso, 1965). The pathogenic effects of asbestos on workers engaged in the mining or processing of this mineral and the utilization of its products became increasingly clarified. The report of Wagner and his associates in 1960 of a striking correlation between exposure to asbestos and the incidence of a previously disputed malignant tumour, mesothelioma of the pleura, generated widespread concern. Stout and Murray (1942) reported long before on the occurrence of this tumour, but many pathologists viewed it still merely as a rarity, or assumed it to have metastasized from another primary source (Willis, 1960). The discovery by Wagner et al. of 33 cases of malignant mesothelioma of the pleura among a small population settled near an asbestos mine was therefore startling. Although in only three of these cases was a direct association apparent with asbestos, either through a work history or as revealed by the presence of asbestosis, the other cases were probably exposed by their proximity to the asbestos mining operations. Between 1956 and 1964 the same authors detected 130 cases of malignant mesothelioma in South Africa in persons living in the vicinity of asbestos fields or having been associated directly with the asbestos industry (Wagner, 1968). Since the initial report of Wagner et al., numerous epidemiological studies

implicated the asbestos minerals beyond any reasonable doubt as constituting the major factor responsible for the high incidence of cancer of the lung and pleura among persons exposed to these mineral dusts. As the volume of this evidence became augmented, a grave anxiety was felt for the welfare of urban populations, because asbestos minerals are so extensively employed in products employed in urban areas. It was genuinely feared that an outbreak of asbestos-related cancers could reach epidemic proportions within 15 or 20 years. Furthermore, retrospective epidemiological studies predicted an increase in asbestos-related cancers in the future. The absence, in many cases of cancer of asbestos etiological origin, of clinical or radiological asbestosis (Thomson, 1962; Elmes, 1966) indicated that a heavy exposure might not be mandatory at all for the development of cancer. This contributed further to the already growing concern. As this problem became known, reports from areas the world over where asbestos industries existed began to appear in the literature, signifying unanimously that a higher risk for the development of cancer of the lung and pleura was apparent in those persons exposed to asbestos. Examples of such reports derived include the following: from the United States (Selikoff et al., 1964; Cordova, 1962; Enterline, 1965; Kleinfeld, 1967; Leiben, 1967; Demy, 1967); Great Britain (Keal, 1960; Buchanan, 1965; Enticknap, 1964; McVittie, 1965; Owen, 1965; Newhouse and Thomson, 1965); Germany (Jacob and Anspach, 1965); South Africa (Webster, 1965;

Wagner, 1965); Australia (McNulty, 1962), and Finland (Hagerstrand, 1968). It became evident from such investigations and observations that a latent period after the first exposure, often of more than 20 years, usually lapsed before the onset of symptomatic malignancy. Inasmuch as the different varieties of asbestos are often intermixed during the processing of asbestos products, an assignment of degree of carcinogenic activity to specific types of asbestos could not usually be allocated. However, in the case of the South African series by Wagner and his associates, it was known that exposure to only a single variety of asbestos occurred at the site of mining. It was consequently proposed initially that the South African variety of crocidolite asbestos (Cape Blue), an amphibole, might be especially carcinogenic. In addition to the difficulty of assessing the effects to mixed exposure there was the further difficulty to ascertain the duration of exposure and the degree to which asbestos workers, comprising cohorts in epidemiological studies, were subjugated to the mineral. These factors could not be determined in many of the epidemiological surveys. However, in the more recent studies by Newhouse (1969) and Newhouse and Wagner (1969) it was observed that a heavy exposure to asbestos of variable duration was associated with a much higher risk of the development of cancer of the lung as compared to a long, moderately light exposure. Moreover, moderate to severe degree of asbestosis occurred in all those workers who had also developed carcinoma of the lung in that particular series.

Many investigators searched routinely for asbestos bodies in autopsy material to ascertain whether the general population was likewise being exposed to asbestos dusts at significant levels. And in fact a high incidence of alleged asbestos bodies was initially reported at autopsy in urban dwellers. Cauna et al. (1965) reported an incidence of 47% in males and 34% in females in Pittsburgh; Thomson and Graves (1966) likewise observed a high incidence of asbestos bodies at random autopsy in Miami - 30% in males and 20% in females. In Montreal, Anjilvel and Thurlbeck (1966) detected asbestos bodies in 57% of males and 34% of females of the general population subjected to autopsy. Other investigators reported similarly a high incidence of asbestos bodies encountered during random autopsy (Ashcroft, 1968; Xipell and Bhathal, 1969; Hagerstrand et al., 1968). These findings seemed to indicate that a high level of non-industrial exposure to asbestos was occurring in the general population as signified by the apparently high incidence of asbestos bodies encountered in routine necropsies. Furthermore, Hagerstrand et al. implied that a positive correlation could exist between the frequency of occurrence of asbestos bodies in the lung and the presence of mesothelioma. In cases with mesothelioma he had always detected large numbers of asbestos bodies. Yet experimentally, Gross and his associates (1967, 1968) demonstrated that asbestos body-like structures could be induced in animals also by non-asbestiform minerals such as fibreglas, commercial talc and other filamentous materials. However, Gross

et al. (1969) noted that in a series of 28 human autopsies in which so-called "asbestos bodies" were demonstrable, asbestos did not form the core of all these structures. Nevertheless, Gold (1969) observed that in extracts from 620 lung biopsies, 336 samples proved positive for asbestos. It is thus apparent that the analysis of "ferruginous bodies" (Gross et al., 1967), when studying the effects of exposure of persons to environmental air-borne materials requires a positive identification of asbestos, to ascertain whether the central core is in fact composed of asbestos. Only then can "ferruginous bodies" be justifiably called "asbestos bodies". Some workers postulated that the levels of asbestos in modern urban communities did not constitute a serious hazard (Enterline and Kendrick, 1967), whereas others (Collins, 1967; Selikoff, 1970) contended that the extent of the hazard of asbestos exposure of the general population is still unknown. Unrecognized heavy exposure constitutes in any case a severe hazard. A person may inadvertently subject himself to a severe exposure to asbestos dust merely in the process of insulating a cottage without an awareness of the danger. School children have been exposed to light doses of asbestos by using asbestos cement products as an arts and craft material.

The influence of other types of pollutants contribute significantly as well to the etiology of asbestos-related disease. Thus, Selikoff et al. (1968) reported that asbestos workers who

smoked had a much higher risk of developing bronchogenic carcinoma than non-smokers under identical circumstances.

Although a great deal of emphasis has been properly placed on asbestos-related disease of the pulmonary system, other body regions were also examined in this regard. For instance, Graham and Graham (1969) observed in a small number of cases the presence of asbestos-like material within the stroma of ovarian neoplasms. It is unfortunate that a positive identity of asbestos was not performed in that study. Cases of peritoneal mesothelioma were likewise reported to be associated with exposure to asbestos (Newhouse and Thomson, 1965; Thomson, 1962).

Notwithstanding the grave concern generated by the problem of asbestos-related disease, Motlagh and Falor (1967) suggested the application of fine asbestos dust as a therapeutic agent in cases of spontaneous pneumothorax; this type of treatment was first utilized by Beck and Leighninger (1954) to induce therapeutic fibrosis in the pericardial sac. The lack of malignancies within 15 years after the former treatment, as reported, does not preclude the potentiality for carcinogenesis at later times, because it is known that the latent period for the development of pleural mesothelioma often exceeded that period. Experimental evidence illustrating the induction of pleural mesothelioma in animals by the intrapleural injection of asbestos

existed prior to the report of Motlagh and Falor (Smith et al., 1965). It is hoped that in view of the accumulating mass of evidence relating asbestos to malignancy, the usage of asbestos in such procedures will be discontinued.

The epidemiological investigations of the past decade clearly established the grave danger of asbestos exposure. These studies are nonetheless subject to severe limitations in that all the contributing parameters cannot be measured. Historically, epidemiology has drawn attention to the reactivity of asbestos within living matter, but it is chiefly through systematic experimental investigation that the etiology of asbestos-related disease can be elucidated.

PULMONARY TISSUE

Introduction

The parenchyma of pulmonary tissue is of necessity constructed extremely delicately to adapt it suitable for efficiently performing its function of gaseous exchange between alveolar air and blood. The pulmonary alveolar tissue, therefore, represents one of the few finely composed cellular and tissue structures of the body that is routinely, as part of its function, exposed to a relatively unaltered external environment. To protect the alveoli, the structural units of alveolar tissue, against foreign matter which may be carried to them by inhaled air, the respiratory system is provided with a complex protective system. This protective system is well prepared to deal with the ordinary variety of stressful agents penetrating to the respiratory portion, but may become overwhelmed by severe insults, for instance, by a gaseous environment that is polluted excessively with noxious substances. Under such stressful conditions the protective system of clearance may become largely, if not totally, compromised, thereby allowing the noxious substances to exert their full pathogenic effects. It is

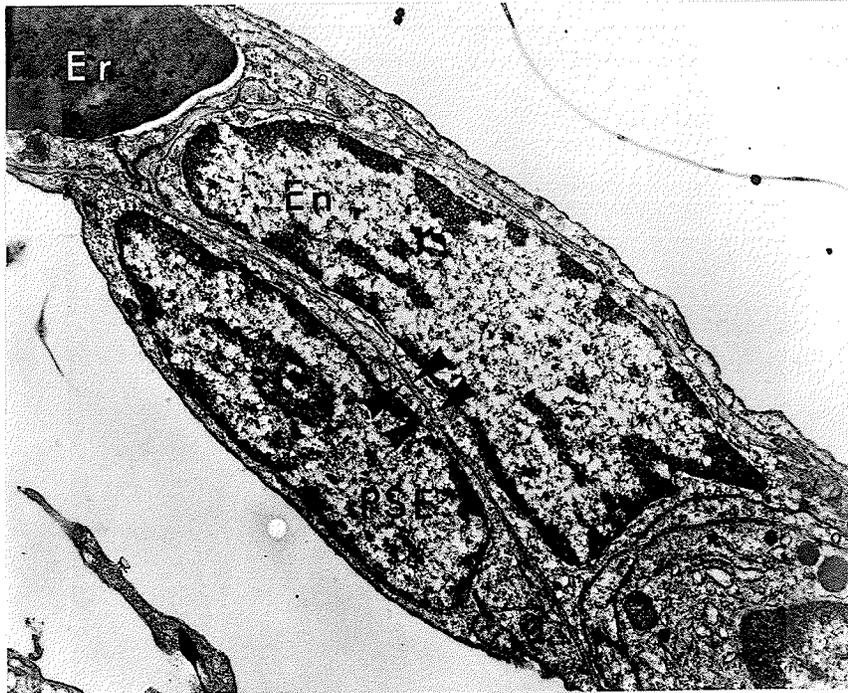
evident that a comprehension of the etiology of such disease processes, ensuing a failure or breakdown of the protective system requires, first, an understanding of the normal protective mechanisms, and second, a delineation of the causes that may lead to their failure. It is therefore the intention of the present treatise to discuss in the following sections the structure and histophysiology of pulmonary tissue, its clearance mechanisms which comprise the protective system of the lung, and finally, the reactivity of the clearance mechanisms to noxious substances.

Pulmonary Alveolar Tissue

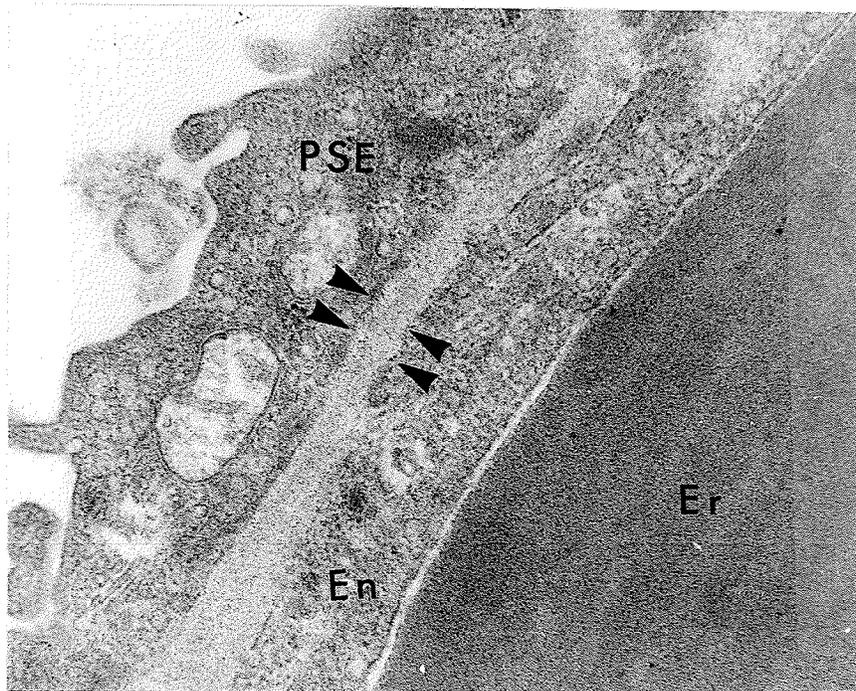
The units of alveolar tissue, the alveoli, consist of complex capillary meshworks on whose external (air) surface is applied an extremely flattened simple squamous epithelium. Disposed between that epithelium and the capillary endothelium are two basement membranes, that of the capillary, the other of the surface epithelium, as demonstrated in Figures 3 and 4. The pulmonary surface epithelial cells exhibit extremely attenuated cytoplasmic extensions covering the surface of the alveoli. These are barely discernable, even in low magnification electron micrographs. In order to facilitate gaseous exchange, the blood-air barrier, composed of the pulmonary surface epithelial cell,

FIGURE 3: Portion of the alveolar wall showing the pulmonary surface epithelial cell (PSE), endothelial cell (En) of the alveolar blood capillary, and the interdisposed basement membranes at the arrows.
Note erythrocyte (Er) in capillary.
Electron micrograph 25,080 x.

FIGURE 4: High magnification of a portion of the alveolar wall.
Note the basement membrane at arrows.
Electron micrograph - Magnification unavailable.



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4

the capillary endothelial cell and the interdisposed two basement membranes, are merely 0.10- - 0.20-micron, and even less in width in the most attenuated portions (Weibel, 1967). Present in the interalveolar septa, as also free in the air spaces, are the alveolar cells. In the following sections, each of the cellular components of the alveolus, save the capillary endothelial cell, will be specifically considered.

The Pulmonary Surface Epithelium (PSE)

Since a long time it has been realized that the pulmonary alveolus was primarily composed of a complex meshwork of blood capillaries. But not until the first electron microscopic studies of alveolar tissue (Low and Daniels, 1952) was the actual presence of a "pulmonary surface epithelium" (Bertalanffy and Leblond, 1955) verified. Earlier it had been suggested by Leblond and Bertalanffy (1951) that such an epithelium might exist, this hypothesis being supported by the observations of reticulin membranes, conceivably basement membranes, covering the entire surface of the alveoli, and demonstrable by the histochemical PA-FSA staining technique. At first, some investigators (Swigert and Kane, 1954; Policard, 1954) doubted the findings of Low and Daniels, but subsequent studies (Karrer, 1956) confirmed

conclusively the presence of the pulmonary surface epithelium. The contiguity of this epithelium with that of the bronchioles indicated the common endodermal origin of both epithelia (Low and Sampaio, 1957). These early studies provided the evidence on which the modern concept of the structure of alveolar tissue is based. The cells of the pulmonary surface epithelium appear to be relatively devoid of cytoplasmic organelles and inclusions, although a limited number of small mitochondria are characteristically present. Histochemical investigations revealed that the glycolytic pathway and the tricarboxylic acid cycle are active in the cells of the pulmonary surface epithelium, as they are also in the capillary endothelial cells (Tyler et al., 1965; Azzopardi and Thurlbeck, 1967).

For a number of years a role for the pulmonary surface epithelium other than possibly its protective contribution for the alveolus could not be assigned. Inasmuch as the process of gaseous exchange between alveolar air and blood is passive, the structural contribution of the pulmonary surface epithelium appeared to be its sole established function. In recent years, however, other activities aside from the afore-mentioned protective contribution have been attributed to the pulmonary surface epithelium. It is generally believed by most workers that the alveolar wall is devoid of lymphatic vessels. However, Pump (1970) presented evidence recently disputing this generally

held view. By the use of corrosion techniques in association with micro-dissection, he believed to be able to demonstrate lymphatic capillary networks on the inner surface of the alveolus which he designated to be true alveolar lymphatics. Nevertheless these findings are not supported by the vast number of electron microscopic investigations conducted on alveolar tissue. It is therefore still highly unlikely that lymphatic vessels exist within the alveolar wall proper. The lack of specific alveolar lymphatics within the alveolar wall implied that some other pathway for protein absorption was necessary to preclude its accumulation within the limited interstitial space of the alveolar wall (Meyer, 1969). In this regard, Drinker and Hardenburgh (1947) observed that dye tagged albumin, instilled intratracheally to dogs, appeared only in small quantities in the right lymphatic duct and not at all in lymph draining from the thoracic duct, or even in the blood plasma. They concluded, therefore, that the alveolar wall was impermeable to proteins and further, that in their series, the albumin became reduced to smaller molecular components capable of passing through the alveolar wall into the interstitial spaces, and hence to proceed to lymphatics at higher levels of the respiratory tract. Courtice and Simmonds (1949), utilizing similar techniques in rabbits in conjunction with precipitin reactions, noted in contrast that intact protein did enter the alveolar wall from the alveolar spaces to pass further directly into the blood circulation. They

asserted in fact that absorption through the alveolar wall represented the major means of removal of protein from the alveolus. That hypothesis was recently strengthened by Meyer et al. (1969). This group employing radioiodinated albumin administered to dogs, ascertained that the pulmonary blood circulation absorbed an 11 times greater quantity of albumin from the alveolar space than did the pulmonary lymphatics. Dominguez et al. (1967) examined likewise the clearance of protein from the alveolar spaces by conducting a comparison between the clearance of radioiodinated albumin and radioactively labelled polyvinylpyrrolidone from the alveolar spaces in guinea pigs. The latter substance was known to be cleared very slowly. Assuming that the clearance of these two substances in the air conducting system and by phagocytosis in the alveoli occurred at a similar rate, these authors centered the cause for a measurable difference in the rate of clearance of these materials from the alveolus on the ability of the alveolar wall to absorb albumin. They too noted a passage of albumin from the alveolar spaces into the blood. The possibility that protein passed between the cellular junctions of the pulmonary surface epithelium and the capillary endothelium was disproven by Bensch et al. (1967) who observed that labelled albumin passed through the epithelial and endothelial cells into the blood capillaries, but not between cell junctions. Regarding the cell junctions, Scheenberger and Karnovsky (1968) demonstrated the impermeability of the cellular

junctions of the pulmonary surface epithelium to horseradish peroxidase, a small molecular weight protein. Thus an additional role, namely that of absorption of protein from the alveolar space, was assigned to the pulmonary surface epithelium. A further significance was postulated for the cells of that epithelium by Dermer (1970) who noted that the cells were active in clearing by pinocytosis amounts of the surfactant. Some workers assigned a phagocytic role to the epithelium (Casarett and Milley, 1964) but it appears that this phenomenon was functionally incidental to pinocytic activity in that the cells ingested concurrently extremely small particulate matter. Thus, a process of phagocytosis, as defined morphologically and histophysiologicaly, cannot be appropriately ascribed to the cells of the pulmonary surface epithelium.

Alveolar Cells

The term "alveolar cell" has been applied to the conspicuous cells constantly encountered in the intercapillary meshworks of the alveolar wall as well as free on the surface of the pulmonary alveolus (Bertalanffy, 1964). These cells were first described by Kolliker in 1881. The alveolar cells were later grouped into two major morphological categories: cells containing numerous vacuoles (vacuolated alveolar cells) and

those in which a paucity of vacuoles was noted (non-vacuolated alveolar cells). To begin with, the opinion was generally held that these two cell forms were merely varieties of the same cell in different stages of development or activity, since intermediate forms with characteristics of both cells were observed (Bertalanffy, 1964). Recent evidence, supplied largely by electron microscopic investigations, rectified the confusion that prevailed for long concerning these cells. It was established that two separate and distinct cell types existed. The terms "vacuolated" and "non-vacuolated" alveolar cell, as introduced by Bertalanffy (1965) remain applicable with regard to light microscope observations. It was recognized, however, that at best only estimations of the actual identity and proportion of these cells based on both cytological characteristics and location can be gained from routine light microscope observations. On the whole, however, it can be safely assumed that under normal circumstances, the bulk of "vacuolated alveolar cells" discernible with the light microscope are the same vacuolated alveolar cells observed with the electron microscope. These are the so-called septal alveolar cells containing osmiophilic lamellated bodies. The "non-vacuolated alveolar cell" can be recognized to represent largely the "alveolar macrophage" of electron microscope observations. The intermediate forms reported conceivably represented alveolar macrophages that phagocytosed osmiophilic lamellated bodies and hence present morphological features common

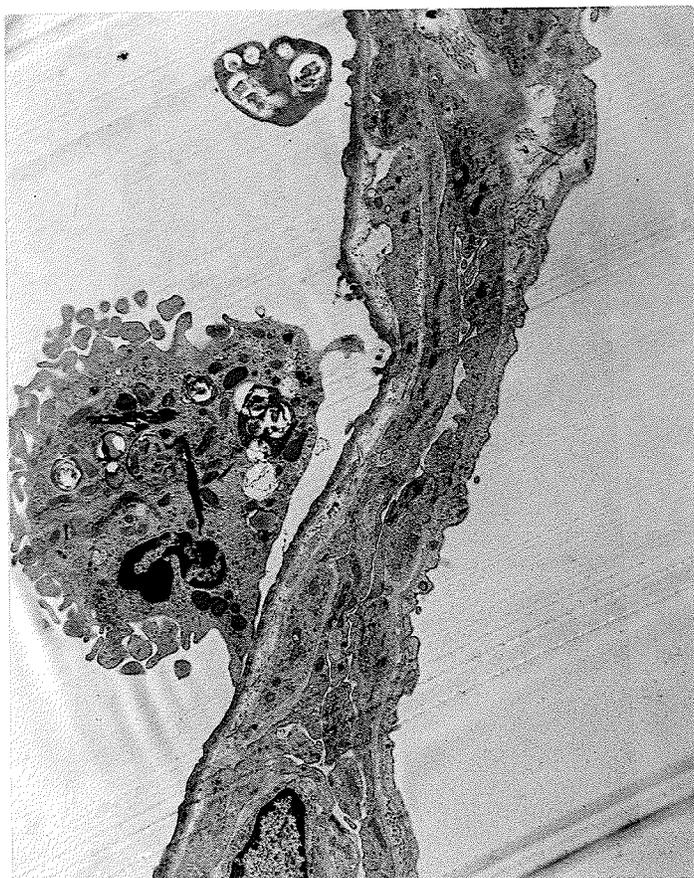
to both cell forms. These cell types have in recent years been the focus of intensive research. Although many aspects of their behavior are yet to be clarified, it is not an understatement that these two cell types are of prime histophysiological importance in maintaining the normal activity of alveolar tissue, as well as in coping with the confrontation of a variety of insults chiefly arising from the environment.

The Alveolar Macrophage

(the non-vacuolated alveolar cell)

As the representatives of the reticulo-endothelial system in respiratory tissue, the population of alveolar macrophages of the lung comprise a highly specialized group of cells that act as scavengers. These cells continually remove by phagocytosis aided by ameboid motility foreign matter and other undesirable debris from the alveolar tissue. A typical example of an alveolar macrophage as seen with the electron microscope is presented in Figure 5. As part of their life cycle, these cells - whether or not containing phagocytosed material - are continually extruded from the lung via the tracheo-bronchial tree. To replenish this continual loss of cells, new cells arise by mitosis in the alveolar walls, thereby maintaining the population of alveolar macrophages constant under ordinary conditions in the alveolar

FIGURE 5: Alveolar macrophage containing ingested asbestos
fibres free on the surface of the alveolar wall.
Electron micrograph 3,876 x.



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tissue. Inasmuch as macrophages are known to arise from many sites (bone marrow, lymphoid tissue and others), the question was raised by several investigators whether the preponderance of alveolar macrophages might conceivably originate from other sites, migrate to the lung and there divide further by mitosis. Alternatively, the alveolar macrophages might arise in situ in alveolar tissue only. In addition, it was pondered as to whether these cells were in fact different from macrophages of other sites aside from their locale and their adaptation to that location. In order to provide answers to these problems, various investigators examined the origin of the alveolar macrophage specifically, as also the particular characteristics of this cell. Pinkett et al. (1966) claimed that in mouse chimeras, produced by the injection of X-irradiated mice with hemopoietic cells of a compatible strain, the large proportion of the alveolar macrophages were of donor origin (as identified by chromosome markers). Because the alveolar macrophages of the recipient mice had been protected from irradiation by shielding, these authors contended that the majority of alveolar macrophages must have originated from sites other than alveolar tissue. Utilizing similar techniques, Virolainen (1968) noted that macrophages from different sites (lung, peritoneum) might be derived from hemopoietic tissues. This study, although quite similar, did not include the protection of the thorax of recipient mice, however.

It was thus conceivable that the alveolar macrophages of the recipient animals were destroyed by irradiation. Furth and Cohn (1968) examined the origin of macrophages by other means. They utilized tritiated thymidine and radioautography in order to trace any migration of macrophages from the bone marrow to the peritoneal cavity via the blood stream. Thus, they observed a high labelling index of the population of peritoneal macrophages that could be correlated to an earlier peak labelling of blood monocytes. This latter peak was preceded by a similar peak in the bone marrow. From these observations the authors postulated that the source of peritoneal macrophages was blood monocytes arising from progenitor cells in the bone marrow. The transit time of the blood monocytes from the bone marrow to the peritoneal cavity was estimated to be 32 hours. On the basis of these findings, the authors hypothesized that macrophages encountered also at other sites including alveolar tissue, were probably also derived from the bone marrow. The report by Howard et al. (1969) conflicted with the report of the afore-mentioned group. These investigators observed that both the peritoneal and alveolar macrophages arose from donor lymphocytes during experimentally induced graft versus host disease in mice. Previously, credence in the origin of the alveolar macrophages from lymphocyte-like cells had been expressed by Collet (1965). It is thus very evident that a great deal of controversy prevails

concerning the origin of the alveolar macrophages and the source of replenishment of these cells. Other workers disagreed with the afore-mentioned theories, implying that alveolar macrophage arose from blood monocytes or lymphocytes. Shorter et al. (1966) reported a labelling index of 1.2% of the alveolar cells within pulmonary tissue, of which the large proportion were macrophages (Bertalanffy, 1964), after a 15 minute pulse label of tritiated thymidine. This evidence contradicted the view of Furth and Cohn that macrophages were incapable of cell division to a great extent once they were removed from bone marrow. Furthermore, the rapidity of uptake of label by the alveolar macrophages precluded the possibility that they comprised a migratory population of monocytes, inasmuch as the transit time stated by Furth and Cohn exceeded greatly the almost instantaneous labelling of the alveolar macrophages. Also, histochemical evidence supported the view that the alveolar macrophages were not directly derived from blood monocytes, and that these cells constitute a highly specialized cell form differing in many respects from macrophages indigenous to other sites. Because of their divergent environment, alveolar macrophages, unlike peritoneal macrophages, depend to a considerable extent on oxidative phosphorylation as a source of energy for phagocytosis, whereas the latter cells depend rather on glycolysis (Oren et al., 1963; Wolfe et al., 1968). Comparison between the

enzyme profiles of alveolar macrophages and peritoneal macrophages signified that marked differences existed between these two cell forms (Dannenberg et al., 1963). The latter authors encountered an enhanced enzymatic activity in alveolar macrophages in association with phagocytosis. Such was not observed to occur to an extent as great in peritoneal macrophages. Furthermore, the alveolar macrophage divided more rapidly, and consumed greater quantities of oxygen in vitro than did the peritoneal macrophage. Maxwell and Marcus (1968) detected also differences in the phagocytic capabilities of alveolar and peritoneal macrophages in vitro. The former cell type demonstrated a greater phagocytic capability in response to Mycobacterium tuberculosis. The differences that exist between alveolar and peritoneal macrophages are certainly not unexpected inasmuch as these cells reside in vastly differing environments. Nevertheless, if these two cell forms were indeed derived from a common stem cell that had already reached a high level of differentiation (i.e., the blood monocyte) it would be anticipated that in vitro these cells would be quite similar in their behavior. A hypothesis, based on experimental evidence, has recently been presented by Bowden et al. (1969), which appears to reconcile many of the seemingly contradictory findings concerning the origin of the alveolar macrophage. Separate groups of mice were X-irradiated either totally or only on one side of the

thorax. The proliferative behavior of the alveolar macrophages was ascertained by tritiated thymidine labelling and also by direct cell counts (from lung washings). The number of blood monocytes was estimated by cell counts. In addition, the DNA content of the bone marrow was determined. These authors observed that whole body irradiation resulted in a sharp decrease in the number of blood monocytes two days after treatment. However, this reduction of monocytes was not accompanied by a concurrent decline in the number of alveolar macrophages. The DNA content of the bone marrow likewise declined sharply at the same time. The number of alveolar macrophages gradually increased and this augmentation could be correlated to a rise of their labelling index within the alveolar tissue. In those animals receiving partial irradiation only, it was observed that the labelling index in the irradiated lung became likewise enhanced. These findings strongly supported the view that alveolar macrophages were not derived from blood monocytes in any direct fashion. On the basis of their observations, Bowden et al. supported the view that alveolar macrophages arose in situ in alveolar tissue from cells within the interstitium. In addition, they speculated that the cells of the interstitium giving rise to the alveolar macrophages might themselves have derived from progenitor cells within the bone marrow.

Apart from being responsible for the clearance of matter from the alveolus, the alveolar macrophage has been implicated also in the clearance of quantities of the surfactant (Gil and Weibel, 1969; Pratt et al., 1969; Dermer, 1970).

The Septal Alveolar Cell

(the vacuolated alveolar cell)

The septal alveolar cell has been the object of intense scrutiny in regard to its role in the metabolism of the surface-active lining layer (surfactant or anti-atelectatic factor) of the lung. Since the early discovery of this surface-active substance by Pattle (1955), and the realization of its physiological significance in maintaining the normal function of alveolar tissue, a great deal of work has been conducted on the characterization of this material, its source and turnover. The septal alveolar cell has been of special interest to many workers with regard to pertinent questions concerning surfactant. This cell, found in the alveolar septa, forms a part of the alveolar wall by mode of its cellular interconnections with the pulmonary surface epithelium. It is characterized by the presence of osmiophilic lamellated bodies in its cytoplasm, removed by routine histological processing, giving the cell its vacuolated

appearance with the light microscope. An example of such a cell as seen with the electron microscope is presented in Figure 6. The osmiophilic lamellated bodies of the septal cell were asserted by one group of workers to constitute the precursors of some portion of surfactant (Balis and Conen, 1964; Sorokin, 1966; Weibel and Gil, 1967; Goldenberg et al., 1967; Kikkawa et al., 1968). This view was contradicted by Niden (1967) when he observed carbon particles within the septal cell and expressed the opinion that it was capable of phagocytosis. He assigned the role of surfactant production rather to the non-ciliated bronchiolar cell (Clara cell) because that cell was demonstrated as being active in lipid synthesis. A similar view was also held by Azzopardi and Thurlbeck (1969). Other workers, however, rejected this opinion. Goldenberg et al. (1967), employing pilocarpine, a parasympathetic stimulator in rat alveolar tissue, were able to induce an increased number of osmiophilic lamellated bodies in septal cells and their subsequent release by the cells. Esterly and Faulkner (1970) refuted the phagocytic role assigned to the septal cell by Niden. Phagocytosis by the septal cell was not observed even after dust loads or India ink, or else polystyrene spheres were imposed on the lungs of rabbits. It seemed likely that the apparent phagocytosis by the septal cell, observed by Niden, may have been the result of a possible physical penetration of dust into these cells. Furthermore, other

FIGURE 6: Portion of rat alveolar wall showing the septal cell.

Note the vacuoles which contained osmiophilic lamellated bodies that have been washed away by fixation.

Electron micrograph - Magnification unavailable.



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evidence supported the concept of a function of surfactant secretion by the septal cell. For instance, Campiche (1960) noted a correlation between the appearance of osmiophilia in the lung and the first detection of surface activity in fetal rats. Along similar lines, Kikkawa et al. (1968) reported that the onset of breathing in fetal rabbits was associated with a great loss of osmiophilia from the septal cells. Furthermore, the first appearance of surface activity in the tracheal washings of fetal rabbits at day 29 of gestation could be associated with the presence of the osmiophilic lamellated bodies at day 28. However, such evidence implicated only circumstantially the septal cell as the producer of surfactant (Buckingham, 1964). Disagreement thus prevails still concerning the specific function of the septal cell in relation to surfactant. Much of this difficulty is ascribable to problems encountered in the chemical characterization of surfactant (Scarpelli, 1968). An association of surface-active pulmonary phospholipid forming with protein a specific pulmonary lipoprotein, has been assumed from the recovery of phospholipids and protein in single fractions after the centrifugation of pulmonary extracts (Abrams, 1966; Klein and Margolis, 1968). However, Scarpelli et al. (1967) did not encounter any lipoprotein in relatively blood free pulmonary extracts, nor in fetal pulmonary fluid (Scarpelli, 1967), nor in pulmonary washings (Scarpelli et al., 1970). Normal surface activity could be

demonstrated of all these phospholipid extracts. It is therefore believed that the surface active property of pulmonary surfactant depends on the phospholipids and that indeed surfactant may be a phospholipid alone. Pattle (1965) had postulated earlier that the surface-active fraction of surfactant consisted of saturated phospholipid. Although Weibel and Gil (1968) demonstrated a thin osmiophilic lining layer, presumably surfactant, overlying the external surface of the alveolar wall, the presumptive identity of this lining layer as surfactant was disputed by other authors (Adamson and Bowden, 1970; Bowden, 1970). Inasmuch as the presence of unsaturated fatty acids is necessary for osmiophilia, the identity of this osmiophilic lining layer or the osmiophilic lamellated bodies of the septal cell as conceivable surfactant material was questioned. Aware of these circumstances, Gil and Weibel (1969) hypothesized that at least a portion of the surfactant lipids may be unsaturated thereby rendering only these portions visible; alternatively, osmium reduced at unsaturated bonds might perhaps be deposited at the polar groups of saturated lipid molecules. Faulkner (1969) added further support to the view that the septal cell was intimately involved in the metabolism of surfactant. He noted that tritium-labelled glycerol was incorporated to a large extent by septal cells. The incorporation of this labelled compound was mainly into the lecithin fraction of pulmonary lipids; this is significant inasmuch

as surfactant is probably composed to a large degree of dipalmitoyl lecithin. More recently, the technique of tricomplex flocculation, specific for saturated phospholipid, was utilized by Dermer (1969, 1970) and also by Bowden and Adamson (1970) to demonstrate the surface-active lining layer of the alveolus. It was observed by these groups that the osmiophilic lamellated bodies of septal cells were unreactive to the tricomplex flocculation test. This indicated that the material in these bodies was not definitive surfactant. Dermer (1970) noted further that reactive material occurred within alveolar macrophages. The reactive portions within alveolar macrophages were generally found in the peripheral portion of these cells. Unreactive material having a similar appearance was observed in the more central portions of the cytoplasm of alveolar macrophages. It was therefore postulated by this investigator that a degradation of the surface-active substance had occurred within the alveolar macrophages. It is also possible that the absence of reactivity to tricomplex flocculation in the osmiophilic lamellated bodies represented a transition form from precursors to veritable surfactant. Although the role of the septal cell in the metabolism of surfactant has still not been clearly defined, the cumulative evidence suggests that this cell may be involved in some fashion in the production of at least some component of the surface-active lining layer of the alveolus.

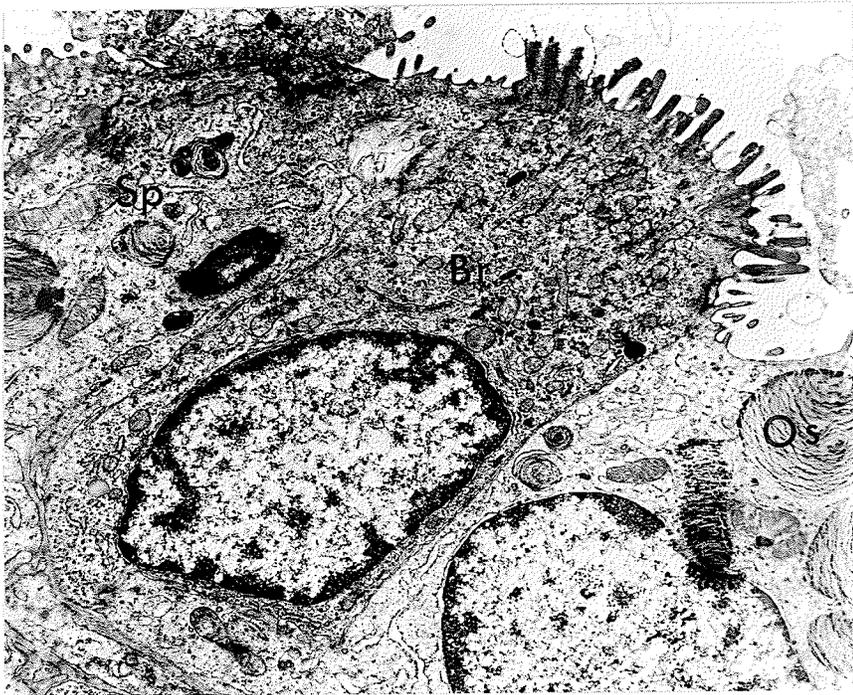
Alveolar Brush Cell

In 1968, Meyrick and Reid reported the existence of a third type of cell in the alveolus. That cell, named the alveolar brush cell, although observed only infrequently, was believed to comprise some five to 10% of all the constituent cells of the alveolus. The specific function of this cell is unknown but it was speculated that it may carry out some absorptive activity, or alternatively it may act as a receptor cell sensitive to chemical or stretch stimuli. An example of this cell type is shown in Figure 7.

Epithelium of the Respiratory Airways

The epithelium of the air-conducting system bears the first insults offered to the respiratory system by foreign matter that has been conveyed by the inhaled air; it thus presents also the initial defenses of the respiratory tissue. The structure of the epithelium of the airways is adapted primarily to the prevention of the penetration of such foreign matter into the deeper portions of the lung. The epithelium of the various segments of the tracheobronchial tree are

FIGURE 7: Alveolar brush cell (Br) in the alveolar wall between two septal alveolar cells (Sp). Note the microvilli projecting from the free surface of the cell
Electron micrograph 6,840 x.



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clearly distinguishable from one another (trachea, bronchus, bronchiole). Yet, the demarcation between one type of epithelium and another is not clearly defined, as the different regions pass gradually from one area into the next. Certain general features are common to all these epithelia. They lie on a thin basement membrane which in turn rests on the lamina propria. This layer is composed of cellular elements, small blood vessels, reticular and elastic elements. The lamina propria is relatively thick in the trachea and bronchi but becomes gradually reduced to an extremely thin layer in the distal orders of bronchioles. Similarly, the epithelium of the airways becomes gradually lower as it passes distally into the depths of the lung. The pseudostratified columnar epithelium of the trachea and bronchi becomes first a simple columnar epithelium in the bronchioles, and then a simple cuboidal in the terminal bronchioles. Ducts of mucous glands open onto the epithelium of the trachea and large bronchi but as the diameter of the airways becomes reduced, the epithelial glands gradually disappear. The epithelium of the larger airways is composed mainly of two cell types: the goblet and ciliated columnar cell. The former cell secretes mucoprotein onto the surface epithelium. The latter cell, by its ciliary beat assists in the movement of the mucous film covering the epithelium towards the oropharynx, thus serving as a mucous escalator. These cell forms are

derived by mitosis from a reserve type cell, the basal cells, that are applied to the basement membrane. Kilburne (1967) claimed the presence of an additional layer of cells that lie above the basal cells, forming an incomplete layer interdispersed between the other cell types. These intermediate cells were stated to give rise to the mature goblet and ciliated columnar cells by differentiation. Presumably they arose from the basal cell layer by mitosis. As the airways decrease in calibre, the goblet cells diminish and disappear gradually from the epithelium. Whereas the function and morphology of the goblet and ciliated columnar cells have been well characterized (Rhodin and Dalhamn, 1956; Rhodin, 1959, 1966; Frasca et al., 1968), the specific function of the non-ciliated bronchiolar cell is unclear. As mentioned previously, some investigators (Niden, 1967; Azzopardi and Thurlbeck, 1969) attributed the secretion of the pulmonary surfactant to this cell but the evidence remains still inconclusive.

Pleura

The pleura serves two major functions in relation to respiration. Firstly, along with the subpleural connective tissue layer, it seals the thoracic cavity and the outer surface

of the lung thereby enabling the volume of the lung to increase and decrease during the respiratory movements of the chest wall. Secondly, the simple squamous epithelium of the pleura, the mesothelium, moistened by the serous pleural transudate facilitates a frictionless movement of the lung against the chest wall by both the parietal and visceral pleural sliding effortlessly over each other. Beneath the mesothelium is the submesothelial connective tissue, richly supplied with a plexus of blood vessels, and lymphatic channels (Hayek, 1953).

CYTODYNAMICS AND HISTOPHYSIOLOGY OF RESPIRATORY TISSUE

DURING HEALTH AND DISEASE

Introduction

The cytodynamic behavior of a cellular population includes such phenomena as cell division, differentiation, migration, exfoliation and others. Of these, the division of the cells of a population by mitosis has been extensively employed to characterize one aspect of cytodynamic activity. Inasmuch as certain of the cellular populations of pulmonary tissue undergo constant renewal which is necessary for the health of the tissue, this approach is especially applicable for the examination of the reactivity of that tissue to irritants. The measurement of the parameter of cell proliferation may present certain difficulties. For instance, if the rate of mitosis is quite slow it may be difficult to accumulate sufficient data from the tissue of untreated animals. In addition, it may be difficult to recognize certain stages of mitosis. Such difficulties were resolved through the employment of two specific techniques that enabled the accumulation of sufficient data concerning the proliferative behavior of cell populations in a facile manner. These methods of examining the

mitotic activity of cells, the colchicine and thymidine techniques, have been thoroughly reviewed by Bertalanffy (1964).

Colchicine Technique

The drug colchicine is an alkaloid extracted from the plant Colchicum autumnale. The ability of this drug to arrest mitosis in the stage of metaphase by inhibiting the formation of the spindle fibres rendered it an invaluable tool for cytodynamic investigations (Eigsti and Dustine, 1955; Leblond and Walker, 1956). If a suitable dosage of colchicine is administered into an animal sacrificed within an appropriate time span after treatment, all the cells entering mitosis and reaching metaphase are arrested in that stage. Determination of the fraction of such arrested cells within a population permits an estimation of the mitotic rate of that cellular population within a certain time span. It is usually calculated as the percentage of metaphases. Thus,

$$\frac{\text{number of arrested metaphases}}{\text{total number of cells tallied}} \times 100 = \text{percent metaphases.}$$

The latter data represents the number of cells that entered cell division during the time span between the injection of colchicine and the sacrifice of the animal. Inasmuch as the number of cells dividing during the particular interval can be expressed as a

fraction of the total population, the time required for all of the cells to divide can be calculated. This is the turnover time (Bertalanffy, 1964). Thus,

if $\frac{M}{100}$ cells divide in time t ,

then for all the cells of a population to divide requires time T or

$$T = \frac{100t}{M}$$

Notwithstanding the great facility that the colchicine technique has provided to investigators, it is not without limitations. The most severe problem presented by this method of cytodynamic investigation is the necessity for the visual recognition of arrested metaphases. This contributes significant human error inasmuch as a large number of decisions must be made during the accumulation of data. Despite this limitation, the colchicine technique, when used carefully, provides much valuable information.

Thymidine Technique

During the DNA synthesis (or S) phase of the cell cycle, when DNA replication proceeds, thymidine, a precursor of DNA, is

taken up as thymidylic acid by the nucleus of the cell. If thymidine labelled with the radioisotope tritium had been administered, it is possible by means of the radioautographic technique to visualize those cells that were in the S phase while the tracer was available, whose nuclei incorporated the tritiated thymidine. This is essentially the basis of the thymidine technique. Incorporation of the labelled thymidine into nuclei following a pulse label occurs maximally for about one hour conceivably less, after the label is introduced (Cronkite, 1959). The remaining thymidine that had not been incorporated is quickly metabolized and excreted (Potter, 1959). By employing this technique it is feasible to estimate that fraction of cells synthesizing DNA while the tracer is available. This value can be expressed as a percentage and is designated the thymidine or labelling index.

The turnover time of a cellular population may be ascertained by two different means employing the thymidine technique (Bertalanffy, 1964). Firstly, cell cycle analysis may be undertaken. This approach, however, was not employed in the present series and therefore is not presented. Alternatively, the transit time, a measure of the turnover time, can be estimated. Groups of animals are sacrificed at different time intervals after the administration of labelled thymidine. The time period

extending from the injection of the drug to the time at which labelled cells are for the first time not evident, represents the transit time of the labelled thymidine. This latter method was employed in a modified fashion in the present series.

The thymidine technique is more sensitive and accurate than the colchicine method inasmuch as much of the human error associated with the latter is removed.

Behavior of Alveolar Tissue

The first study of the cytodynamic behavior of alveolar tissue utilizing modern technique was by Bertalanffy and Leblond (1955). Employing the colchicine technique these authors determined that the percent metaphases of rat alveolar tissue six hours after the administration of colchicine was .90. The daily mitotic rate of this tissue was 3.66%. Through specific morphological observations it was established that two distinct cellular populations were constantly renewed by mitosis in alveolar tissue. They were the vacuolated and non-vacuolated alveolar cells. The vacuolated alveolar cell population was renewed completely about every 28 days, whereas the population of non-vacuolated alveolar cells had a shorter renewal time, approximately seven days. These observations were confirmed

subsequently by the thymidine technique (Spencer and Shorter, 1962; Shorter et al., 1966; Bowden et al., 1968). Inasmuch as the most rapidly dividing cellular population of the alveolar tissue, the alveolar macrophages, constitute the major defense mechanism of this tissue, investigators began to examine the mitotic responses of these cells to various irritations. For instance, Casarett and Milley (1964) reported that the mitotic rate of rat alveolar tissue was significantly increased from normal after the imposition of a dust load of iron-oxide by intratracheal instillation. Streker (1965) observed a five-fold increase in the mitotic activity of rat alveolar tissue after the exposure of the animals to quartz dust. The parenteral injection of irritating substances into animals evoked similar mitotic responses in alveolar tissue. In this regard, Casarett et al. (1967) found that the parenteral injection of Freund's adjuvant into rats brought about a significant increase in the mitotic rate of alveolar tissue. In addition, an augmentation of the mitotic activity of mouse alveolar tissue in response to parenterally injected benzopyrene was reported by Kaufmann (1969). That author utilized both the colchicine and thymidine techniques in order to ascertain the mitotic responsiveness of the alveolar tissue and observed a greatly increased labelling index of the alveolar tissue. More recently, Boren et al. (1970) reported that the labelling index of hamster alveolar tissue became augmented significantly after intratracheal instillation of hematite or a

mixture of hematite and benzopyrene. The latter treatment stimulated an even greater response than the former. Cigarette smoke was also demonstrated to enhance the mitotic rate of hamster alveolar tissue (Boren, 1970). In contrast, Bowden et al. (1968) reported that a high oxygen concentration did not appreciably affect the labelling index of mouse alveolar tissue. Previous to this, Evans et al. (1969) observed a significant decline of the labelling index of the alveolar tissue of mice that had been subjected to high levels of oxygen. It must be noted, however, that the latter group used a 100% oxygen atmosphere whereas the former workers employed a less concentrated oxygen atmosphere of 90%. Evans and his co-workers reported additionally that a high level of labelling of the endothelial cells of the alveolar capillaries was observed under similar conditions. Bowden and associates did not observe such enhanced labelling of the endothelial cells. However, these two groups did concur in the observation that the endothelial cells of the alveolar capillary were severely affected by the treatment.

The cytodynamic activity of alveolar tissue has also been examined by other means. For example, the number of free alveolar cells within the tissue was measured by counting the free alveolar cells in lung washings (Brain, 1970). Bowden and his associates (1969) employed this technique extensively for correlating the

labelling indices of alveolar tissue with the incidence of free cells. They observed that the number of free alveolar cells was not reduced significantly by the whole body irradiation of mice. This technique was also employed to examine the changes in the number of free alveolar macrophages after the exposure of animals to various dusts. Along these lines, Labelle and Brieger (1960) observed that the number of these cells became greatly augmented after the exposure of rats to dusts of various types. With the cessation of dust exposure, the number of exfoliated cells gradually returned to normal levels. Similar responses of even greater magnitude were recorded in those rats that had been treated with the dusts by intratracheal injection. Furthermore, Labelle and Brieger reported a positive correlation between the quantity of dust administered and the number of free cells. Yet, that correlation did not hold true when the differences in the quantities of dust administered were merely small. Gross et al. (1969) examined likewise the effect of various dusts at different concentrations administered intratracheally in rats on the number of free alveolar macrophages. Although an increase in the number of these cells was discernable after dust administration, nevertheless correlations could not be established between the number of cells and the quantity or type of dust administered. Other investigators utilized merely visual estimates to characterize changes in the number of free alveolar macrophages. Bhagwat and

Conen (1969) for example, described an increase in the number of free alveolar macrophages in the lungs of rabbits in which pneumonia was induced by artificial means. In contrast, Dowell et al. (1970) observed that the exposure of rabbits to ozone resulted in a decrease of free alveolar macrophages in the air spaces. The osmotic fragility of the macrophages was measured as well and was noted to increase in those animals exposed to ozone. The authors stated that this may have accounted for the decrease in the number of free macrophages, inasmuch as the fragile cells ruptured more readily than the macrophages not so affected in untreated animals.

The histochemical activity of alveolar macrophages was examined by Sherwin et al. (1967). Free alveolar macrophages were collected from the lungs of guinea pigs that had been exposed to nitrogen dioxide. The number of these cells exhibiting a positive histochemical response for lactic dehydrogenase in vitro was markedly increased. This suggested that the cells had chosen an alternative metabolic pathway for energy production in the presence of the irritating gas. Hurst et al. (1970) exposed alveolar macrophages in vitro to ozone and reported a decrease in the activity of acid hydrolases that was histochemically demonstrable. This finding suggested that the ozone was exerting cytotoxic effects on the cells.

The phenomenon of macrophage congregation; the grouping together of alveolar macrophages, was examined by Sherwin et al. (1968) in vitro. A higher degree of congregation was observed of alveolar macrophages derived from guinea pigs exposed to nitrogen dioxide gas than of similar cells from untreated animals. This phenomenon may represent a cooperative action on the part of alveolar macrophages in response to an irritating substance.

Although the septal cell has been little alluded to with regard to its reactivity to noxious agents, some reports have recently appeared in the literature concerning the behavior of that cell under stressful conditions. The paucity of literature on this aspect of the behavior of the septal cell is partly ascribable to the general preoccupation of many investigators with the role of that cell in relation to surfactant. Likewise, a similar preoccupation of workers with the alveolar macrophage and its response to noxious materials has limited the number of studies on the reactivity of the septal cell. Moreover, the difficulty of specifically measuring the cytodynamic responses of this cell presented a formidable barrier. However, certain investigators examined recently the septal cell in this regard. Kapancini et al. (1969) observed that severe alveolar injury in monkeys induced by oxygen toxicity was followed by a remarkable proliferation of septal cells. Likewise, Carrington

and Green (1970) reported a similar reaction of septal cells in the lungs of dogs that had been exposed to either mercury fumes or to cadmium salt aerosols. Similarly, Yuen and Sherwin (1971) observed the replacement of the pulmonary surface epithelium by septal cells in guinea pig lung after the exposure of the animals to nitrogen dioxide gas.

Although the research on the cytodynamic behavior of the cell populations in alveolar tissue to date has yielded much information on the reactivity of these cells in respiratory tissue, many conflicts still exist. It is believed that further cytodynamic investigations will clarify some of the disease processes to which the alveolar tissue is subjected.

Epithelium of the Respiratory Airways

The mitotic rate of the epithelium of the airways can be more readily ascertained because the cells comprising it are regularly arranged and the cell populations are more uniform, controversy has lately arisen concerning the proliferative behavior of this epithelium. Utilizing the colchicine technique, Leblond and Walker (1956) estimated the turnover time of rat tracheal epithelium to be 47.6 days. The use of the thymidine

technique to estimate the turnover time of this epithelium has yielded much lower values (Koburg and Maurer, 1962; Shorter et al., 1966). Much of this discrepancy is probably due to the difficulty inherent in the colchicine technique of recognizing the arrested metaphases of the basal cells of the epithelium. Such discrepancies of cytodynamic estimations exist for other portions of the epithelium of the airways as well (Bertalanffy, 1964; Blenkinsopp, 1967). In this regard, Bertalanffy (1964) proposed that these discrepancies between the data of various groups on the cytodynamics of the airway epithelia might be the result of the variation of turnover times of various parts of the epithelium, even those regions in close proximity. This phenomenon was previously reported by Koburg and Maurer (1962). In contrast, Blenkinsopp (1967) expressed the opinion that some of the discrepancies, at least, were based on the use of different techniques to calculate the turnover time. Whereas Shorter et al. employed the transit time of tritiated thymidine labelled cells within the epithelium to estimate the turnover time, Blenkinsopp held the view that such estimations were erroneous in that the transit time of label in cells perhaps only expressed the turnover time of a discrete cellular population but was not representative of the entire epithelium. Utilizing the data of Shorter and his group of the thymidine index after a short pulse label, Blenkinsopp was able to calculate turnover times agreeing with

his own data. It was also asserted by Blenkinsopp that the epithelium of the airways is composed of more than one renewing cell population. He was of the opinion that basal cells and superficially located cells of the epithelium should be considered as separate renewing populations. This opinion, however, was not colluded by Bertalanffy (1971). The many differences between the findings of different groups concerning the normal cytodynamic activity of the airway epithelium are probably based on the variation of techniques. It is therefore of more importance to consider the absolute changes in the proliferative activity of this epithelium from the reported normal in response to a variety of insults. In this fashion, regardless of the discrepancies between the normal values reported by various workers, the alterations from the normal caused by different treatments can be examined. In this regard investigators examined the cytodynamic response of airway epithelium to irritating materials. For instance, Lamb and Reid (1967) exposed rats to sulfur dioxide and ascertained that the mitotic activity of the airway epithelium (excluding the most terminal airways) was raised to levels significantly greater than in untreated animals by one week after exposure. This high level of mitotic activity gradually decreased, but in the proximal airways normal levels were not re-established even after six weeks. Significant increased goblet cell production in the epithelium was also noted; in fact

goblet cells appeared even in the distal airways where they were not encountered under normal conditions. Similarly, Boren (1970) examined in separate series the effect of cigarette smoke or high levels of oxygen on the cytodynamics of the airway epithelium of hamsters. Although an increase of the thymidine labelling of cells per area along the length of the epithelium was noted in both instances, a greater response was observed in those animals that had been exposed to cigarette smoke. In the case of chronic respiratory disease in rats, Wells (1970) reported that the turnover time of the epithelium of these animals was much shorter than that observed in normal animals. These studies clearly showed that the epithelium of the airways is highly responsive to noxious substances in general. Inasmuch as this epithelium represents the major part of upper respiratory tract clearance, it will again be considered in relation to this portion of the clearance mechanisms of the respiratory system.

Pleural Mesothelium

The cytodynamic behavior of the mesothelium of the pleura has not been investigated extensively. It was reported by Bertalanffy and Lau (1962) that the turnover time of this epithelium is approximately 33.8 days; this being of the same order as that of

the pulmonary surface epithelium (Bertalanffy, 1964). The pleural mesothelium, however, is of great importance in the present investigation because of its reactivity in response to asbestos.

PULMONARY CLEARANCE

General Considerations

Pulmonary clearance, the ability of the respiratory system to clear foreign matter from its environs, may be identified by two major components: upper and lower respiratory tract clearance (Gross, 1964; Kilburne, 1968). These two levels of pulmonary clearance are intimately connected both structurally and functionally. The upper respiratory tract clearance involves structurally the epithelium of the airways. The mucous blanket secreted onto the epithelium "captures" foreign matter that settles upon it from the inspired air. The movement of the mucous film by the beating of cilia, or mucous escalator, transports such foreign matter to the oropharynx from which it is expectorated or swallowed (Kilburne, 1968). The clearance component of the lower respiratory tract is largely represented by the population of alveolar macrophages in the alveolar tissue (Bertalanffy, 1967). This population of cells phagocytoses any foreign matter that has entered the alveoli. Clearance of the foreign matter carried by the alveolar macrophages from the lung can be executed by two means. Firstly, the macrophages can move

to the airways epithelium thereby gaining access to the upper respiratory tract component and the ingested foreign matter is then cleared from the lung through the air-conducting system. Secondly, a less effective means of clearance operates if the alveolar macrophages gain access to the lymphatic system (Brundelet, 1965, 1967). In this case clearance from the alveoli may be successful but the ultimate removal of foreign matter from the body is less certain. In addition, the film of fluid upon the external surface of the alveolar wall is also believed to be active in lower respiratory tract clearance. By physical means related to respiratory movements of the lung, this fluid film is moved to the mucous film of the airways. Macrophages containing ingested matter as well as free foreign matter are therefore carried to the tracheobronchial tree (Gross, 1964).

Failure of pulmonary clearance may be the result of various factors. The quantity of foreign matter presented to the clearance mechanisms may be too excessive to render the clearance mechanism effective. Toxic foreign substances may cause severe damage to one or another of the clearance mechanisms, thereby preventing clearance. Finally, disease processes may impair clearance mechanisms to such a degree that adequate clearance of even innocuous substances cannot be successfully achieved.

Upper Respiratory Tract Clearance

Under normal physiological conditions the upper respiratory tract component of the clearance mechanisms is able to clear the bulk of inhaled foreign matter. The effectiveness of this clearance component under such conditions is largely dependent upon the size of inhaled particles. Hatch (1961) reported that the depth to which inhaled particles penetrate into the respiratory tract and the site of deposition of particles within the respiratory tissue varied with the aerodynamic particle size and the breathing pattern. Upper tract clearance became increasingly ineffective as the size of particles decreased. Against dust particles ranging in size from 1 - 2 micra, the effectiveness of the upper clearance component approached zero. Conversely, for particles larger than 10 micra, the upper tract component was theoretically 100% effective. These findings were based on theoretical considerations taking into account the dimensions of the respiratory tract and the settling characteristics of dust particles of different sizes. Morrow and Casarett (1960) substantiated these theoretical considerations. They reported that 90% of the dust particles retained in the lungs of rats after inhalation exposure were less than 0.2 micra in diameter. Thus, as postulated by Hatch, particles of small

size were cleared most inefficiently. Larger particles were cleared by the epithelium of the airways inasmuch as these particles settled onto the mucous film before attaining the alveolar spaces. Severe impairment of this upper tract clearance mechanism can be caused by injury to the airway epithelium (Kilburne, 1970), as also by the imposition of an extremely heavy dust load in air. For instance, a marked reduction in the flow of mucous along the tracheal epithelium of rats exposed to sulfur dioxide was observed by Kilburne and Williams (1969). As mentioned previously, Lamb and Reid (1967) recorded enhanced mitotic activity in the airway epithelia of rats exposed to that same gas. Likewise, cigarette smoke was implicated as a causative agent of severe damage to the airway epithelium (Frasca et al., 1968). A hyperplastic thickening of dog bronchial epithelium by cell proliferation was the result of exposure of the animals to the cigarette smoke. Ciliated columnar cells and goblet cells were absent, having been replaced by columnar and cuboidal cells whose free surfaces were covered only by stubby projections. It can be readily comprehended that any substance damaging the epithelium of the airways will compromise upper respiratory tract clearance. The connection between the upper and lower tract clearance components is highly significant in executing efficient pulmonary clearance. For instance, Spritzer et al. (1968) observed that 79.3% of cells

cleared by the upper respiratory tract component were mononuclear. Presumably the bulk of these cells were alveolar macrophages. More than one million alveolar macrophages were cleared each hour by this route. It follows then that a severe impairment of upper respiratory tract clearance will place additional stress on the lower tract clearance mechanisms.

Lower Respiratory Tract Clearance

The major constituent of the lower respiratory tract clearance mechanism is represented by the population of alveolar macrophages that is constantly renewed by cell division, and is cleared by extrusion via the upper respiratory tract clearance component. Lower tract clearance is especially effective in preventing bacteria from damaging pulmonary tissue. Laurenzini et al. (1963) examined the efficiency of the lower tract clearance of rats when challenged by viable non-pathogenic bacteria. Homogenates of the lung tissue of rats exposed to a bacteria-laden aerosol were spread on culture media. The number of colonies of bacteria found in the culture media after incubation represented the surviving bacteria within the rat alveolar tissue. Utilizing this technique, it was ascertained that fully 95% of bacteria were rendered inviable by the lower respiratory tract clearance

component within six hours. Impairment of such efficient clearance was caused by various agents: alcohol, hypoxia and cigarette smoke. In a similar fashion, Green and Kass (1964) exposed rats to an aerosol containing radioactively labelled bacteria. Although bacterial viability was reduced by 80 - 90% within four hours, radioactive label declined by only 14 - 20% within this time span. This indicated that the bulk of clearance was not by way of the upper respiratory tract but was mainly brought about by the bactericidal activity of the lung; this being largely executed by the alveolar macrophages.

Failure of the lower respiratory tract clearance component of alveolar macrophage presents a serious problem to the continued functional portions of the respiratory system. Such failure can be brought about by two major means: the incapability of lower tract clearance to cope with a heavy load of foreign matter resultant from a failure of the upper tract clearance component; or else, the lower tract component can be directly impaired by the cytotoxic action of foreign material on the alveolar macrophages. The action of silica dust on the respiratory tissue is an example of the combined effect of these two modes of impairment of lower respiratory tract clearance. Gross and Brieger (1965) injected silica dust intratracheally into rats. This dust induced bronchiolitis obliterans in the respiratory tissue, thereby

causing a blockage of the respiratory bronchioles which extended to adjoining alveolar ducts. In this fashion, the upper respiratory tract component of clearance was sealed from intimate contact with the lower tract component. Thus any silica remaining in the alveolar tissue could not be efficiently removed. In addition, the cytotoxicity of silica on alveolar macrophages has been well demonstrated (Allison et al., 1965; Vigliani et al., 1969). These workers observed that in vitro silica dust killed alveolar macrophages. Thus the destruction of macrophages by this action of silica further favoured the impairment of lower respiratory tract clearance.

In the eventuality that upper tract clearance is blocked but the lower tract clearance component has remained intact, an alternative route for clearance does exist. As previously described, clearance of foreign substances by the alveolar macrophages by way of the lymphatic system is possible. For example, Brundelet (1965, 1967) observed in rats that imposed burdens of carmin and trypan blue were carried by alveolar macrophages to peribronchiolar lymphoid foci. From here the macrophages gained access to the epithelium of the airways by ameboid movement. It was postulated by Moolten (1967) that such movement of macrophages through the lymphatics was probably mediated by the negative pressure within interstitial fluid. Thus macrophages

laden with foreign matter or the foreign matter alone could be drawn by the sump-like action of the interstitial fluid to lymphatic channels. Regarding this mechanism however, various workers believe that it represents a very minor, highly inefficient means of pulmonary clearance (Gross, 1964; Thiart and Engelbrecht, 1964).

It was generally established that the pulmonary clearance of experimentally imposed dust loads occurred in two phases (Gross, 1964). The initial phase of clearance was rapid and reached a maximum after a few days (Le Bouffant, 1960; Thiart and Engelbrecht, 1964); thereafter, clearance proceeded slowly (Thomas and Stannarck, 1964; Morrow et al., 1964). The quantity of foreign matter removed during each phase of clearance varied depending on the nature of the burden (particle size, cytotoxicity) as well as on the mode of imposition (air-route, intratracheal instillation). For instance, Labelle and Brieger (1960) noted that 40% of a dust load of uranium dioxide was cleared after two days by rat lung. Conversely, Reeves and Vorwald (1967) observed that fully 50% of an imposed dust load of beryllium sulfate remained in the lungs of rats after 52 weeks.

It is evident that the etiology of disease caused by airborne agents in the respiratory tissue depends to a large degree

on the interaction of such substances with the clearance mechanisms of the tissue. A diagrammatic representation of the various components of pulmonary clearance and the factors affecting them is shown in Figure 8. In the following section, a consideration of the literature on experimentally induced asbestos-related disease of the respiratory system, is presented.

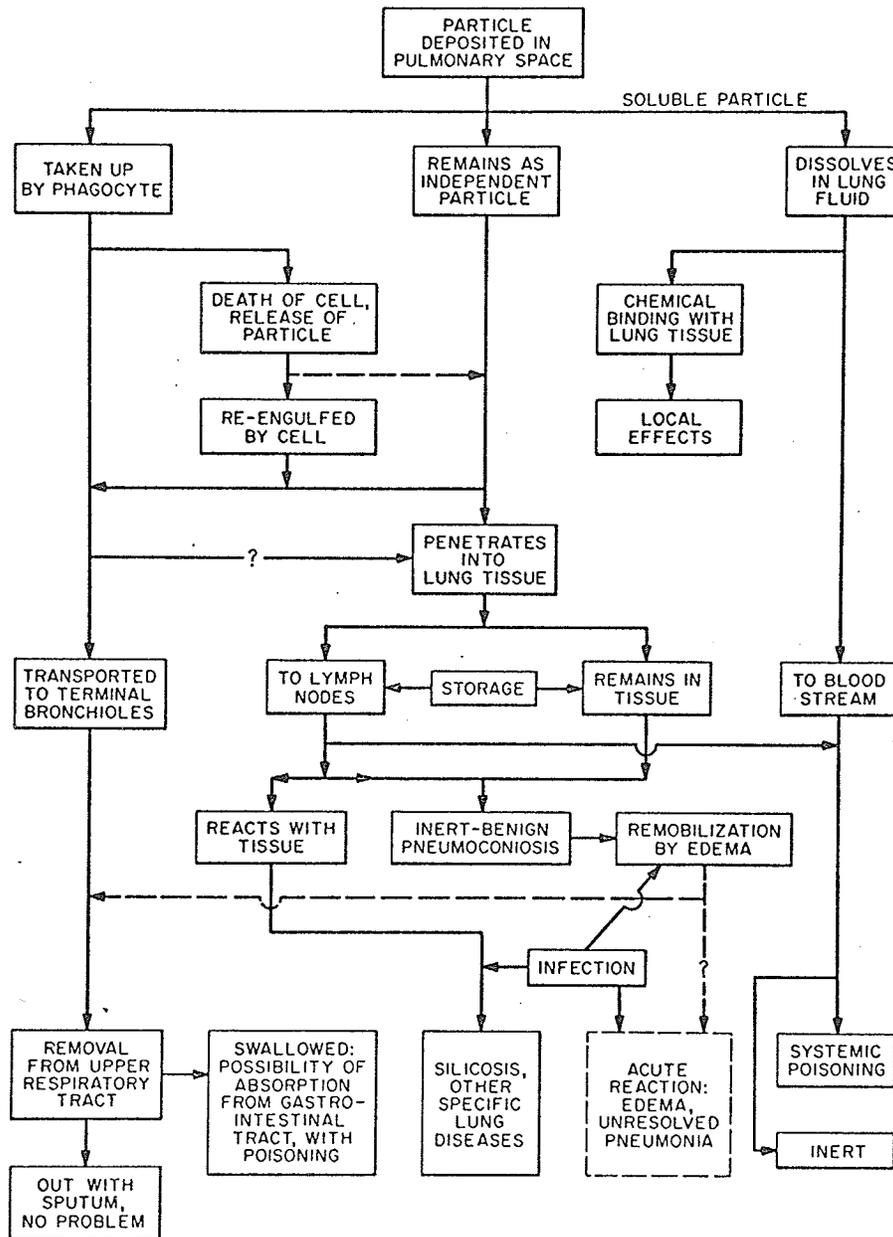


Figure 8. - Diagrammatic representation of pulmonary clearance mechanisms (after Hatch, 1959).

EXPERIMENTAL ASBESTOS-RELATED DISEASE OF THE PULMONARY SYSTEM

The major objectives of scientists examining experimentally asbestos-related diseases of the pulmonary system have been to establish by experimental series in animals the carcinogenic activity of asbestos within pulmonary tissue and to delineate the etiology of asbestos-related disease in the pulmonary system. Nordman and Serge (1941) reported the initiation of lung tumours in mice after nine months of inhalation exposure to asbestos. These workers observed that in two of their experimental animals, adenomas were evident within the lung tissue. Yet, this finding was generally not accepted inasmuch as one of the tumours was recognizable as occurring commonly even in untreated mice, and the validity of the other tumour was questioned (Smith et al., 1965). The first extensive experimental series conducted on the biological effects of asbestos on pulmonary tissue, however, was by Vorwald and his associates (1951). These workers hoped to induce lung malignancies in rats and guinea pigs by the exposure of these animals to asbestos dusts. The animals were administered asbestos both by inhalation exposure and intratracheal instillation. Yet even after extended periods of post-treatment, these workers did not observe neoplasia of the respiratory system within any of their

experimental animals. They further reported that long fibre asbestos (chrysotile) was more effective in inducing fibrosis of the lung tissue than short fibres of similar material. However, the validity of these observations was limited in that the quantities of asbestos in the two samples utilized were unequal. The sample of short fibre chrysotile contained larger amounts of impurities of other particles from the native ore; in fact, this sample was largely comprised of the native ore, and short asbestos fibres comprised less than 50% of the total sample. Similar studies were conducted by Behrens (1951) and Smith (1951), but these authors were likewise unsuccessful in inducing neoplasms within the experimental animals. Later, Lynch and his associates (1957) were able to induce pulmonary adenomas in mice after extended periods of exposure to chrysotile (up to two years). But in view of the spontaneity of this tumour, notwithstanding the higher incidence observed in exposed mice, these workers did not regard their results as having established the carcinogenic behavior of asbestos. The work of Wagner (1962) also met with a similar difficulty. Although pleural mesothelioma was successfully induced in three rats injected intrathoracically two years previously with asbestos, the low incidence of tumours and the occurrence of a mesothelioma in a rat treated with silica (a non-asbestiform dust) precluded these experiments from signifying a specific carcinogenic behavior of asbestos.

A similar study was conducted by Smith et al. (1965), who successfully induced pleural mesothelioma in five hamsters by the intrathoracic injection of asbestos into these animals. Both harsh chrysotile and amosite brought about the formation of mesothelioma, but soft chrysotile did not. Other animals in this series were administered asbestos dusts by intratracheal instillation, but in none of these animals was there any evidence of neoplasms. Similarly, Miller et al. (1965) attempted to induce neoplasms in the lungs of hamsters by intratracheal injection of chrysotile and amosite dust. Some of the animals were injected in addition with benzopyrene. Although the incidence of lung tumours in those animals injected with both asbestos and benzopyrene was higher, yet because of the small overall incidence within the groups, the findings were not statistically significant. Although a carcinogenic activity of some types of asbestos in the induction of pleural mesothelioma was demonstrated by Smith et al. as mentioned previously, nevertheless the successful initiation of cancers within the lung parenchyma by asbestos was not achieved. The investigation by Gross et al. (1967), with extensive experimental series, provided experimental evidence that asbestos could in fact induce cancer of the lung in animals. Rats were exposed to chrysotile asbestos dust clouds six hours per day, five days per week, for as long as 62 weeks. Some of the rats were previously injected

intratracheally with a dilute solution of NaOH in order to prevent upper respiratory tract clearance, and thereby facilitate the retention of asbestos in the lungs. A 48% incidence of lung tumours was observed in the lungs of animals exposed to chrysotile asbestos dust clouds after the intratracheal instillation of the dilute NaOH solution into the airways. Those rats exposed to dust clouds of asbestos only showed an incidence of 24% of cancers of the lung. Finally, another group of rats treated by the intratracheal instillation of chrysotile exhibited a 16% incidence of lung cancers. These findings, however, were only partly conclusive. The hammer-mill device utilized to produce dust clouds of asbestos was subjected to considerable wear by the chrysotile. Inasmuch as one of the contaminants of wear from this device was nickel (a known carcinogen) (Sunderman et al., 1959; Sunderman, 1967), Gross and his group cautioned that at least part of the induction of tumours may have been attributed to this nickel contamination of the chrysotile. Notwithstanding the role of nickel in this series, at least a co-carcinogenic activity of chrysotile asbestos was likely. More recently, Wagner and Berry (1969) reported a high degree of success in the induction of pleural mesothelioma in rats after the intrathoracic inoculation of various asbestos forms into these animals. An incidence of nearly 50% of mesothelioma of the pleura was observed in this series. These investigations supported beyond reasonable

doubt that the asbestos minerals were able to induce neoplasia in the pulmonary system.

At the same time other workers were examining the etiology of asbestosis with the long term objective of providing information on the mode of action of the asbestos minerals in bringing about carcinogenesis within the pulmonary tissue. In this regard, Holt and Young (1960), Holt et al. (1964) and Holt et al. (1965) differed with Vorwald in the opinion that long fibres of chrysotile were more fibrogenic than short fibres. These workers observed in inhalation exposure series that the short fibres of chrysotile gained access to the lungs of rats more readily and therefore exerted their fibrogenic action on the pulmonary tissue. It was concluded that in view of the greater accessibility of short fibres to the pulmonary tissue, they should be more fibrogenic than long fibres. With regard to this contradiction, it is recalled that the sample of short fibre chrysotile employed by Vorwald and his group was highly impure as it contained less than 50% of true asbestos fibres. Conversely, Holt and his associates utilized pure chrysotile which was hammer-milled to very small fibre sizes. This question of the relative fibrogenicity of different sizes of asbestos fibres arose again recently in the literature. For example, Klosterkotter (1969) and the group at the Pneumoconiosis Research Unit of South

Africa (1969) both reported that long asbestos fibres were more active in inducing fibrous reactions within pulmonary tissue than short fibres. It became clear on the examination of experimental technique why such discrepancies occurred. Whereas Holt and Young exposed animals to asbestos by means of inhalation, Klosterkotter and the South African group utilized the intratracheal instillation means of exposure. It is evident then that long fibres of chrysotile were indeed more fibrogenic than short fibres once they gained access to the lung tissue. The greater fibrogenicity of short fibres as observed by Holt and his group depended more on the capability of these fibres to penetrate into the lung tissue by the inhalation route than on a greater fibrogenic activity per se.

The clearance of asbestos from the respiratory system was likewise a subject of extensive investigation but the technical difficulties encountered were responsible for the paucity of literature on this aspect of asbestos-related disease. Labelle and Brieger (1960) examined the effect of a superimposed dust load of asbestos on the clearance of uranium dioxide from the lungs of rats. They reported that the asbestos markedly inhibited the clearance of the uranium dioxide but a direct calculation of the clearance rate of asbestos was not undertaken. Wagner and Skidmore (1965) attempted a direct estimation of the clearance of

of asbestos from rat lungs, but their method of measuring the quantity of asbestos retained was indirect and subject to question. They calculated the amount of silica remaining within the lungs of the experimental animals presuming that this represented by rough proportion the quantity of asbestos because asbestos contains silica as an impurity. A direct attempt at measuring the amount of retained asbestos within the lungs of rats was made by Morgan and Holmes (1968). They utilized neutron activated crocidolite and chrysotile asbestos. The clearance of this material from the lungs could be ascertained by scintillation counting techniques inasmuch as the asbestos was labelled with radioisotopes. After four months more than half of an intratracheally instilled dose of this asbestos was still retained in respiratory tissue. The loss of label from the asbestos by leaching, however, did limit the accuracy of their findings to some extent. Pylev et al. (1969), as had Labelle and Brieger (1960) previously, examined the effect of asbestos on the clearance of another imposed substance. Crocidolite asbestos was intratracheally instilled into hamsters that had previously received tritiated 3,4 benzopyrene by the same route. The presence of the asbestos inhibited the clearance of the labelled benzopyrene only after 21 days. An increase in the total number of alveolar macrophages in the lungs of animals so treated was also noted. Both of these phenomena were also evident in animals that had

received carbon black rather than asbestos as the second intratracheally instilled material. Kanazawa et al. (1969) examined the clearance of asbestos from pulmonary tissue from a qualitative standpoint rather than quantitative, as the above series. Utilizing conventional light microscopy techniques and phase contrast microscopy, these workers observed asbestos fibres within extrapulmonary lymphoid tissue. The asbestos had been intratracheally instilled in rats. This work was affected severely by technical difficulties, and only large asbestos fibres could be localized by this means.

Various theories on the mode of action of asbestos on pulmonary tissue were proposed. For example, some workers believed that the mode of action was largely a chemical one, whereas other groups postulated that a physical irritation of lung tissue by asbestos was decisive (Whipple, 1965). In recent years both of these theories were more closely examined. For instance, Holt et al. (1965) suggested that the phagocytosis of chrysotile asbestos fibres by alveolar macrophages was a prerequisite for pulmonary fibrosis. In this regard, Allison et al. (1965) reported on the cytotoxic action of asbestos fibres on alveolar macrophages in vitro. Also Davis (1963, 1965) examined the asbestotic lesions of both animals and man, but reported that numerous asbestos fibres not visible with the light microscope

could be discerned in electron micrography of lung tissue. It was hypothesized by that author that alveolar macrophages laden with asbestos fibres were capable of transformation into fibroblast-like cells. Davis, like Holt and his group, asserted that the development of asbestosis was essentially an intracellular process involving alveolar macrophages and their derivatives. Regarding the development of asbestosis, Holt et al. (1965) delineated the early stages in guinea pigs. The initial reaction was a bronchiolitis which extended from the terminal portions of the airways to adjacent alveoli. Subsequently, a widespread and progressive fibrosis of the lung as well as an adenoid proliferation of the bronchiolar epithelium occurred. A similar response was observed in rats by Wagner and Skidmore (1965). However, Gross and Treville (1967) did not concur with these findings. They reported that asbestosis was self-limiting in rats, whereas in guinea pigs a progressive fibrosis ensued from asbestosis. It was noted by these workers, as also by others (Holt et al., 1965), that unlike in hamsters or guinea pigs, asbestos bodies formed only rarely in rats. Since the asbestos body occurs in high incidence in humans, the difference of reactivity between rats and the afore-mentioned animals was of great interest. The formation of the asbestos body itself generated a great deal of interest and investigation. Davis (1965) was the first worker to examine the asbestos body

with the electron microscope, and corroborated the early hypothesis of Gloyne (1929) that the body was composed of blood pigment deposited onto asbestos fibres. Beattie (1961) had postulated, on the other hand, that the asbestos body was composed to a large extent of collagen but this view was again refuted by Blount et al. (1966) who stated that the outer coating was definitely not collagen. It was asserted by Holt and Young (1967) that the asbestos body was essentially derived from the cytoplasm of macrophages that attempted to ingest asbestos fibres. They proposed that the adsorption of protein from the cytoplasm of the macrophage led to a shrinkage of the cell membrane. With time this shrinkage gave rise to the characteristic appearance of the definitive asbestos body. The ferritin derived from blood pigment and believed to comprise the bulk of the coating, was not ascertained by these investigators. Botham and Holt (1968) provided finally a more complete account on the formation of the asbestos body. Large asbestos fibres ingested by alveolar macrophages became coated with iron-containing protein (presumably hematogenous ferritin) that had been derived from hemolyzed erythrocytes ingested at some previous time by the alveolar macrophages. With the death of the alveolar macrophages containing the protein-coated asbestos fibres, the latter, with fragments of the cellular membrane of the macrophage attached, were released as presumptive asbestos bodies. Subsequently

the shrinkage of the membrane fragments resulted in the formation of the definitive asbestos bodies. These findings were essentially confirmed by Suzuki et al. (1969), examining the development of the asbestos body with the electron microscope. It was reported that the death of the alveolar macrophage, as earlier purported by Botham and Holt, was not a necessary prerequisite for the formation of the asbestos body. Moreover, a striking hyperplasia of cell organelles was observed in alveolar macrophages containing ingested asbestos fibres (Suzuki and Churg, 1970). This was indicative of the active participation of this cell type in the production of the asbestos body. The asbestos body probably represented a protective mechanism of the lung against the cytotoxic action of asbestos fibres, but it is as yet not clearly understood why in some species of animals, for instance in the rat, asbestos bodies do not arise but rarely.

Concerning the chemical theory of action of asbestos, Gross et al. (1970) demonstrated recently that a pure synthetically produced chrysotile asbestos of small fibre size did not initiate significant asbestosis in the respiratory tissue of rats, whereas the pathogenic action of natural chrysotile asbestos was considerable. Yet, inasmuch as fibrous non-asbestiform substances do not duplicate the pathogenic effect of asbestosis, fibre size alone can hardly be responsible and some credence for

a chemical mode of activity of asbestos still remains. As it is evident from reports cited in this section, the relative significance of fibre size, chemical properties, and physical properties of the asbestos minerals in determining their pathological effect remains still obscure. But it has become obvious from the experimental investigations of the past 10 years that the asbestos minerals are definitely carcinogenic. This evidence certainly substantiated the findings of epidemiological investigations on this problem. It is likely that further experimental consideration of the problem of asbestos-related disease of the respiratory system will provide answers to the questions still remaining in the asbestosis problem.

OBJECTIVES OF THE PRESENT INVESTIGATION

At the time the author first began to design the protocols of the present investigation, other scientists had not as yet reported the successful induction of significant numbers of neoplastic lesions in the lungs of experimental animals exposed to asbestos. It was therefore proposed for the present investigation that one of the primary objectives would be to successfully induce such lung cancers by the exposure of rats to asbestos. Furthermore, a cytodynamic examination of alveolar tissue during the course of the development of neoplasia was included in the proposed series. It became apparent, however, from the work of Gross et al. (1967), successfully achieving induction of lung cancers by exposure to asbestos, that carcinogenesis in the lung required extensive periods of dust exposure. Yet, suitable inhalation exposure equipment was unavailable at the outset and was not ready for some time thereafter. As well, it was realized that a cytodynamic investigation of the long-term induction of cancers by asbestos would require most elaborate animal housing facilities - to preclude extraneous pulmonary disease - which also unfortunately were unavailable. Therefore it was decided to conduct short-term series to study in detail the early development of asbestosis in rats, including cytodynamic investigations.

Two primary objectives formed the basis of the design of the experimental series. First, it was proposed to examine the specific nature of asbestos in relation to the mineral's effects in pulmonary tissue, that result in the drastic pathology of asbestosis. Second, inasmuch as it is evident from most of the observations of previous workers, as reported, that cytodynamic activity of pulmonary tissue constituted the major contributory factor in the disease processes as asbestos dusts altered the clearance mechanisms of the lung, the responses of lung tissue to asbestos as also to other varieties of control dusts were determined. These two objectives were the first to be developed at the onset of this study. During the course of the experimental series, other objectives presented themselves and further means of examining the problem of asbestosis were pursued.

As the work progressed and more literature on the problem of asbestos-related disease became available, it became apparent that the investigation of the cytodynamic reactivity of the pulmonary tissue to asbestos (or to any other noxious substance) constituted a most facile and sound means of clarification of the etiology of asbestosis (and related pulmonary diseases). The work reported and discussed in the following sections support this premise.

METHODS AND MATERIALS

ANIMAL MATERIAL

Male albino rats of the Sprague-Dawley strain (Holtzman Company) served as the experimental animals. Although in other types of studies it may seem preferable to utilize younger adult rats to alleviate the possibility of extraneous disease conditions acquired with age, this was found to be impractical in the present series. Rats with less than 200 grams of body weight were unable to cope with the stress of intratracheal injection. This treatment frequently proved fatal to such smaller animals. In order to preclude mortalities resultant from that treatment, rats ranging from 200 to 300 grams were utilized. The animals usually weighed less than 200 grams when received, and were placed in holding cages until adapted to the new surrounding while their minimum weight requirement attained that most expedient for treatment. As a prophylactic measure to forestall chronic pulmonary diseases common to rats, the animals were administered aureomycin (Cyanamid Ltd.) in their drinking water. A regimen of three days aureomycin-water alternated with three days of ordinary tap water was maintained until three days before the commencement of the experimental

treatment when the antibiotic was permanently removed. The animals were fed pellets of Purina Fox Food. They were routinely weighed at least at the time of treatment and also sacrifice.

DUST MATERIALS

Asbestos Dusts

Natural Chrysotile Asbestos

At the onset of this investigation it became evident that much of the confusion in the literature concerning the specific biological effects of asbestos in pulmonary tissue had arisen because of the lack of properly standardized and researched asbestos dust samples. Inasmuch as the composition of the dust samples of asbestos employed by different workers varied considerably in such factors as fibre size, natural and artificial contaminants, as others, it is not at all surprising that uniformity was lacking between some reports. In order to clearly define the composition of the asbestos samples employed in the present study, pure asbestos fibre in native ore was obtained from

the Johns Manville Company. This asbestos material, unlike the commercially prepared samples many workers employed, was not contaminated by various extraneous substances that are commonly mixed with the fibres during commercial processing. To begin with, the pure asbestos fibres were removed from the native ore by forceps and cut into smaller lengths with scissors, attempting to reproduce the procedure of Badollet and Gantt (1965) designed to produce small fibres. However, that approach proved unsatisfactory as a general laboratory technique, and the procedure was inefficient in breaking down the long asbestos fibres into sufficiently small particles suitable for biological investigations. A further attempt to produce suitable fibres utilized a mineral grinding machine in the Department of Earth Sciences, University of Manitoba. Although this device readily ground hard rocks into powder, it was still unable to successfully yield sufficiently fine asbestos dust. Shortly thereafter it was learned (Timbrell et al., 1968) that uncontaminated asbestos samples of extremely fine fibre size, prepared by commercial grinding equipment specifically for biological use, were available to investigators upon request. Unlike in the commercial processes, only asbestos fibres were processed by the equipment, thereby omitting any of the usual extraneous contaminants. These dust samples were prepared by the Pneumoconiosis Research Unit of South Africa for the International Union Against Cancer. The properties of these

dusts (IUCC dust samples of asbestos) had been thoroughly analyzed and researched, and concise reports were available. Canadian chrysotile asbestos (IUCC asbestos dust sample Chrysotile B) was employed throughout the present investigation; it is heretofore identified as "natural chrysotile".

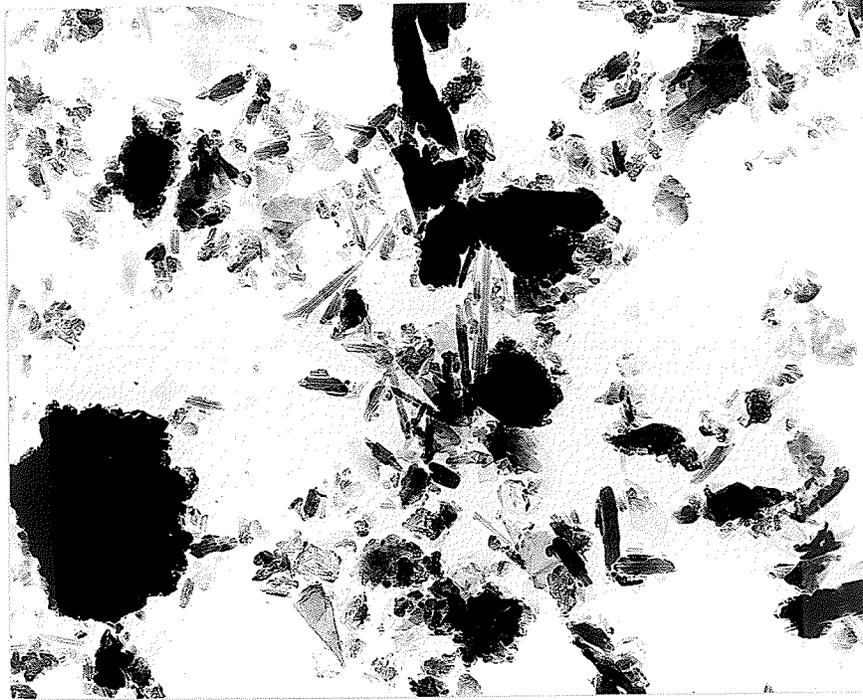
Synthetic Chrysotile Asbestos

Because the natural contaminants (native ore, trace elements) present within and between the fibres of asbestos may conceivably contribute to its total effect on biological matter, it was desirable to keep apart the specific effects of chrysotile from those produced by such contaminants that do not contribute to its crystalline structure. Because chrysotile can be fully synthesized from pure compounds, it was thus decided to produce the mineral from its basic components. The availability of synthetic chrysotile free from contaminating materials allows comparisons to be made between the biological effects in respiratory tissue of the pure synthetic mineral and those of natural chrysotile. For asbestos synthesis, the method of Bowen and Tuttle (1949) was successfully employed by Dr. A. C. Turnock, Department of Earth Sciences, University of Manitoba. This procedure required the placement of the following combination of substances into a

precious metal container: MgO as periclase in one micron particles, and SiO₂ in the form of amorphous precipitated gel. These substances were placed into the container in amounts proportionate to their occurrence as components of chrysotile. Water was subsequently added slightly in excess to allow for possible evaporation during the synthetic process. The container was welded shut and placed in a special apparatus which subjected the contents to controlled extreme hydrothermal conditions. The charge was first heated at 300°C for seven days and then at 400°C for an additional seven days, at pressures of 2,000 atmosphere (the pressure being applied through the malleable walls of the precious-metal container by a surrounding pressure film). At the completion of synthesis the container was opened and the contents removed. The synthetic product was then dried by heat. A fibrous structure could not be detected in that synthetic material even up to a magnification of 1,000 with the light microscope. It appeared initially, therefore, that chrysotile had not been successfully synthesized. However, examination of the synthetic product with the electron microscope revealed the presence of definite minute fibres resembling morphologically those of chrysotile (Figures 9, 14). A comparison of the X-ray diffraction pattern between this synthetic material and that of the chrysotile B IUCC asbestos dust sample verified that the two minerals were indeed of a very similar structure

FIGURE 9: Synthetic chrysotile asbestos. Note the numerous fibres interspersed among the amorphous material. Electron micrograph 20,520 x.

FIGURE 10: Improved synthetic chrysotile asbestos produced according to the method of Yang (1961). Note that the fibres are arranged in a definite bundle. Electron micrograph 15,960 x.



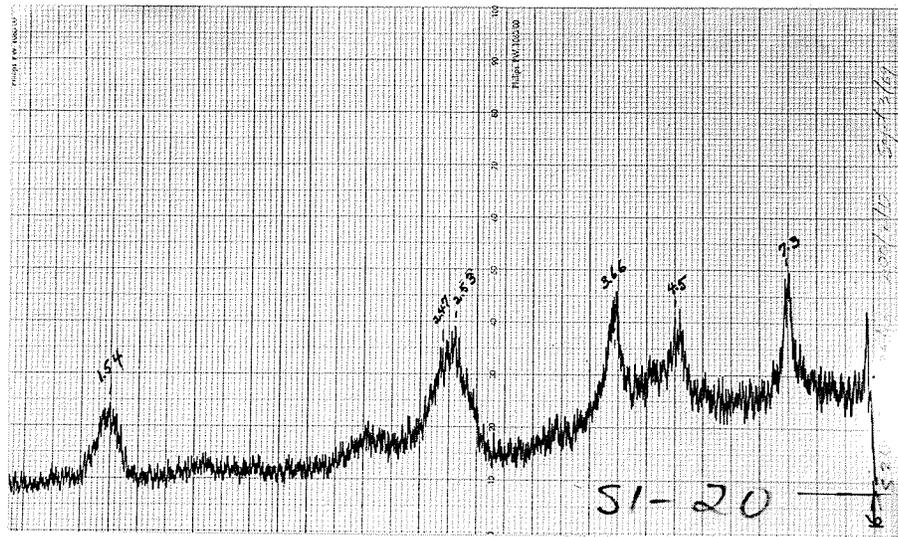
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FIGURE 11: X-ray diffraction pattern of synthetic chrysotile (S1-20).

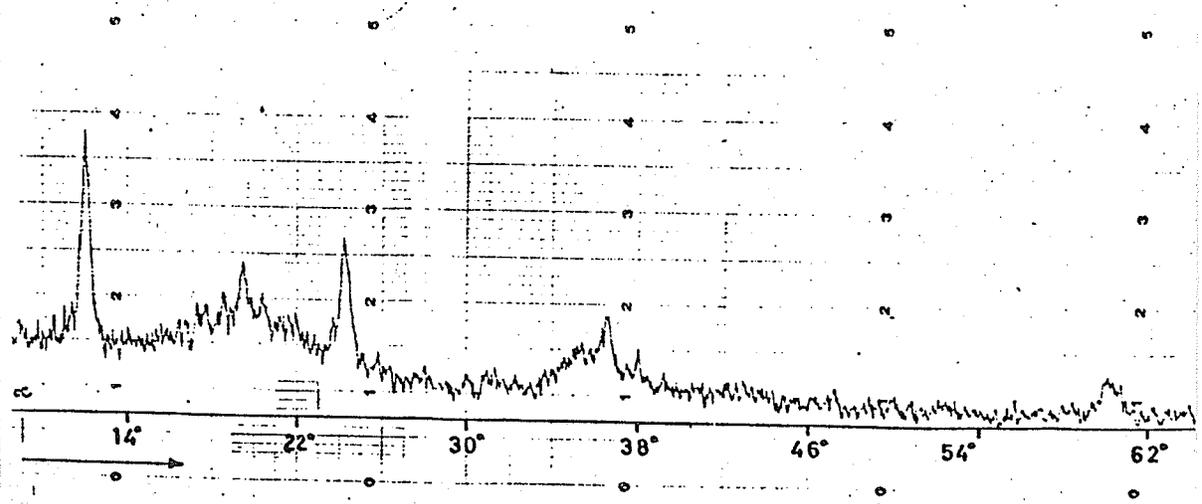
FIGURE 12: X-ray diffraction pattern of Chrysotile B (IUCC asbestos dust sample - Canadian Chrysotile). (From data sheets on IUCC dust samples, Pneumoconiosis Research Unit of South Africa.)



11

PNEUMOCONIOSIS RESEARCH UNIT, JOHANNESBURG

X-RAY DIFFRACTION PATTERN
OF U.I.C.C. STANDARD ASBESTOS SAMPLE.
5. CHRYSOTILE B



12

FIGURE 13: Tritium labelled natural chrysotile asbestos.

The chrysotile structure remained unaltered by the technical procedure of tritium labelling.

Electron micrograph 10,260 x.

FIGURE 14: Natural unlabelled chrysotile asbestos.

Compare with Figure 13.

Electron micrograph 10,260 x.



13



14

(Figures 11, 12). Yet, it is known that serpentine may appear in other forms as well; thus, despite a similarity of X-ray diffraction patterns, the possibility existed that much of the synthetic material was not chrysotile but that other forms of serpentine, having a very similar crystalline arrangement, might contribute to the nature of the synthetic product. It was then estimated from electron micrographs that chrysotile comprised about 40% of the synthetic mineral. Despite the low proportion of chrysotile, this preparation served expediently for comparisons between its effects in pulmonary tissue and those of natural chrysotile. For instance, the purely chemical effect of chrysotile, and to a lesser extent, also the significance of fibre size in pathogenicity, could be examined.

While improving the technical approach of chrysotile synthesis it became evident that the modification of the technique according to Yang (1961) yielded a much higher quantity of pure chrysotile fibre to such a degree that fully 90% of the synthetic product was unequivocally composed of chrysotile. Furthermore, utilizing that improved procedure it became possible to form true fibre bundles (Figure 10). Such bundles had been the most difficult to synthesize (Speil and Leinweber, 1969). Inasmuch as this improved chrysotile preparation was obtained only recently, it could not be utilized in the present series of experiments.

However, it should serve in future studies as an excellent means of investigating a variety of factors contributing to the pathogenic properties of chrysotile asbestos.

Tritium-Labelled Chrysotile Asbestos

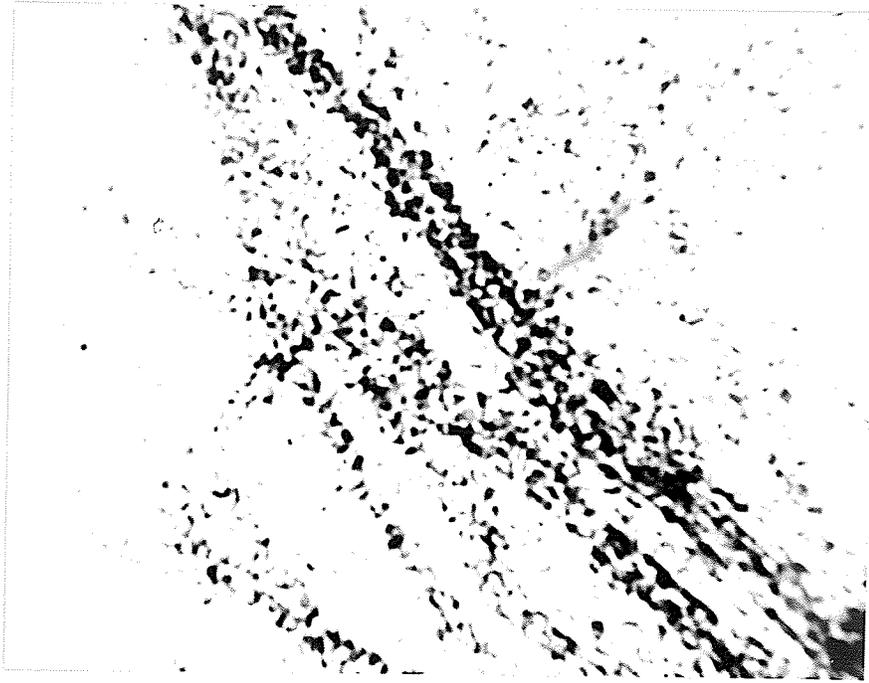
It is quite evident that the precise localization of asbestos fibres in routine histological sections of experimental animals would be a most expedient approach in elucidating the mechanisms implicated in asbestos-related disease. Moreover, the feasibility to measure the clearance of asbestos from the lungs of animals exposed to this dust could clarify the relative efficiency of the pulmonary clearance mechanism of asbestos in relation to various factors, such as quantity inhaled or fibre size. The previously available methods for the localization of asbestos fibres within pulmonary tissue and for the measurement of the pulmonary clearance of asbestos are severely limited. It became thus evident that a preparation of radioactively labelled chrysotile asbestos would be highly expedient for the present investigation. The method of neutron activation, later reported by Morgan and Holmes (1968), was suggested to the author, but it was not attempted in view of the non-specificity of the labelling achieved, the infeasibility of localizing asbestos fibres by

radioautography (because of the strong gamma-radiation emitted by the resultant radioisotopes), and also because of the possibility of extraneous biological effects resulting from such potent radiation. An entirely new approach was therefore developed by the author in collaboration with Dr. A. C. Turnock. Since tritium is an ideal label for both radioautography and also scintillation counting, it was attempted to incorporate that radioisotope into chrysotile. Inasmuch as water comprises a portion of the crystal structure of chrysotile, the compound being a hydrated magnesium silicate, tritiated water with a specific activity of 0.2 C/gm. (from the New England Nuclear Corporation) was substituted for the distilled water employed when producing the synthetic chrysotile material. The other steps in the procedure remained identical as previously detailed. On completion of the synthesis, the product was rinsed thoroughly with distilled water and heated at 105°C for 16 hours to remove any free traces of tritiated water that had not been incorporated into the crystalline structure. To test this resulting product for suitable labelling it was suspended in water, smeared on glass slides, heated over an open flame repeatedly, and coated with Nuclear Track Emulsion (Kodak NTB2) for radioautography. Similar samples of non-labelled synthetic chrysotile were simultaneously prepared for comparison. After one week of exposure conspicuous grain formation was evident in the emulsion overlying the labelled

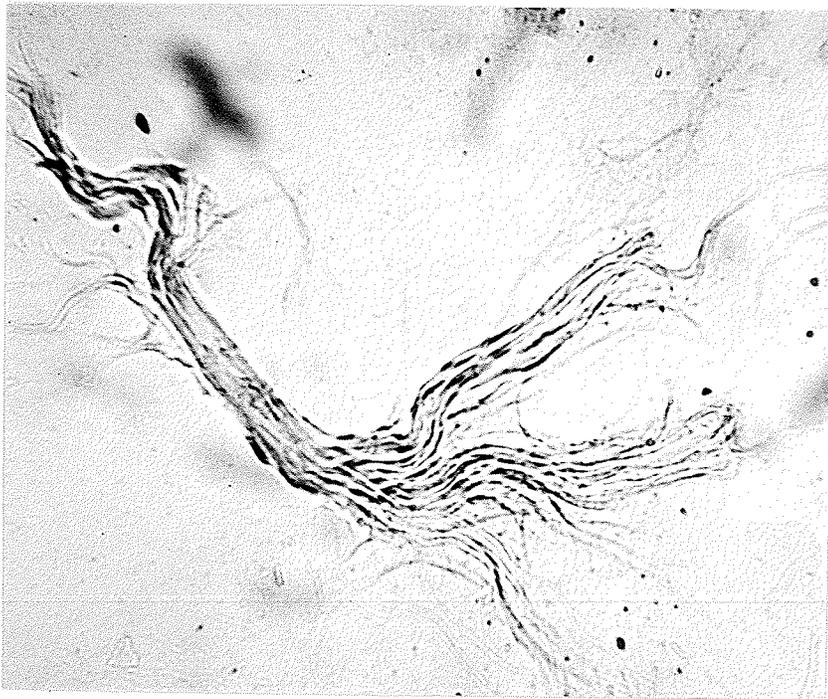
synthetic chrysotile. Conversely unlabelled synthetic chrysotile exhibited merely the usual background grain formation. The success of this endeavour with the synthetic compound suggested that labelling of natural chrysotile with tritium might be accomplished by similar means. Therefore, natural chrysotile asbestos in the presence of tritiated water was subjected to 305°C at 1,000 atmosphere of pressure for 28 days. Pilot experiments had indicated that at higher temperatures and pressures extensive dehydration of natural chrysotile occurred. An exchange of hydroxyls from the natural chrysotile with tritium-oxyls from tritiated water was then achieved. Electron microscopic examination of the chrysotile thus treated signified that destruction of the fibre pattern had not occurred (Figures 13, 14). As in the case of the synthetic labelled chrysotile, the suitability of the preparation for biological investigation was confirmed by radioautography. A photomicrograph of such a preparation as it appeared in the radioautography is shown in Figure 15. In contrast, the radioautographs of unlabelled natural chrysotile did not show significant grain formation in the Nuclear Track Emulsion (Figure 16). The techniques employed in producing these labelled asbestos dusts, as some of the biological observations made in pulmonary tissue after their administration, were reported in a publication now in press (Turnock et al., 1971).

FIGURE 15: Radioautograph of labelled natural chrysotile asbestos.
The delineation of the asbestos fibres and fibre bundles
is clearly evident by the grain formation in the radio-
autographic emulsion.
Radioautograph 1000 x.

FIGURE 16: Radioautograph of natural unlabelled chrysotile asbestos.
Merely the scanty background grain formation is evident.
Radioautograph 400 x.



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Control Dusts

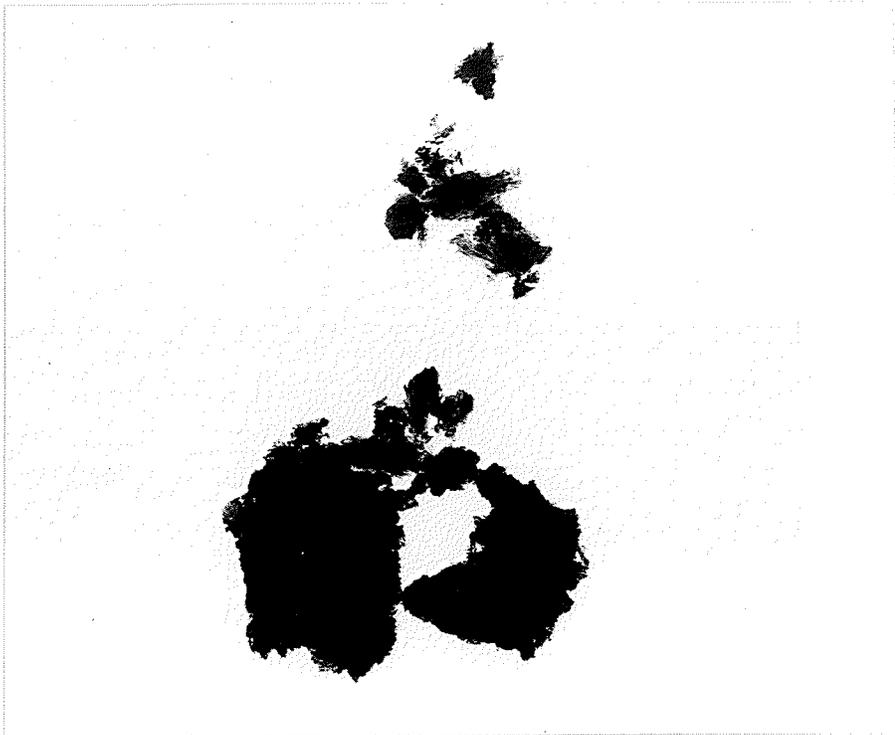
To serve as control materials in the asbestos dust experiments, two other types of dusts were employed. Comparisons were made between the effects produced by these inert dusts in pulmonary tissue, and the asbestos dusts. The first variety was a fine carbon dust utilized to ascertain the reactivity of pulmonary tissue to a non-fibrous inert particulate matter. Inasmuch as the conceivable significance of particle size in the development of pulmonary lesions was not realized at the outset of the experimental series, a characterization of the precise particle size distribution of the carbon dust was not performed. Electron microscopic examination of this dust indicated, however, that it was composed to a large proportion of ultramicroscopic particles (Figure 17) forming larger grains by cohesion. The second variety of inert dust was finely ground fibreglas (obtained from the Pneumoconiosis Research Unit of South Africa) with a particle size ranging from 0.5 - 1 micron (Figure 18).

FIGURE 17: Carbon dust. The carbonaceous mass in the centre of the electron micrograph is composed of numerous small ultramicroscopic particles.

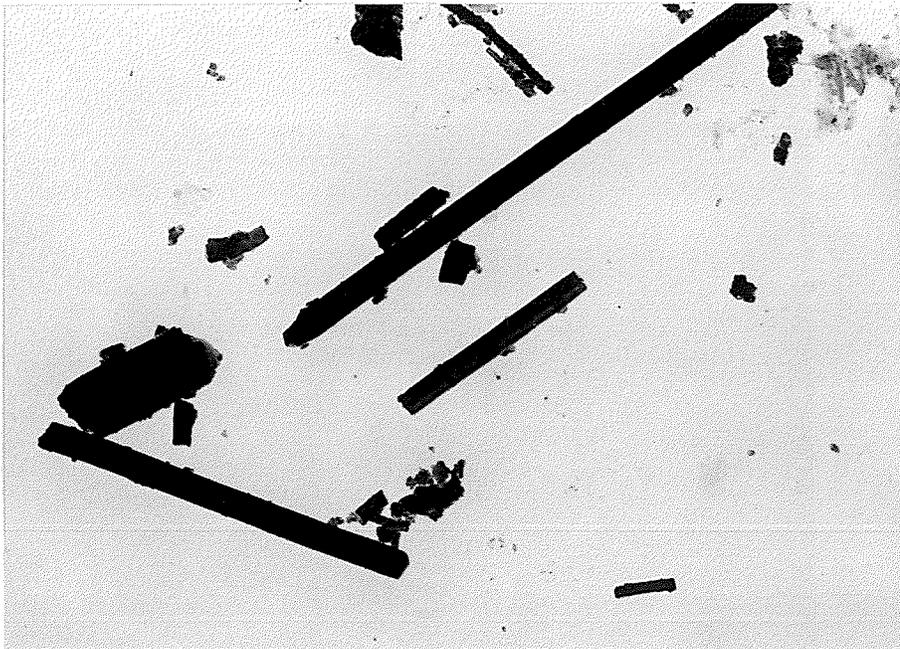
Electron micrograph 10,260 x.

FIGURE 18: Fibreglas dust. This material served as a control dust of a fibrous, non-asbestiform nature.

Electron micrograph 12,540 x.



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TREATMENT OF ANIMALS

General Considerations

It is possible to use various modes of treatment to induce experimentally asbestos-related pulmonary lesions in animals. The most commonly employed treatments are: intratracheal or intrathoracic injection of dust suspensions, and further, inhalation exposure to dust clouds. Each of these techniques of exposure has advantages and disadvantages. The best mode of treatment is, of course, that of inhalation exposure because extraneous effects inherent to the injection techniques are excluded; further, that a natural mode of exposure is comparable to human exposure results. Yet, elaborate equipment and long time spans of exposure are necessary before significant pathological changes in the tissues become evident. Conversely, injection techniques are readily performed and require only short periods for the development of severe pulmonary pathology. In the absence of elaborate inhalation exposure equipment and sufficient time for the consideration of the usual development of disease, the injection techniques offer more advantages than disadvantages. At the same time it is essential that the experimental series in which

injection techniques are employed are most carefully watched so that any effects resulting from injection treatment alone can be clearly separated from the specific effects of the administered dust materials.

Intratracheal Injection Technique

Two major factors were considered in the selection of a specific intratracheal injection technique for the present series of experiments. These were: (1) the efficiency of injection, and (2) the degree of trauma resulting from injection. A great deal of effort was expended to minimize the severity of the injection technique since the mere injection of fluid serving as the vehicle for the dust materials into the trachea constitutes alone a severe insult. Of the available methods that were considered, that of Gross (1958) appeared to be the most expedient and also the least traumatic to animals. A laryngoscopic speculum was constructed, based on a design developed by Gross. The modified technique of Gross was performed while the experimental animal was under deep ether anaesthesia. The speculum was placed into the animal's mouth, depressing the tongue. By raising the upper jaw to hook the canine teeth onto the upper aspect of the speculum, the animal could be suspended completely by the speculum

alone. A modified intubation tube was then inserted through the speculum into the pharynx. Subsequently, the tube was pushed sufficiently deep until it approached the tracheal opening. At this point of the procedure, the proper anaesthesia of the animal was of critical importance in determining the success or failure of the intratracheal injection of suspension. If anaesthesia was insufficient, the insertion of the tube into the trachea became extremely difficult because of the vehement gagging reflex and the tightening of the epiglottis associated with this reflex. Such a difficulty did not arise if the animal was properly anaesthetized and the tube was readily inserted into the trachea. It was initially feared that the insertion of the tube too deep into the trachea might result in some tracheal damage, as it was indeed the case in earlier experimental series. In the latter, a few animals died from a punctured trachea. The situation was rectified by practice, however. Once the intubation tube was properly inserted into the trachea, assurance of a successful inertion could be gained by a gentle rubbing of the tube against the anterior portions of the tracheal rings. The depth of insertion of the tube was determined by slight pressure of the finger against the neck of the animal by an assistant. Eventually a critical zone of safety was established. Superior to the level of that zone an injection was prone to failure, as much of the inoculant was coughed up by the animal. Inferior to that zone the

injection could bring about severe tracheal injury. Well over 100 rats were successfully injected in this fashion even before the experiments described in this thesis were begun. Each animal was closely observed after injection for signs of respiratory failure until it had recovered from anaesthesia. On occasion an animal might stop breathing as a result of the combination of injection and anaesthesia. In such cases, the animal was assisted by artificial resuscitation until it was breathing once more. The technique of intratracheal injection is graphically illustrated in Figure 19.

The dusts administered were sterilized by heat and suspended in sterile saline to forestall respiratory infections. The intubation tube was dipped in 70% ethanol and flamed in order to prevent the possibility of cross-infection. The laryngoscopic speculum was also treated in this fashion between injections. The apparatus employed in this procedure is illustrated in Figure 20.

The intratracheal injection technique proved to be highly efficient and minimally traumatic. Examination of the lungs from animals thus treated signified that only a small proportion of animals were improperly injected, as into the oesophagus. Post-mortem examination of those animals that succumbed during

(Pen and ink drawing from original by R. Price,
Medical Illustrator.)

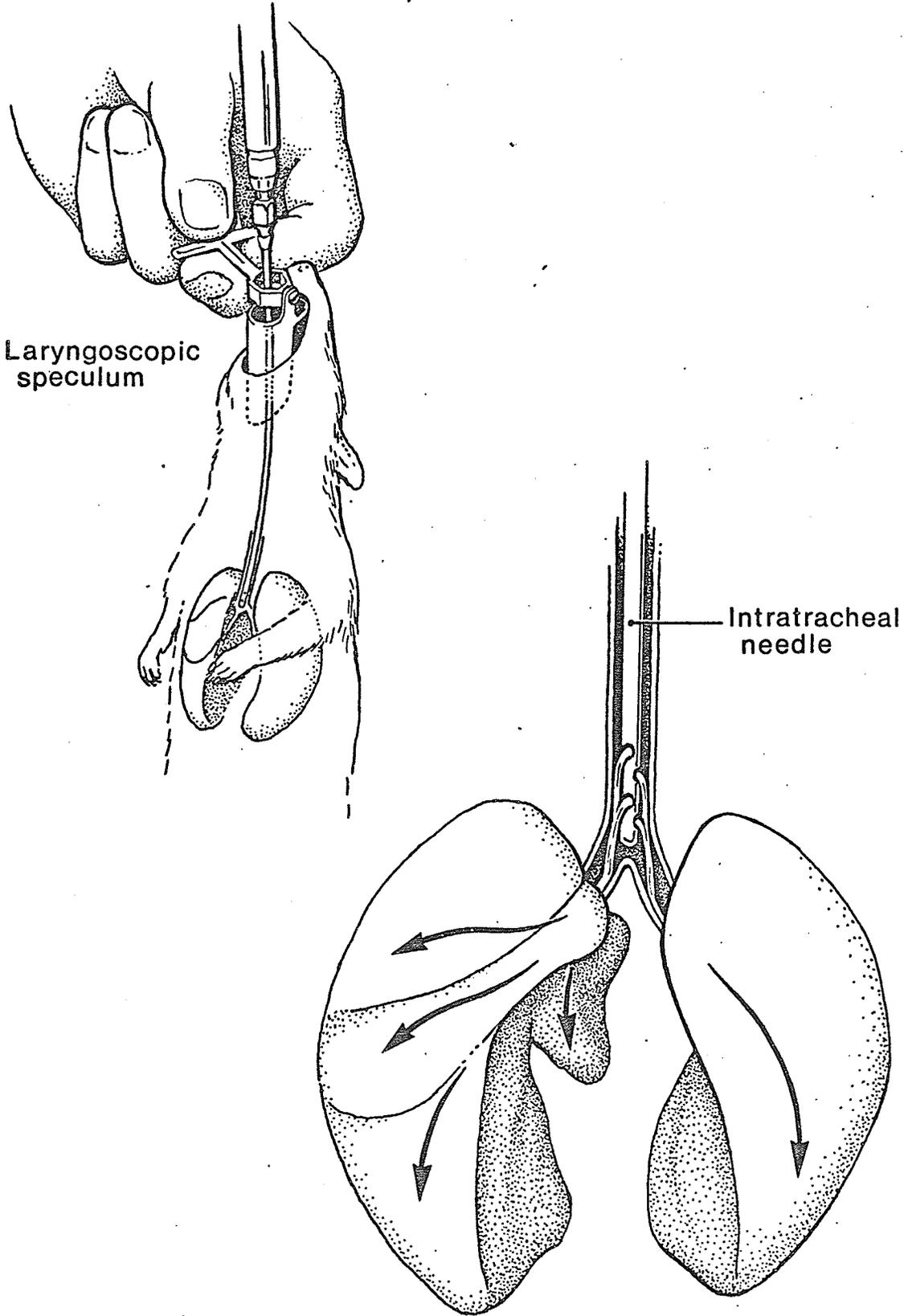


FIGURE 20: Apparatus for intratracheal injection.

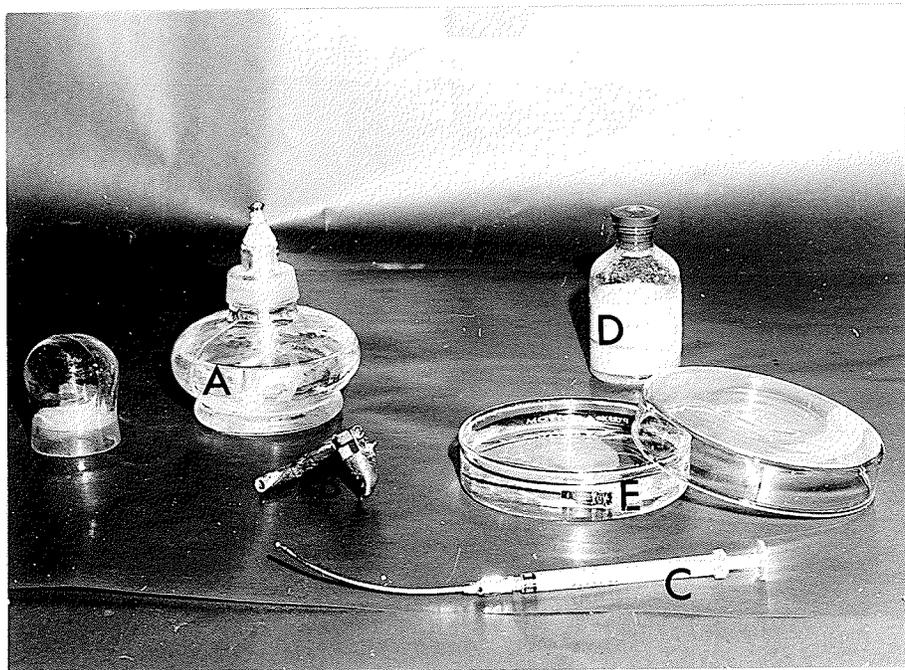
A - Alcohol Burner

B - Laryngoscopic Speculum

C - Syringe with Modified Intubation Tube

D - Sterile Dust Suspension in Saline

E - Petri Dish with 70% Ethanol for Flaming Needle and speculum.



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the procedure revealed that the great majority had already been afflicted with severe pulmonary disease by the time they were treated.

Intrapleural Injection Technique

This technique was utilized merely in a limited pilot study. The method of Smith et al. (1965) of directly injecting materials through the thoracic wall into the pleural cavity was initially considered, but then rejected because of the severe trauma produced. Also, the danger of lung damage was inherent to this method. The animals were therefore injected indirectly into the pleural cavity. A large bore needle was inserted through the raised skin of the abdomen into the abdominal cavity. The needle was then pushed through the diaphragm into the pleural cavity where the suspension was injected. Assurance of the proper insertion of the needle was gained by gently rubbing it against the chest wall. In the small series (eight animals) treated in this fashion, fatalities did not occur nor was there any grossly visible evidence of severe damage to either the lungs or the abdominal viscera.

Inhalation Exposure Technique

Certain difficulties are associated with any inhalation exposure technique. Inasmuch as commercially produced inhalation exposure chambers of suitable design are unavailable, it was necessary to design and have constructed a suitable inhalation exposure environmental chamber especially for the present series. Two factors in particular were considered in the design of that chamber: (1) the environmental comfort of the animals, and (2) the production of dust clouds of asbestos.

The Inhalation Exposure Environmental Chamber

The guiding principle in designing the chamber was a minimal disruption of the living conditions of the experimental animals apart from their exposure to asbestos dust clouds as the sole aberrant factor. Numerous types of exposure chambers described in the literature (Hinners et al., 1967; Stuart and Beasley, 1967; Hoffmann and Wynder, 1969) present severe spatial restrictions to animals; they thus facilitate only very short periods of exposure to irritants. For instance, Adamson et al. (1969) reported that the mere confinement of mice to a restricted area inherent in the design of one type of exposure chamber resulted in measurable

alterations from normal of the body weights of mice even in the absence of any irritating substance in the air. It was therefore decided to design the chamber in such a manner as to minimize any interference with environmental conditions that might measurably alter the various biological parameters.

The overall dimensions of the specially constructed chamber were four feet in length, by three feet in width, by two feet in height. The body of the chamber was constructed of plexiglas, supported by an angle iron frame whose legs were on wheels, thus facilitating easy movement. The chamber was divided into upper and lower levels continuous in terms of conditions such as temperature and relative humidity. The floor of each level consisted of four triangular plexiglas sheets joined in such a manner that the floors gently sloped towards the centre. A wire mesh rested on supports at each level and served as the floor for the movement and reposition of the animals. At the centre of each floor was an effluent duct; the upper leading into the lower; the lower duct leading into an outflow flexible rubber hose which terminated in a sewer (Figure 21). Air-tight sample ports were installed at each level, facilitating the periodic sampling of air by an electrostatic precipitator (Mine Safety Appliance Company) to ascertain the concentration of dust. Two circular doors at each level served for the deposition or

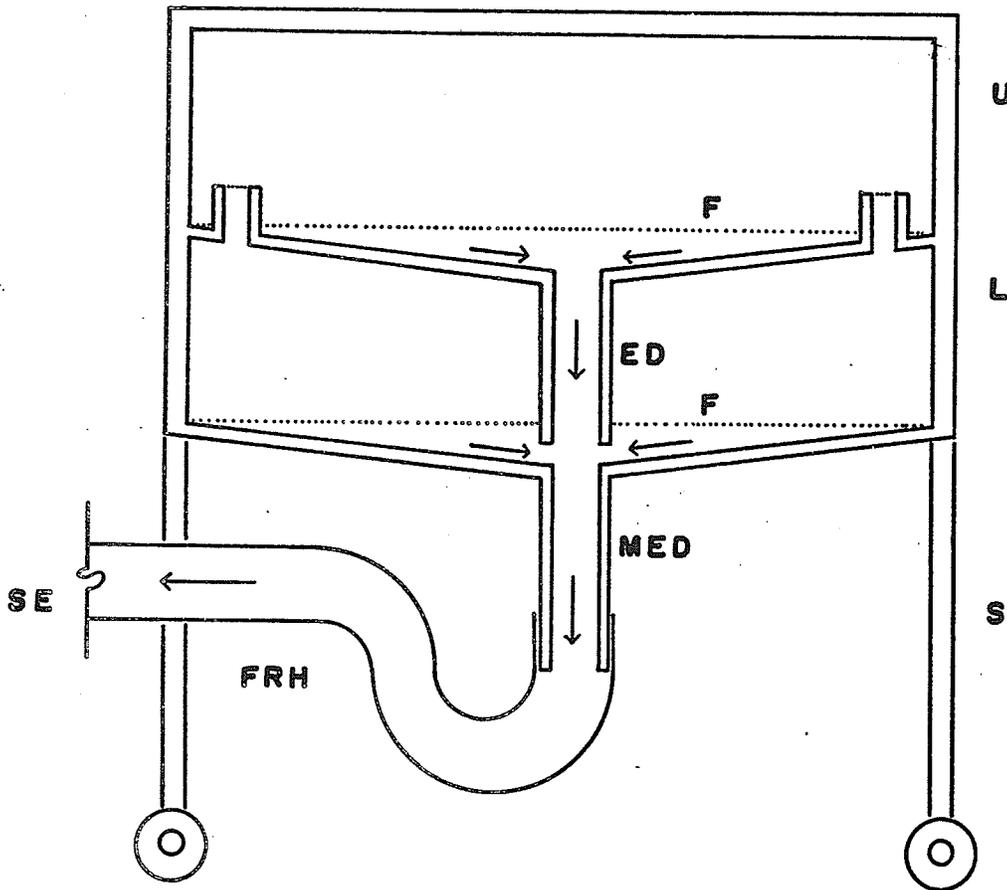


FIGURE 21: Schematic view of cross section of Inhalation Exposure Environmental Chamber.

U - Upper Level

L - Lower Level

F - Screen Floors

ED - Effluent Duct

MED - Major Effluent Duct

S - Supporting Angle Iron Frame

FRH - Flexible Rubber Hose to Sewer (SE)

removal of animals from the chamber. The animals were able to obtain drinking water ad libitum from glass dishes in each level. Food was provided in two receptacles on each level that could be filled through small doors in the walls of the chamber. The periodic cleaning of the chamber had originally been designed to be an automatic sprinkling device which would wash waste from the floors of each level through the effluent ducts into the sewer. It became apparent, however, that the water pressure in the general water lines of the entire building was insufficient to facilitate such cleaning. It was therefore necessary to wash the two floors twice daily manually with a water hose.

The environment of the chamber was controlled by three devices: (1) a humidistat (Honeywell) responding to a fall in humidity and thereby activating a humidification device, (2) a dehumidistat (Honeywell) responding to a rise in humidity and causing the evacuation of humid air from the chamber by a domestic vacuum cleaner (Viking), and finally, (3) a thermostat (White-Rogers) which, responding to fluctuations in temperature, completed the electrical circuitry to a domestic hair drier heating coil (Shick). Inasmuch as the fan of the hair drier continually blew fresh air into the chamber, the action of the thermostat ensured the supply of warm air on demand (Figure 22). By this means the air temperature was maintained at $75^{\circ} \pm 10^{\circ}$ F. The

FIGURE 22: Schema of Temperature Control.

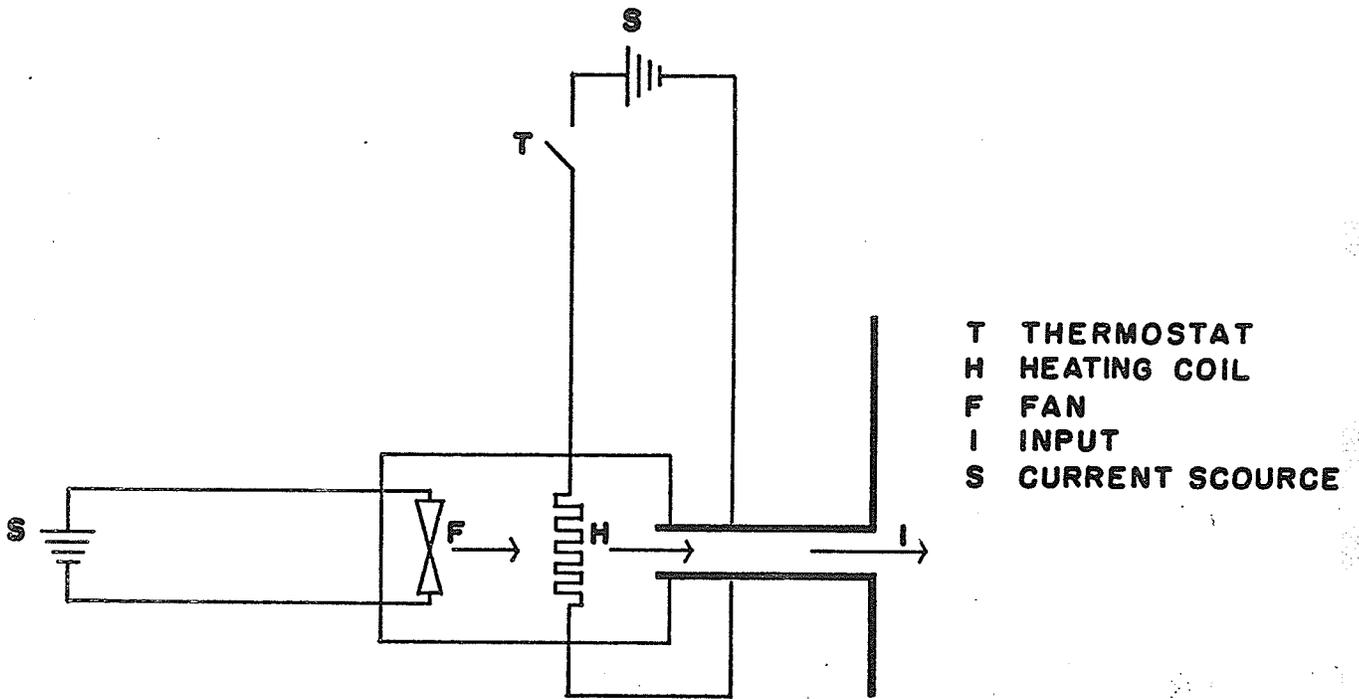
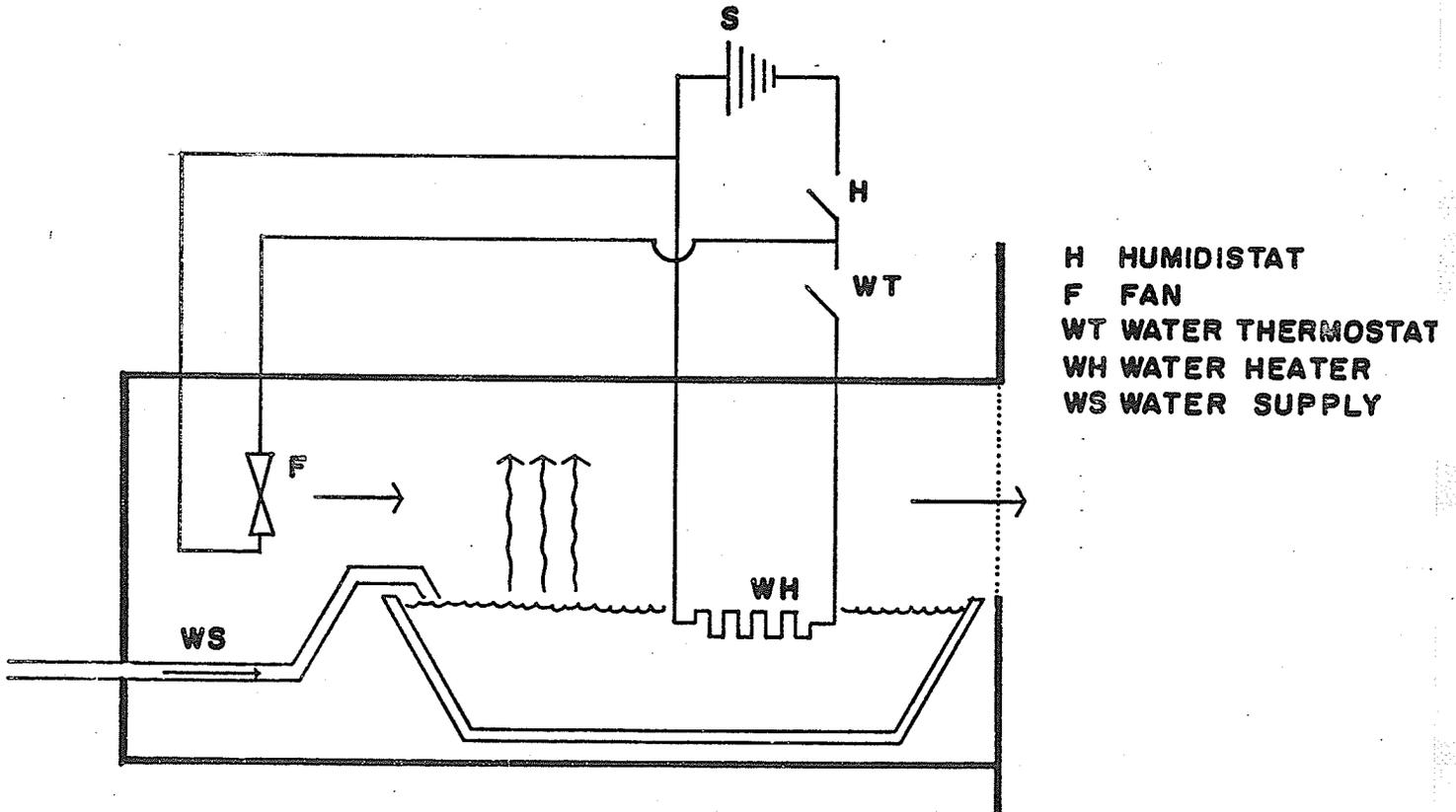


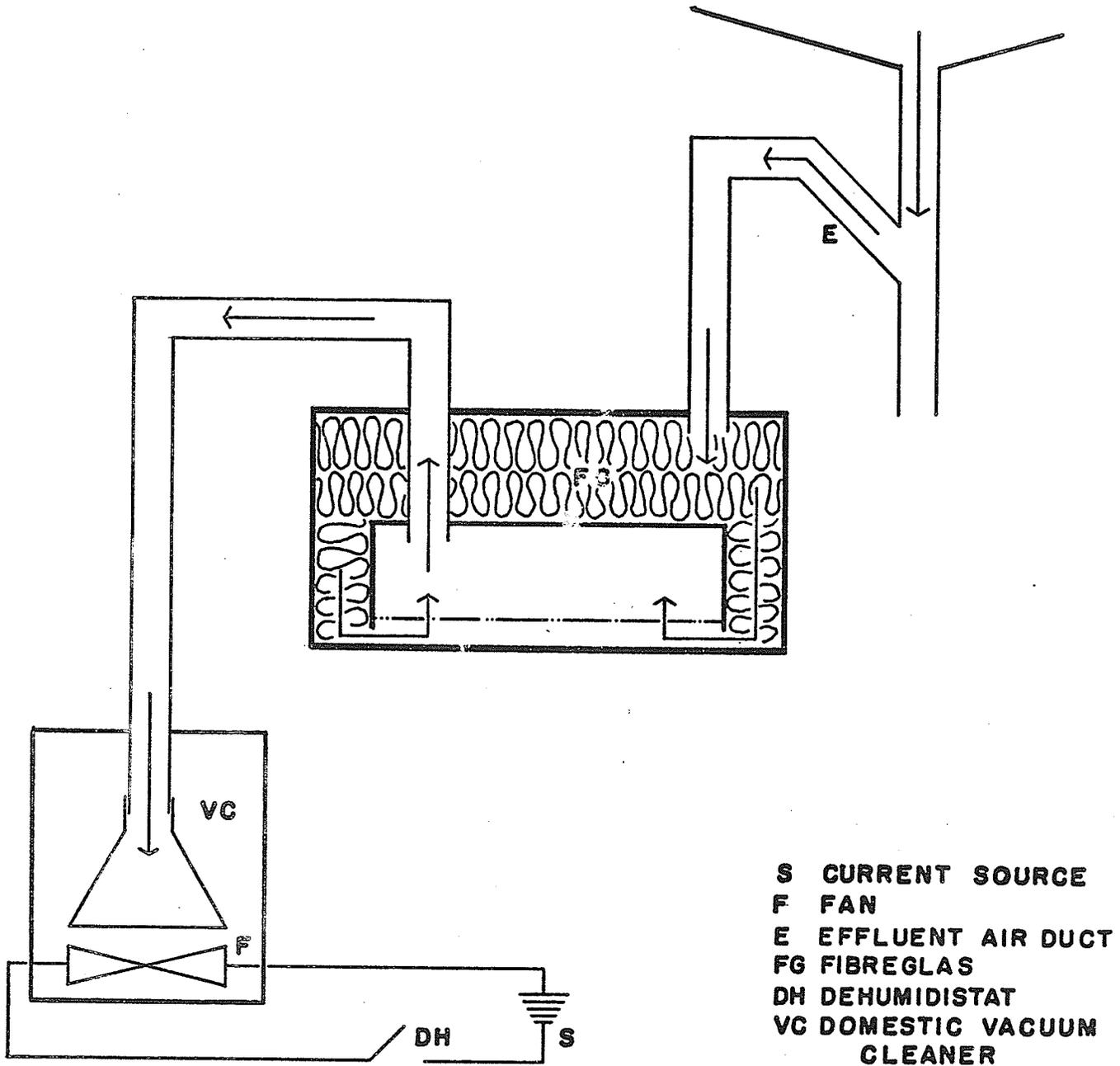
FIGURE 23: Schema of Humidification Apparatus.



humidification device was the conventional domestic furnace humidifier which had been suitably modified. This device maintained automatically the level of water in a dish. Within the dish was placed a heating coil operated by a water thermostat (Honeywell). In response to a fall in humidity below 40%, the humidistat completed the circuitry of both an air fan and the water thermostat. The water was then heated to 100° F by the heating coil and the humidified air above the dish was blown into the chamber by the air fan. The schematic operation of this device is shown in Figure 23. The prevention of the backflow of air at the input of these devices was achieved by rubber valves which closed the input on the attainment of a positive pressure within the chamber.

Dehumidification was achieved through the filtering system of the chamber. When the humidity rose to more than 50%, the dehumidistat effected the operation of the domestic vacuum cleaner. The filtering system is schematically represented in Figure 24. It was composed of three stages. First, the dust-rich air was drawn through a filter box containing oiled fibreglas which removed the bulk of dust from the air. Second, the vacuum cleaner filtered any remaining dust particles from the air. Finally, an electronic air cleaner (Honeywell) operated in the general environment of the animal room. That cleaner, an electrostatic

FIGURE 24: Schema of Filtering System and Dehumidification.

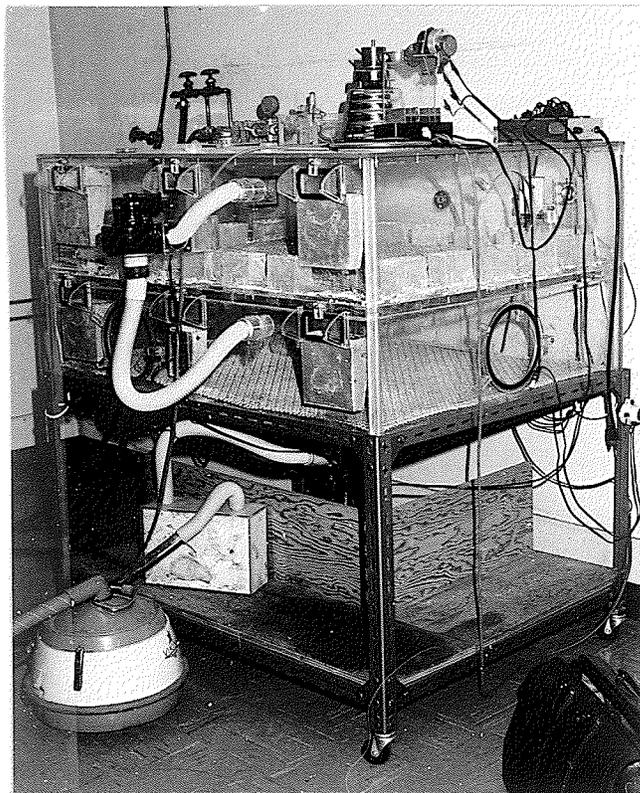


precipitator, removed 99% of the air-borne particles in the ambient air with three complete turnovers of air per hour. Through the use of these devices the environment of the chamber was maintained at $75^{\circ} \pm 10^{\circ}$ F, and $40 \pm 10\%$ relative humidity. Forty rats could be maintained on each level simultaneously and were able to move about quite freely. In fact, the total surface area available to each animal was greater than in conventional caging facilities. Moreover, the animals had free run within the large chamber, practically impossible in the usual housing facilities. The inhalation exposure environmental chamber is presented in Figure 25.

The Production of Dust Clouds

Perhaps the greatest difficulty of inhalation exposure experiments was the production of adequate dust clouds. For example, Vorwald et al. (1951) reported on that problem in the course of their investigations. These authors utilized a paddle-wheel device set above animal cages, this device beating dust contained in large pans. This procedure required large quantities of dust and also extensive areas in which to keep the animals. The device developed by Wagner and Skidmore (1965) operated on the principle of dust agitation by air but severely limited the range of dust concentrations possible. Holt et al. (1965) solved

FIGURE 25: Inhalation Exposure Environmental Chamber.



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the problem of dust generation partially through the use of a hammer-mill device which carried out the double function of dust dispersal and the production of fine asbestos dust particles; yet, that device by its nature contributed significant contaminants to the dust clouds (Gross et al., 1967). Two factors underlie the problem of the production of asbestos dust clouds: (1) the milling of asbestos to attain suitable ranges of particle size, and (2) their dispersal. Inasmuch as asbestos is extremely difficult to grind to small particle sizes, most earlier investigations were limited to the utilization of commercial grades that, as mentioned previously, contained many contaminants. The availability of the IUCC standard samples of asbestos alleviated this difficulty and confined the problem of dust production to that of dispersal only. Nevertheless commercially available dispersal devices could not be used for asbestos because of the hygroscopic and fibrous nature of this mineral. It was hoped that the Wright's Dust Machine (Wright, 1950) (Rank Bros. Ltd.) would be suitable, yet after much effort and modification of that device, it became evident that it would not efficiently disperse even the very finely ground IUCC samples without continuous attention to the device. Lately, Timbrell et al. (1968) reported on a design of a dust generating device specifically for the dispersal of the IUCC samples of asbestos. This design was employed in the present investigation to construct

a suitable dust generator. The generator consisted basically of a domestic blender (Osterizer) in which the glassware was replaced by a brass housing. This housing fitted the glassware receptacle by means of a screw mount. The blade of the blender also was replaced. A blade (after Timbrell et al.) specially constructed of mild steel replaced the original. Entering the side of the housing at a 60 degree angle was an input tube, whereas the output tube left the housing directly from its top. On the side of the housing opposite the input tube was an opening receptacle for a modified plastic syringe. The syringe, containing asbestos dust, fitted tightly into the receptacle and in turn accepted a teflon plunger driven by an electric motor. The driven plunger continually pushed forth small quantities of asbestos from the syringe into the brass housing. The asbestos was then agitated by the action of the blade and subsequently carried by air via the output airstream into both levels of the chamber. To facilitate this, the output airstream was divided by a "Y" tube into two portions, one leading to the upper and the other to the lower level of the chamber. The input airstream, derived from an air compressor, was filtered by an air filter and dried partially by calcium chloride contained within the air filter. The pressure and rate of flow of the air were regulated respectively by a pressure gauge and a flowmeter. The speed of revolution of the blade motor was controlled by a motor speed control (J & J

Electronics) which provided constant revolutions per minute despite the variation of torque presented to the motor by the force of the asbestos against the blade. Inasmuch as the airstream could be regulated and the quantity of asbestos presented to the blade could be varied by a gear arrangement on the driven plunger, it was possible to vary the quantity of asbestos within the air entering the chamber. During the present series the maximum dust concentration (5 mgm./L.) that could be achieved with this device was employed. The dust generating device and its operation are illustrated in Figures 26 and 27.

PREPARATION OF TISSUES

Although the method of fixation by perfusion (Forsmann, 1967) had been used in earlier series, it was found to be too complex and problematic for the fixation of tissues from the lungs of at least four animals within a short period of time. Consequently fixation was achieved by injecting the fixative intratracheally into rats that had been freshly killed by chloroform. First, a large incision was made through the abdominal wall of the rat. The portal vein and abdominal aorta were sectioned in order to allow exsanguination. The trachea was

FIGURE 26: Dust Generator.

FIGURE 27: Schema of Operation of Dust Generator.

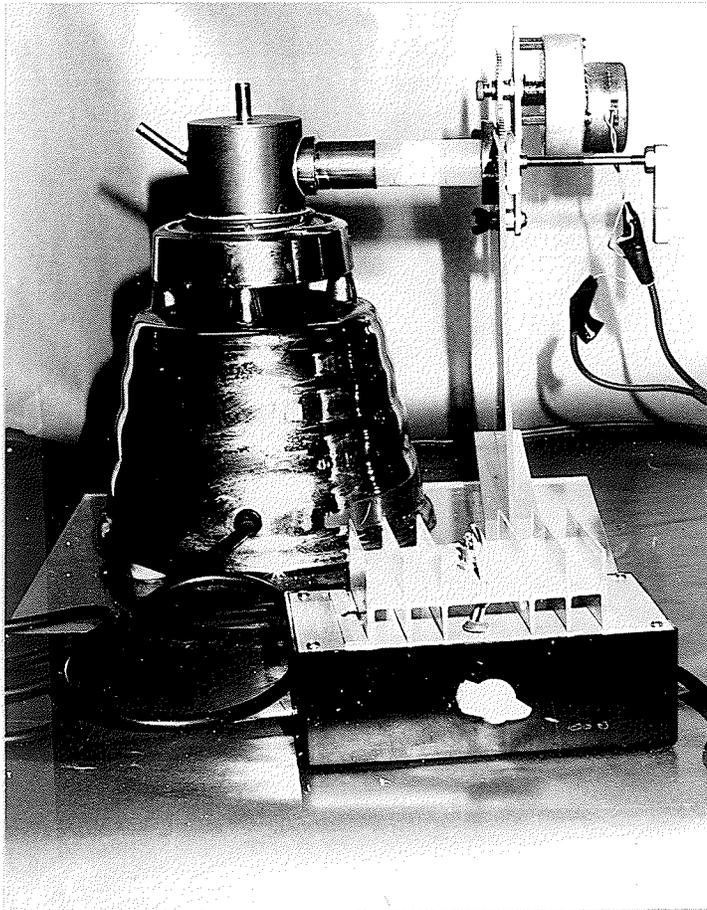
IP - Input Airstream

OP - Output Airstream

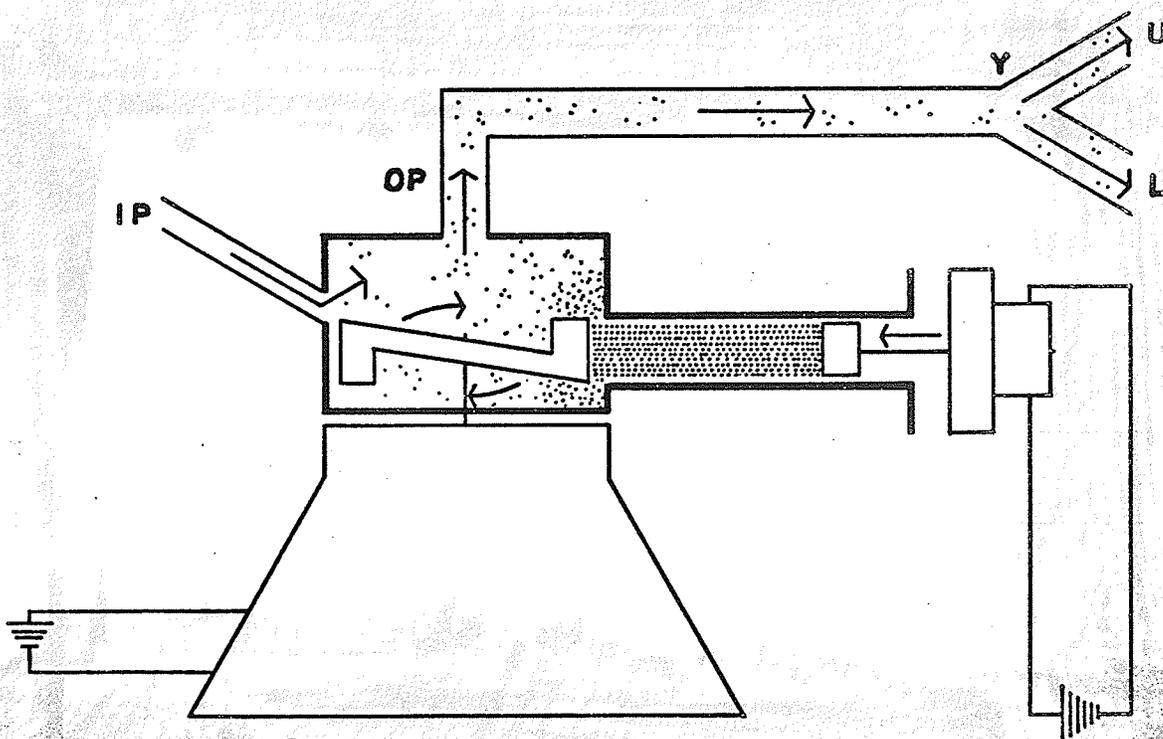
Y - "Y" Tube

U - Plastic Tube to Upper Levels

L - Plastic Tube to Lower Levels



(26)



(27)

exposed by an incision through the skin of the neck and the cutting of the strap muscles. The trachea was clamped superiorly with a hemostat. Below this clamp a needle was inserted into the lumen of the trachea. Ten ml. of 2.5% glutaraldehyde in phosphate buffer was injected into the lungs via the trachea. On completion of the injection, the hemostat was removed from its original site and placed below the site of injection to prevent the escape of fixative from the lungs. Over-inflation of the lungs during the course of injection was prevented by the visualization of their expansion through the previously cut diaphragm. Once the experimental animals were treated in this fashion, their lungs along with the heart, trachea and thymus were removed as one mass and placed in jars containing similar fixative. The hemostats on the tracheae were removed after 10 minutes by which time the fixation of the lung tissue prevented the expulsion of injected fixative.

Glutaraldehyde was utilized rather than conventional fixatives because this agent provides not only excellent fixation (Chambers et al., 1968) of respiratory tissue, but also allows the tissue to be used for both light and electron microscopic examination. The hardening of tissues by glutaraldehyde is minimal and therefore storage for extended periods is possible. Furthermore, glutaraldehyde does not cause an appreciable shrinkage of tissues as do some other routine fixatives.

For light microscopy, blocks of lung tissue were sectioned through the inferior lobe of the right lung. In addition, cross sections of trachea in certain series were also prepared. The blocks were dehydrated and embedded in paraffin. Two sections, five micra in thickness, at least 10 micra apart, were stained routinely with hematoxylin and eosin. They were utilized for compilation of the cytodynamical data.

For electron microscopy, small blocks were cut from lung tissue. They were rinsed in phosphate buffer, post-fixed in osmium tetroxide, dehydrated and embedded in either epon or methacrylate. Thick one-half micron sections were cut for light microscopy and stained with toluidine blue. Thin sections prepared for electron microscope examination were stained with uranyl acetate and lead citrate.

RADIOAUTOGRAPHICAL PREPARATIONS

Radioautography was employed both for the purpose of localizing those cells that had incorporated tritiated thymidine while in DNA synthesis, and also to localize labelled asbestos fibres within tissue and cells. Moreover, the same radioautographic technique was also used to verify the labelling of

asbestos with tritium during the preparation of the labelled asbestos dusts. Slides of either tissue sections or dust preparations were covered by dipping with Nuclear Track Emulsion (Kodak NTB2). The exposure times varied from as brief as three days to as long as eight weeks, depending on the material used. After exposure the emulsions were developed in D-19 and fixed with hypo. The preparations were then thoroughly washed, and stained with hematoxylin and eosin (paraffin sections) or else toluidine blue (epon, methacrylate sections). The dust preparations remained unstained.

TECHNIQUES OF CYTODYNAMIC MEASUREMENT

Colchicine Technique

The experimental animals were administered 0.10 mgm./100 grams body weight of a 1 mg./cc. solution of colchicine (Inland Alkaloid) six hours before sacrifice. The dosage and time of colchicine actions were the same as suggested by Bertalanffy (1966). Animals were routinely injected at 10 a.m. and sacrificed at 4 p.m. of the same day. Although diurnal variations have been

observed in cell populations of other sites, such variations have never been noted in the cell populations of either the epithelium of the airways or the alveolar lung tissue (Bertalanffy, 1964). In view of this fact and the desire to obtain samples from a range of periods after treatment, the standard six-hour period was utilized, and 24 hour estimates of the cytodynamics of the cellular population were not performed.

Although Bertalanffy (1964) was able to present data on the rate of cell division of the individual cellular populations of alveolar tissue by estimating the frequency of cell types in alveolar tissue and by differential counts, this was a most difficult endeavour not practically feasible in the present investigation. The reason was the disruption of the normal populations of cells in alveolar tissue by the treatment and the concurrent increase of abnormal morphological forms that could not be readily categorized. Therefore, differential counts or estimations of cell frequencies could not be accomplished (Figure 28). In view of these difficulties the mitotic counts of alveolar tissue included all the cellular populations within one cumulative estimate. These estimates included only the cellular constituents of the alveolar tissue, excluding polymorphonuclear elements and the cells of the connective tissue of bronchioles and blood vessels. Random counts of 2,000 cells from two lung sections were carried out. Identical counts were made of the epithelium of certain series. Metaphases,

FIGURE 28: Epon section $\frac{1}{2}$ micron thick of alveolar tissue.
Photomicrograph 400 x.

Note: Colchicine metaphase at M.

Cell with granules at G.

Distinguishing these two cells would be difficult in ordinary paraffin 5 micra sections. Also note vacuolated alveolar cells at V. Even with the improvement in resolution provided by this section, endothelial cells and pulmonary surface epithelial cells cannot be distinguished.



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that were also included in the total number of cells scored, were separately tallied. Although it was possible to recognize certain experimental groups morphologically by sections, the specific times of treatment could not thus be ascertained. Therefore, in order to preclude the personal prejudice of the observer, slides of the tissue sections of specific animals from specific groups were covered by numbers on a label hiding the identity of the section while the counts were performed. When the latter was completed for all of the sections, the assignment of the values thus obtained to specific groups was carried out.

Tritiated Thymidine Technique

The thymidine technique of measuring the cytodynamic activity of cellular populations was employed in two series of experiments. Firstly, groups of animals were injected with a pulse label of tritiated thymidine (New England Nuclear) of 1 $\mu\text{C.}$ /gram of body weight. These animals were killed one hour later. Secondly, groups of animals were administered the same dosage of tritiated thymidine but were not sacrificed until one or two weeks after the injection. To ascertain the labelling index of the epithelium of alveolar tissue, 1,000 cells were counted from two sections for each animal. Labelled cells were separately

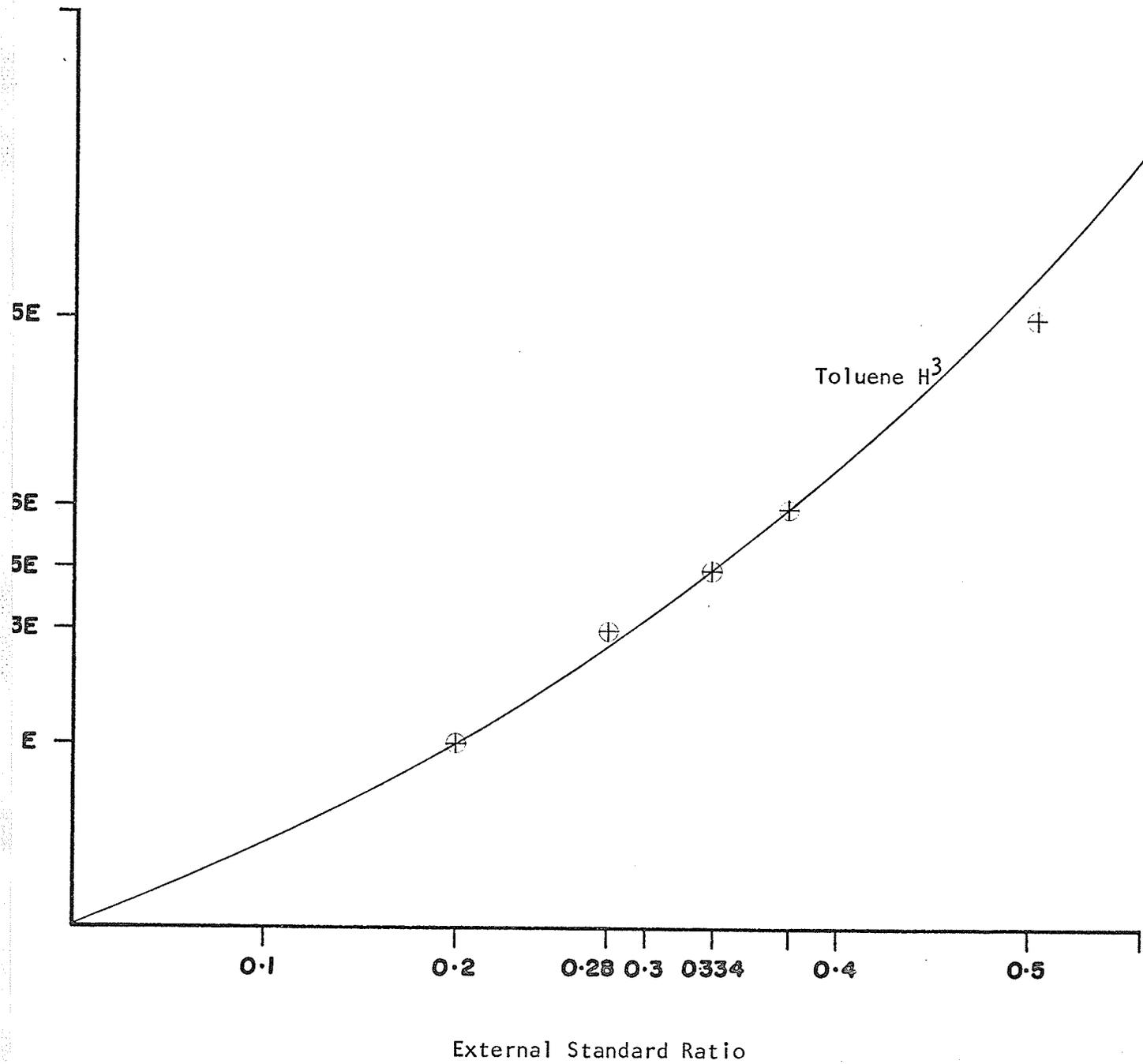
tallied. In the case of the pleural mesothelium, all the cells on two sections were scored. The first experimental series provided information on the number of cells that incorporated tritiated thymidine during a pulse label of one hour. The second type of experiment, the degree of clearance of label from the cellular populations between the times of injection and sacrifice of the animals, was estimated.

SCINTILLATION COUNTING TECHNIQUE

Notwithstanding the great advantages offered by the radioactively labelled asbestos samples for the measurement of the clearance capabilities of the respiratory tissue in response to the administration of these materials, technical difficulties were encountered in achieving valid scintillation counts of the radioactive emissions from these labelled substances within lung tissue. Firstly, because of the limited quantity of labelled asbestos dusts available, the same experimental series served both for radioautographical analysis and for scintillation counting. This necessitated the fixation of the lung tissues for histology, a most undesirable condition for scintillation counting. The tissue remaining after suitable tissue samples were removed for

radioautography was stored in the fixative for an extended period of time until the tissue sections were available. This prevented the loss of specific tissues for radioautographical study because of technical difficulties. When all the sections for radioautography had been found suitable, the scintillation counting of the asbestos within the remaining tissue specimens was undertaken. The tissue was thoroughly rinsed in phosphate buffer to remove the glutaraldehyde, and subsequently placed in 100% ethanol and allowed to air-dry. The tissue was then further dried thoroughly in an oven. This dried tissue proved unsuitable, however, for the conventional techniques of scintillation counting of dry tissue. Numerous difficulties were encountered and were ascribed to the residue of glutaraldehyde within the tissue and also to the difficulty of properly digesting such fixed tissue. The technical approach was then altered by first neutralizing the effect of the glutaraldehyde and then attempting the tissue digestion. Hydrogen peroxide (10 ul./3 mgm. tissue) was added to small quantities of tissue in scintillation vials to bring about the oxidation of the glutaraldehyde to glutaric acid. It was hoped that this treatment would produce a breaking of bonds between the glutaraldehyde and the protein of the tissue. The vials were stoppered well and placed in an incubation bath at 50° C to facilitate that reaction. Inasmuch as peroxide markedly influences the efficiency of scintillation counting, the liquid

contents of the vial were allowed to evaporate after a half-hour of incubation. To the dry contents of the vial 20 ul. of water and 300 ul. of NCS (Nuclear Chicago Solubilizer) was added. The water enhanced the digestive action of NCS. The vial was stoppered again and placed in an agitating water bath at 50° C to assist digestion. Digestion of the tissue protein was completed within an hour. The contents were allowed to cool and 20 ul. of 6N glacial acetic acid was added to neutralize the alkalinity of the suspension brought about by the NCS (quaternary ammonium hydroxide base). Finally 10 ml. of scintillation cocktail (6 gm. PPO/litre toluene) was added and the activity in the vials was counted in the Philips Liquid Scintillation Spectrophotometer (courtesy of the Department of Pharmacology, University of Manitoba). Each vial was subjected to an External Standard, so that the efficiency in counting could be determined, and any necessary corrections for decreased counting efficiency to be made. Preliminary experiments to determine that efficiency were conducted on the basis of External Standard Ratio, using saturated picric acid as a colour quencher and tritiated toluene as the isotope. The efficiency and External Standard Ratio were employed to construct the curve in Figure 29. Since all of the samples were counted with very nearly the same efficiency, correction for quenching was not necessary.

FIGURE 29: Toluene H³ Colour Quench Curve.

STATISTICAL METHODS

The statistical analysis of the data was carried out using the facilities of the Computer Department for Health Sciences of the University of Manitoba. The IBM 360/65 computer was employed throughout, either directly or by means of the teletype facilities available. The following programmes were utilized:

- Factorial Analysis of Variance - ST 43, 44 (Teletype statistics package), or
STATS 12 (directly on IBM 360/65, from Computer Library);
- Duncan's Multiple Range Test - ST 45 (Teletype statistics package);
- Covariance Analysis - STATS 15 (on IBM 360/65, from Computer Library).

The 0.05% level of significance was used throughout to determine statistical differences within the data.

RESULTS

EXPERIMENT 1

The purpose of this experiment was to test the specific effects of chrysotile asbestos fibres (both natural and synthetic) in rat lung tissue. These effects were investigated both morphologically and also in terms of the cytodynamic activity of the cellular populations of the alveolar tissue. Groups of rats were intratracheally injected with 1 cc. of a sterile saline suspension of 3.5 mgm. of one or the other type of asbestos. The control animals of this experimental series were injected in an identical fashion with 1 cc. of sterile saline suspension containing 3.5 mgm. of carbon or fibreglas, or else with 1 cc. of the saline alone. The colchicine technique was employed in all the different groups to accumulate data on the mitotic activity of the cellular populations of alveolar tissue. Groups of rats, each composed of four animals from a specific treatment category, were sacrificed at one, three, five, seven, 14, 21, 28 and 56 days after the administration of the particular type of material.

Observations of the Living Animals

The treated animals were examined daily for any visible effects of the insult upon them during the entire course of the experiment. Whereas those animals that received natural chrysotile were markedly affected, the animals treated with the other materials did not appear physically affected. In contrast, the animals treated with natural chrysotile were markedly emaciated. The latter animals experienced much respiratory difficulty for nearly a week after having been administered the material. Also, the highest incidence of mortality occurred in that group. Although the losses were compensated by additional animals, the fatalities were sufficiently high so that some groups remained, in the end, incomplete. The rats that survived the treatment with natural chrysotile did gradually recover.

Histological Observations

Saline Treated Series

Definite inflammatory reactions involving especially the

epithelium of the airways were initially observed in the lung tissue of saline treated rats. But by two weeks after the treatment there was little evidence of significant pathological alterations of the lung tissue. It was noted, however, in later groups (28 and 56 days) that lung disease of a chronic form was common. However, that finding was unremarkable inasmuch as such disease conditions are also frequent even in normal, untreated animals.

Carbon Treated Series

At early stages after the treatment of the animals, numerous carbonaceous particles were observed both free in the alveolar spaces and also within the alveolar walls and alveolar macrophages. Some larger particles were occasionally lodged within the alveolar tissue and surrounded by inflammatory cells (neutrophils, macrophages). As early as one week after treatment very few particles remained in the alveolar tissue, apart from the occasional large particle previously mentioned. Even intensive search revealed merely a few carbon-laden macrophages. At later stages of the experiment, the only evidence of treatment was the lodgement of a limited number of large carbon particles within the alveolar tissue. Although regularly observed in the

lungs of carbon treated animals, the larger particles appeared to involve the alveolar tissue only to a limited extent. An example of such an entrapped particle of carbon within rat alveolar tissue is seen in Figure 30.

Fibreglas Treated Series

Initially it was feared that the intratracheal injection of fibreglas had failed to be successful in the animals thus treated. Yet this seemed peculiar as it would have been the only group administered unsuccessfully because few failures were generally experienced with the injection technique. But then it became apparent that conventional light microscopy was grossly unsuitable for the visualization of fibreglas in lung tissue. Inasmuch as the refractive index of fibreglas is identical to that of glass, it is clear why this difficulty arose. Nevertheless, occasionally fibreglas fibres could be observed (Figure 31), but in general the visualization of this material was difficult. The effect of the fibreglas was a more severe inflammatory reaction in the lungs than it was encountered in animals treated with either carbon or saline. This reaction was as transitory as in the latter groups, however.

FIGURE 30: Rat alveolar tissue two months after intratracheal injection of carbon dust.

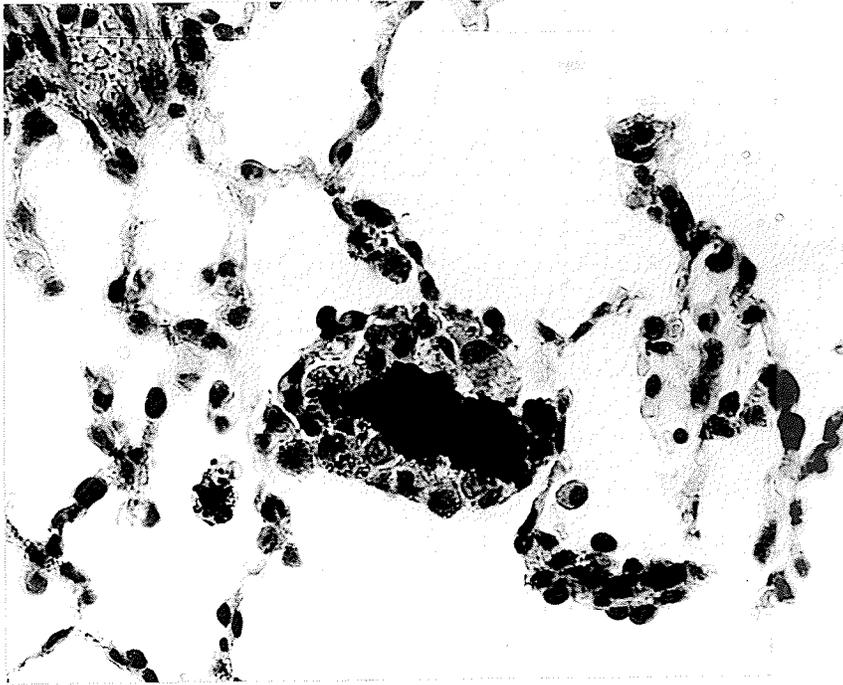
Note large aggregation of carbon dust in a nodule-like formation.

Photomicrograph 160 x.

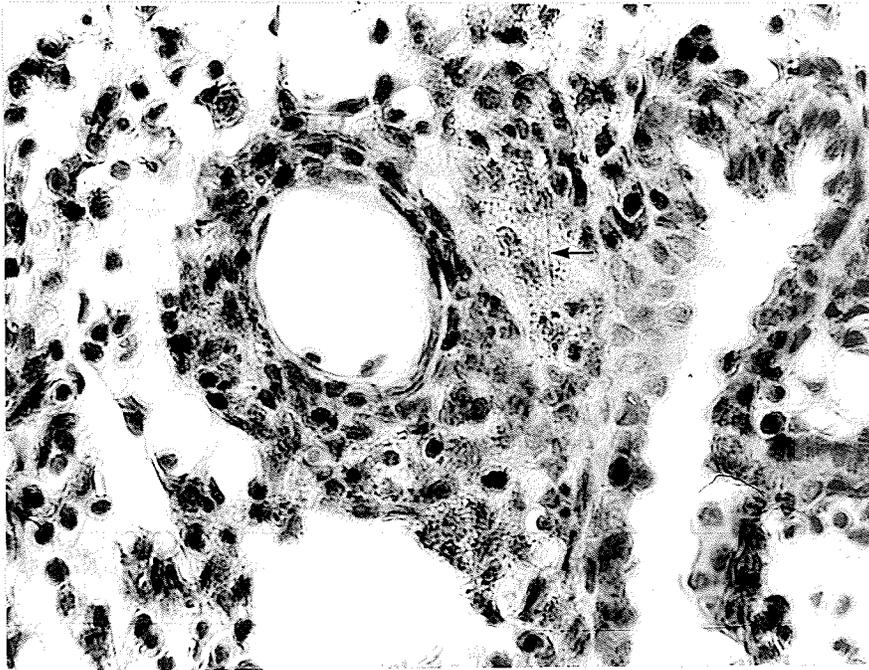
FIGURE 31: Rat alveolar tissue two months after intratracheal injection of fibreglas.

Note fibre of fibreglas at arrow.

Photomicrograph 160 x.



30



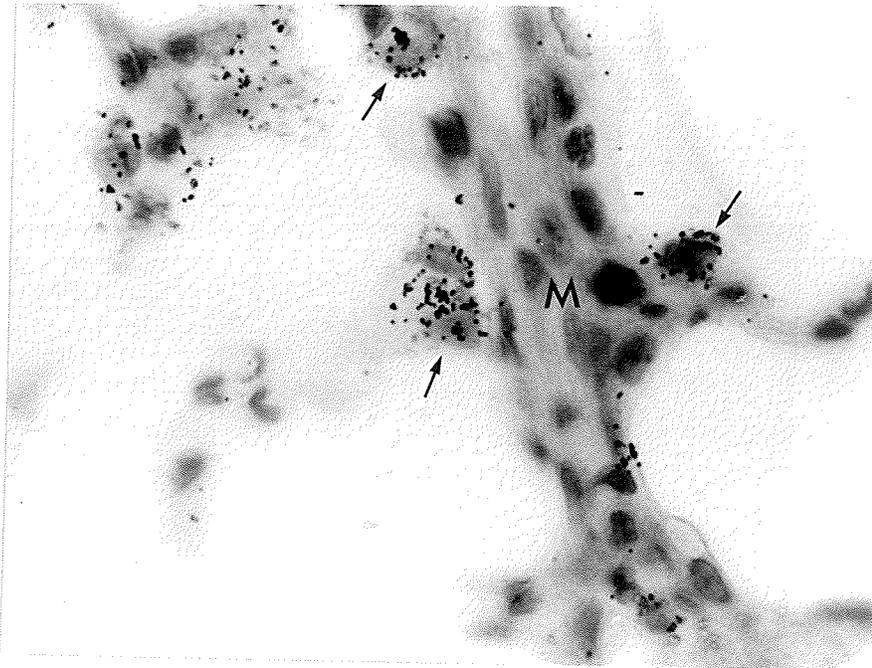
31

Synthetic Chrysotile Treated Series

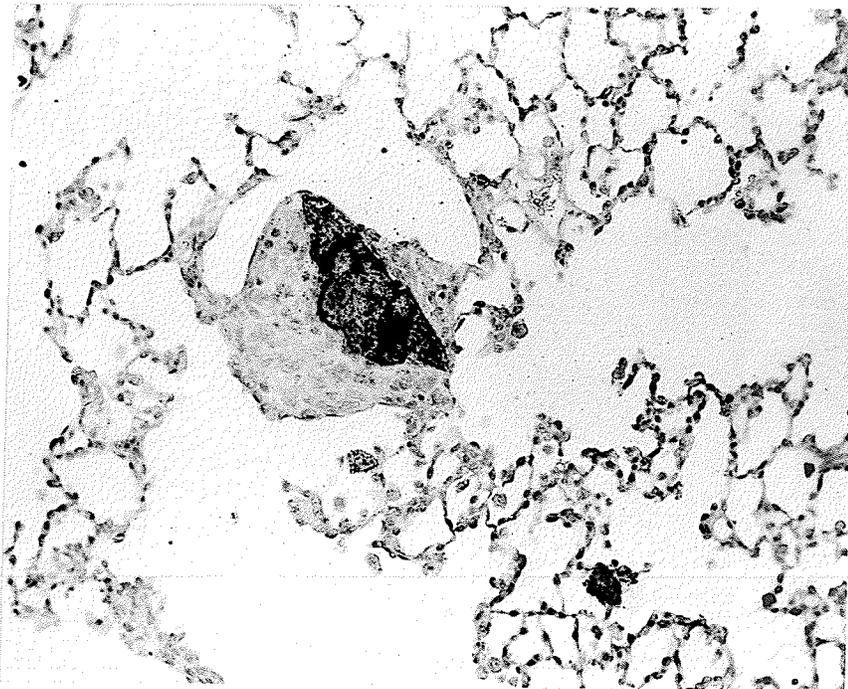
The reaction of the rat lung to synthetic chrysotile was not unlike its response to carbon or fibreglas. Inasmuch as the synthetic asbestos was tritium labelled, it could be readily localized in the lung tissue by radioautography. In the latter preparations, numerous chrysotile laden macrophages were observed throughout the alveolar tissue. Most of the cells occurred free or were located superficially in the alveolar wall. By one week after treatment very little free chrysotile dust was noted within the alveolar spaces. Figure 32 presents some chrysotile laden macrophages in a radioautograph of alveolar tissue, one day after administration. Although a large proportion of the dust was of a small size, occasional large aggregates of chrysotile which appeared to be of an amorphous variety could be visualized. These aggregates were apparently too large to be successfully removed by the cells during the duration of the experiment, and eventually appeared within discrete fibrotic nodules (Figure 33). Such nodules represented the major pathological feature in alveolar tissue affected by synthetic chrysotile. Labelling indicating the presence of synthetic chrysotile was also observed within the peribronchiolar and periarteriolar connective tissue. Moreover, labelled particles were noted close to visceral pleura both within alveolar macrophages and also as free particles.

FIGURE 32: Labelled synthetic chrysotile within alveolar tissue macrophages of rat lung one hour after treatment. Note labelling at arrows which appears to be intracellular. An excellent example of a colchicine metaphase is evident at M. Radioautograph 400 x.

FIGURE 33: Large aggregate of platy synthetic serpentine (part of synthetic chrysotile asbestos sample) within rat alveolar tissue 56 days after treatment. Radioautograph 40 x.



32



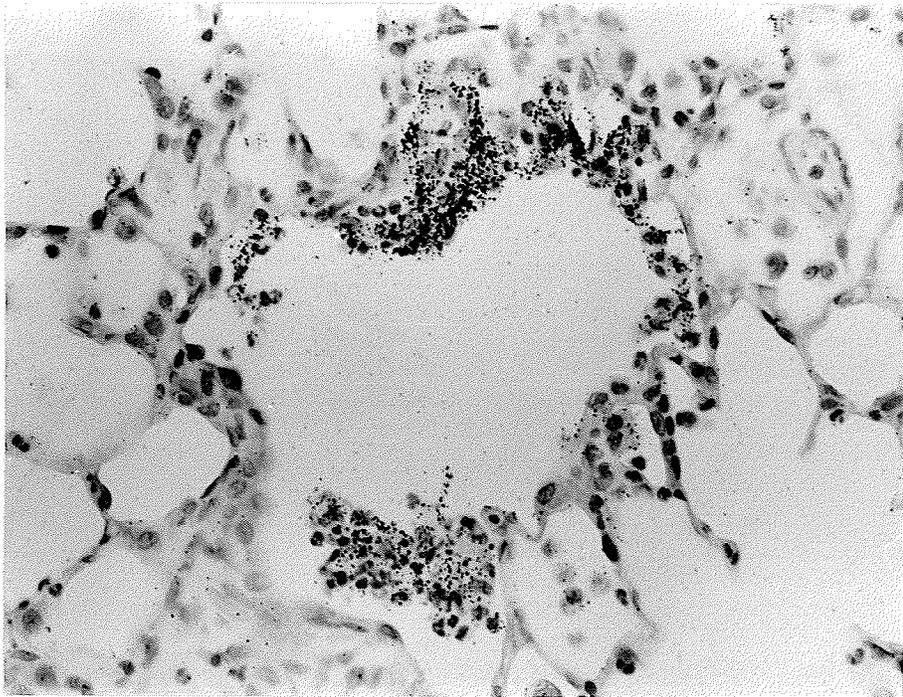
33

Intracellular particles were, however, by far the most frequent occurrence. The remaining alveolar tissue, aside from the aforementioned nodules, appeared largely unaffected by the synthetic chrysotile.

Natural Chrysotile Treated Series

In contrast to the other types of dusts tested, natural chrysotile exerted a drastic effect on the rat lung tissue. One day after administration, the asbestos was observed both in the airways and within the alveolar tissue. Aggregations of fibres coated the lining of the bronchioles (Figure 34). The asbestos fibres in the bronchial luminae were largely intermixed with cells that had responded to this insult. Many bronchioles were plugged by this mixture of fibres and cells (Figure 35). In addition, the epithelium of the bronchioles was to a large extent damaged, and in certain regions the airways were even denuded of epithelium. The alveolar tissue was likewise greatly affected. Labelled particles were observed along large portions of the surface of alveolar walls (Figure 36). Also, aggregations of asbestos fibres intermixed with cells were present in the alveolar spaces (Figure 37). Innumerable dust-laden alveolar macrophages, exhibiting labelled asbestos particles in the cytoplasm, were observed throughout the alveolar tissue. Labelled asbestos fibres were

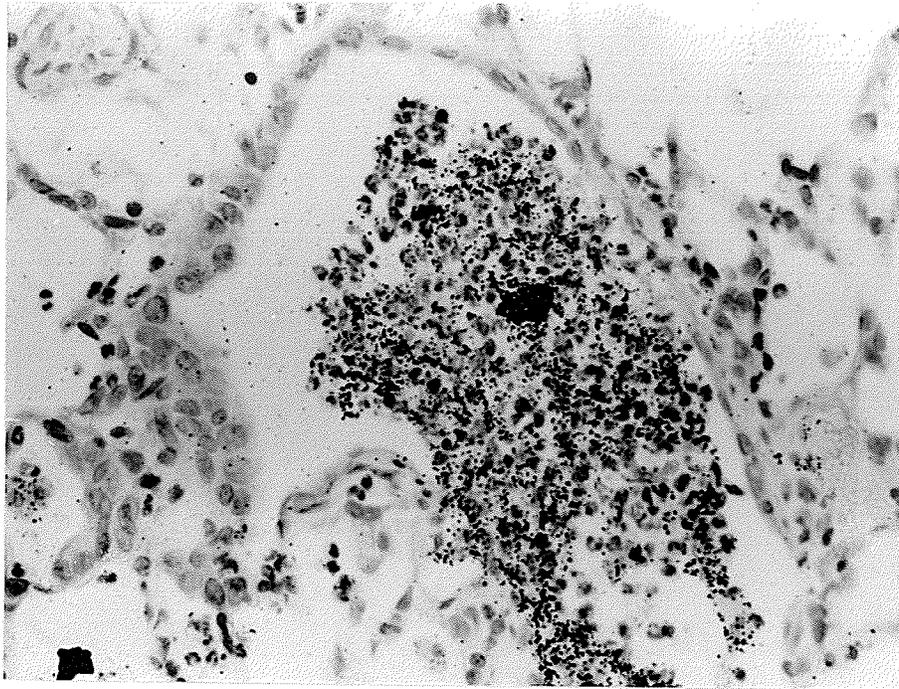
FIGURE 34: Labelled natural asbestos coating a terminal bronchiole
and antrum of rat lung.
Three days after treatment.
Radioautograph 100 x.



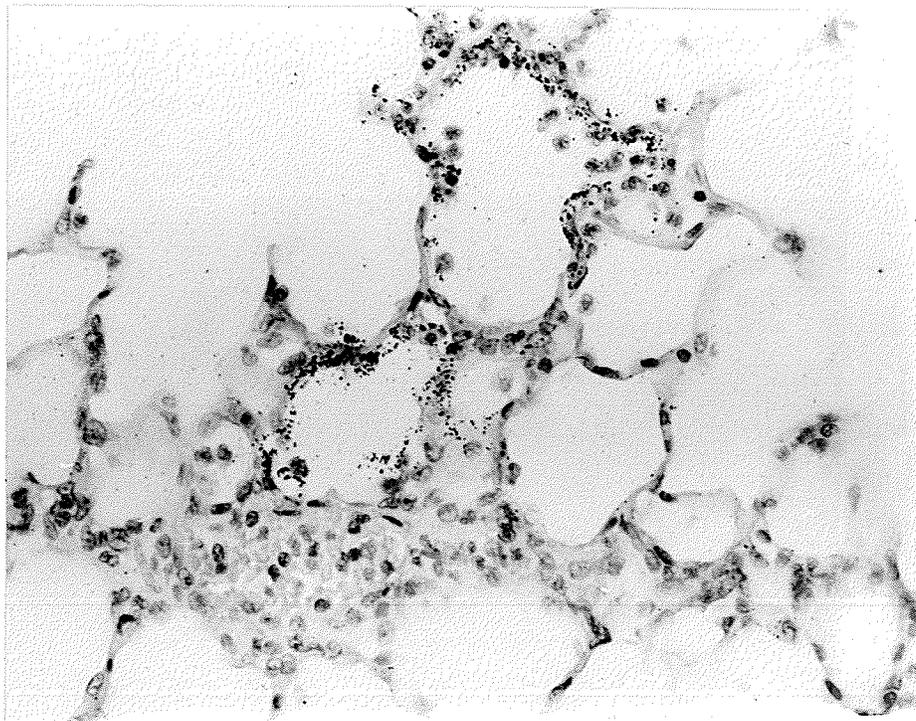
34

FIGURE 35: Bronchiole obliterated by a mixture of asbestos
fibres and cells.
Radioautograph 100 x.

FIGURE 36: Labelled asbestos fibres coating the alveolar walls.
Radioautograph 100 x.



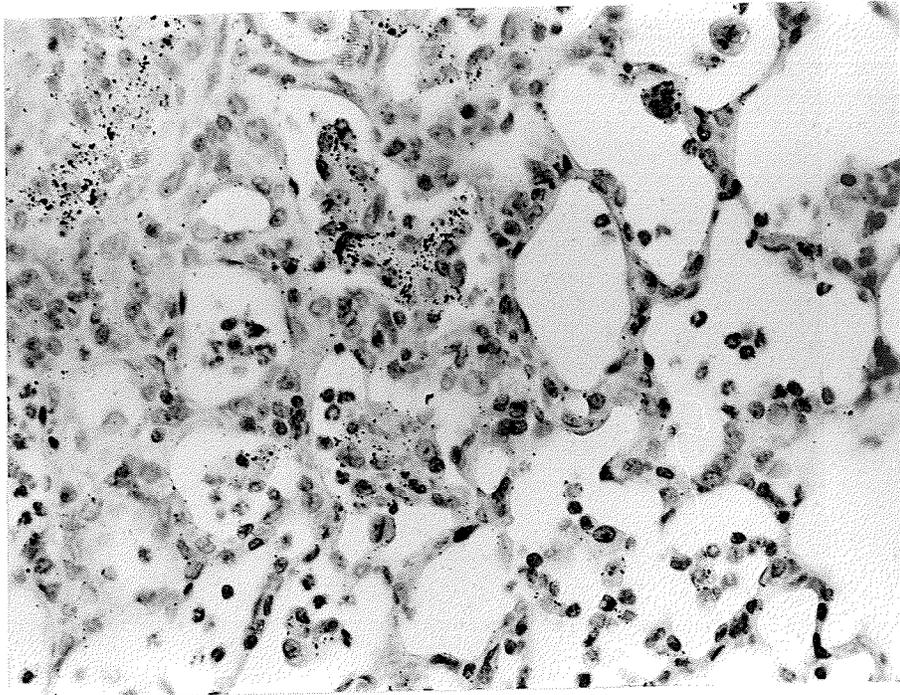
35



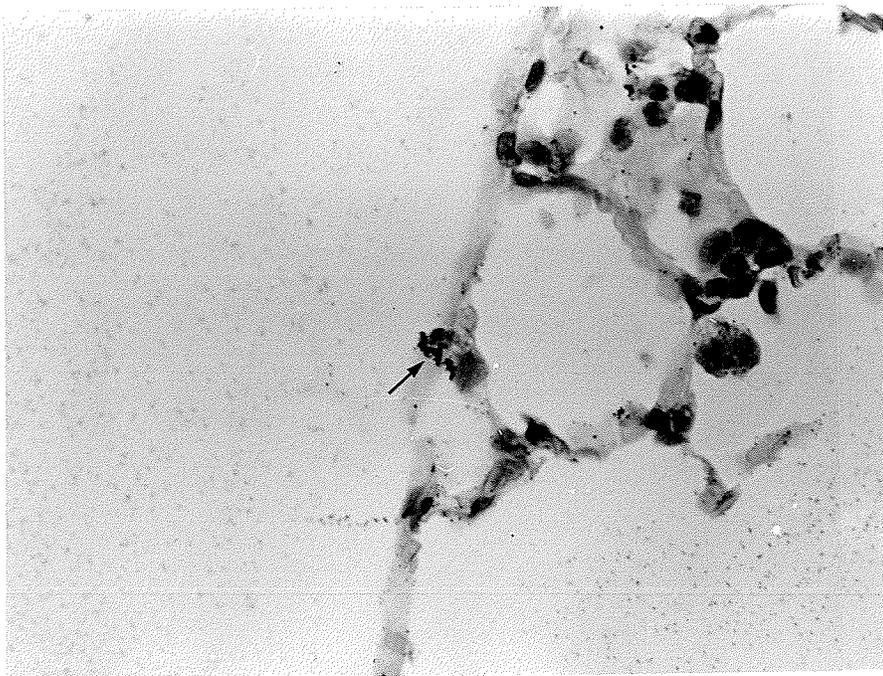
36

FIGURE 37: Aggregations of alveolar macrophages containing labelled natural asbestos particles and free labelled asbestos fibres within the alveolar tissue. Five days after treatment. Radioautograph 100 x.

FIGURE 38: Pleural mesothelium. At the arrow is an asbestos fibre that has penetrated the visceral pleura. Three days after treatment. Radioautograph 160 x.



37



38

also noted in the visceral pleura (Figure 38). With increasing time after administration, characteristic permanent lesions developed. Such lesions involved the bronchial tree at all levels but particularly the terminal and respiratory bronchioles with the smallest calibre. In the larger bronchioles, the mixture of asbestos fibres and cells became frequently overgrown by the bronchiolar epithelium. As early as one week after treatment, encapsulated asbestos-cellular plugs had been formed by the airway epithelium. The asbestos plug had initially denuded the airway epithelium beneath it, coming thereby in contact with the underlying connective tissue; the bronchiolar epithelium subsequently invested the plug. The result was a characteristic polypoid outgrowth ensuing in an obliterating bronchiolitis (bronchiolitis obliterans). The cellular core of the polypoid nodule became gradually replaced largely by collagenous connective tissue, thereby enclosing the asbestos fibres within the nodule (Figure 39). Apart from such characteristic lesions in the airways, similar lesions developed also at the level of the alveolar ducts. Such nodules extending into the respiratory tissue contained likewise large quantities of asbestos fibres (Figures 40 and 41). These lesions blocked that alveolar tissue lying distally to the affected duct. In addition, diffuse fibrosis developed in the alveolar tissue of the lungs of chrysotile treated animals. It extended generally from nodules at the level of the respiratory

FIGURE 39: Nodule of bronchiolitis obliterans.

Note labelling of natural asbestos fibres in the centre of the nodule. It is lined by a hyperplastic epithelium and contains a central core of connective tissue.

Twenty-one days after intratracheal injection.

Radioautograph 160 x.



39

FIGURE 40: Solitary fibrotic nodule formed within a terminal bronchiole. Note labelled asbestos.

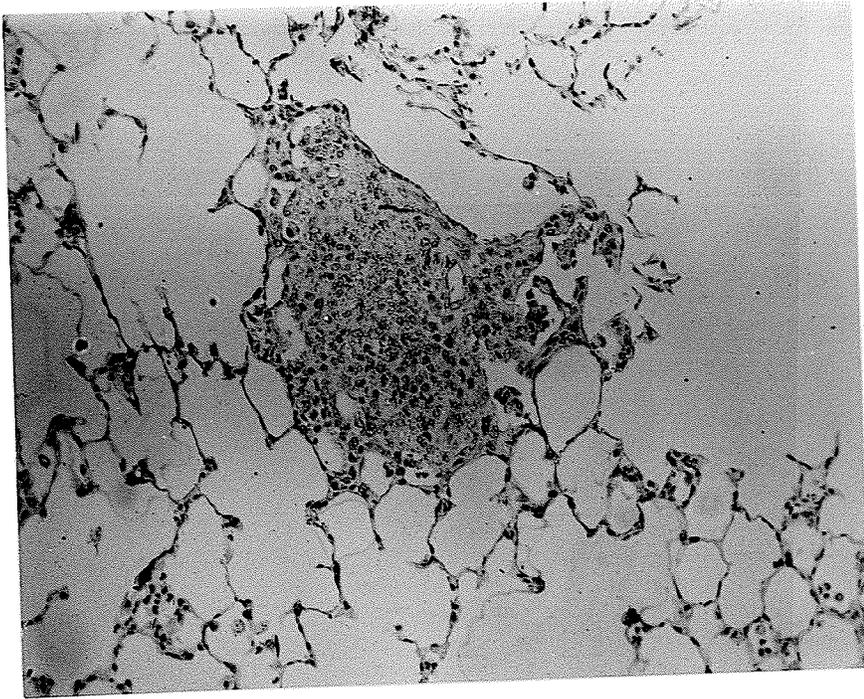
Twenty-eight days after treatment with labelled natural chrysotile.

Radioautograph 40 x.

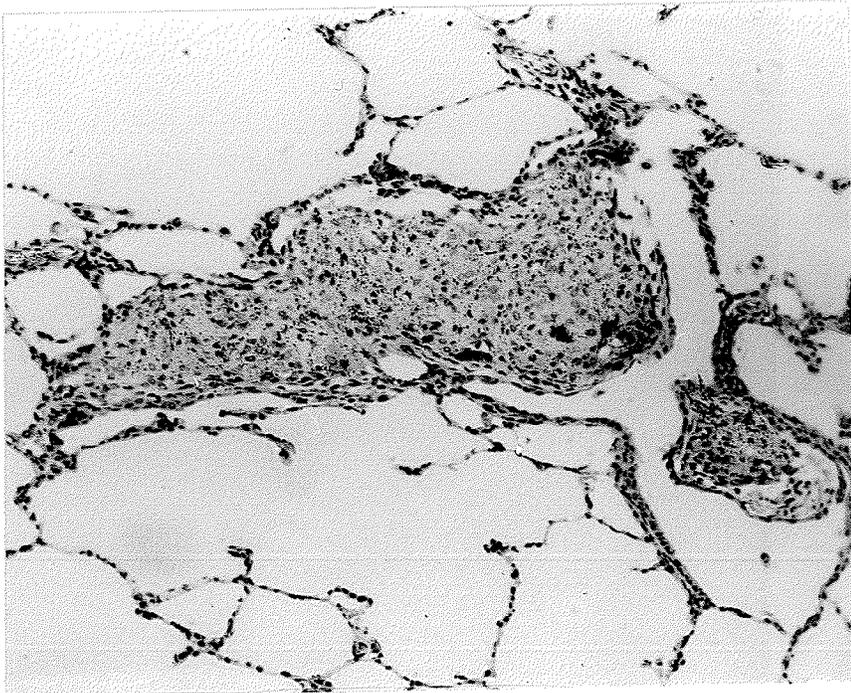
FIGURE 41: Longitudinal section of fibrotic nodule completely blocking the bronchiole.

Twenty-one days after treatment with labelled natural chrysotile.

Radioautograph 40 x.



40



41

bronchiole deep into the respiratory tissue (Figure 42). At later stages the alveolar walls became gradually thickened (Figure 43) in some regions, this obviously diminishing greatly their physiological activity.

The bronchial epithelium itself also was affected by the experimental treatment. It became thickened and metaplastic. The normal columnar ciliated and goblet cells became gradually replaced totally in some regions, first by cuboidal, then by squamous cells. Moreover, the underlying connective tissue stroma became markedly increased. Such pathological alterations of the bronchi are exemplified in Figures 44, 45 and 46. Radioautography revealed frequently labelled asbestos fibres to occur also within peribronchiolar and periarteriolar connective tissue adventitiae (Figures 44, 45 and 47).

The described lesions were largely circumscribed by about a month after treatment. The polypoid nodules did not continue to extend further into the air passages, and were covered by normal appearing epithelium, replacing gradually the initial metaplastic epithelium. Nodules at the level of the respiratory bronchiole often extended into the adjacent alveoli but such involvement did not progress further by two months after treatment. The involvement of the alveolar tissue was not widespread but generally restricted

FIGURE 42: Fibrosis of terminal bronchiole involving the surrounding alveolar tissue.

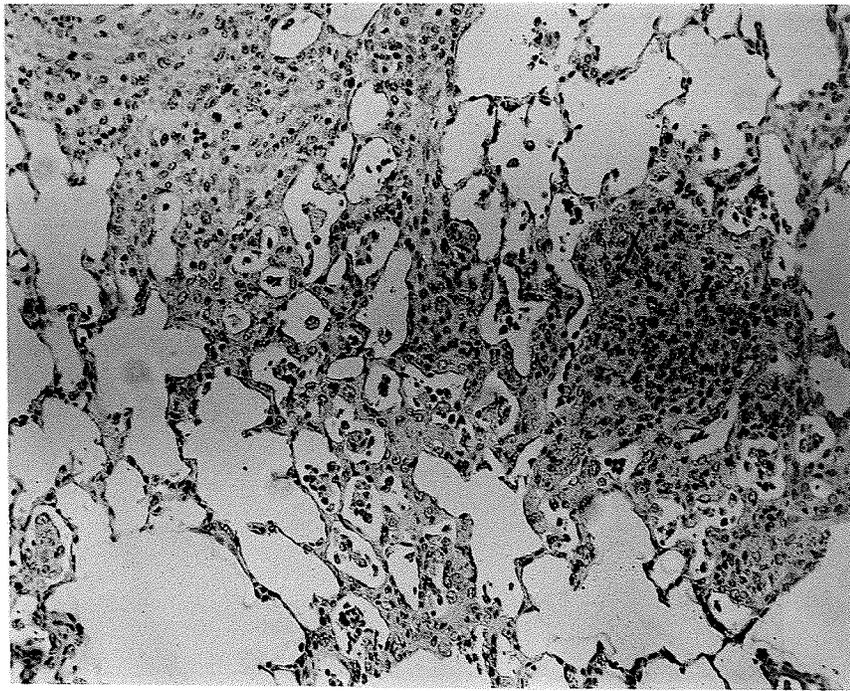
Five days after treatment with labelled natural chrysotile.

Radioautograph 40 x.

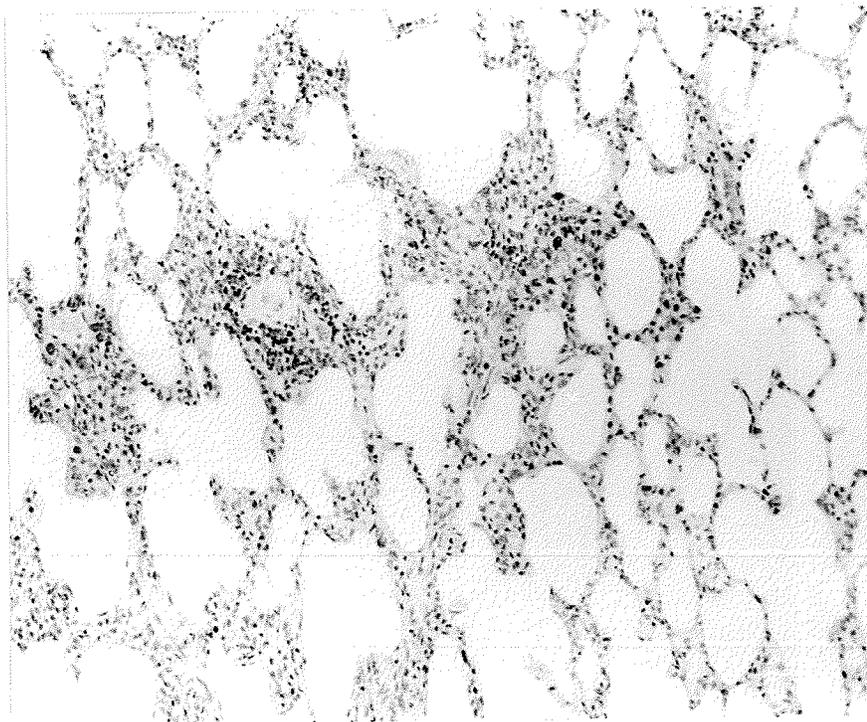
FIGURE 43: Fibrous thickening of alveolar wall produced by natural asbestos.

Twenty-eight days after treatment.

Photomicrograph 40 x.



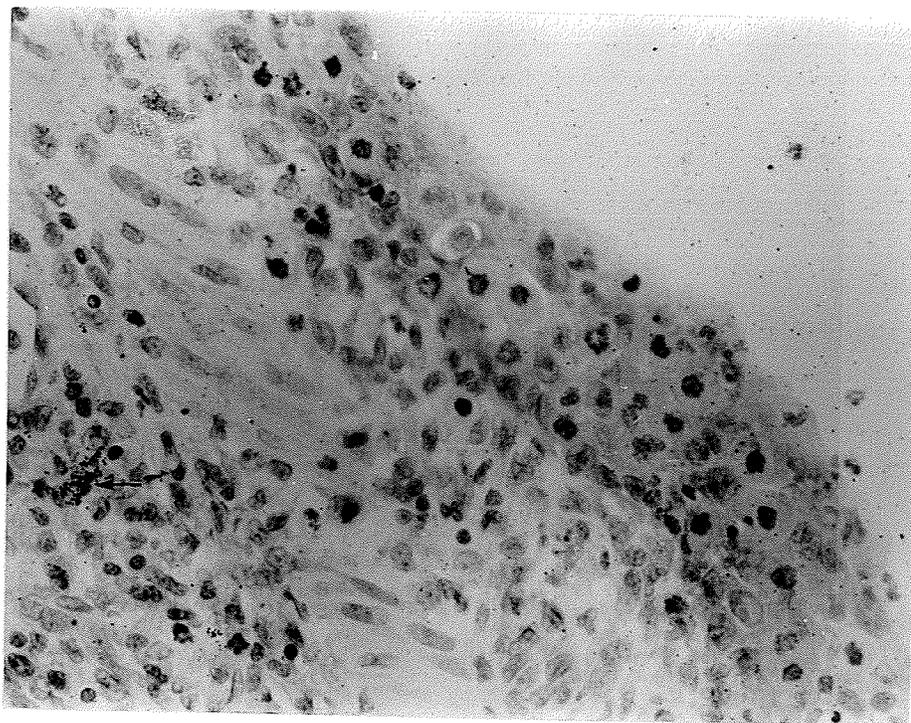
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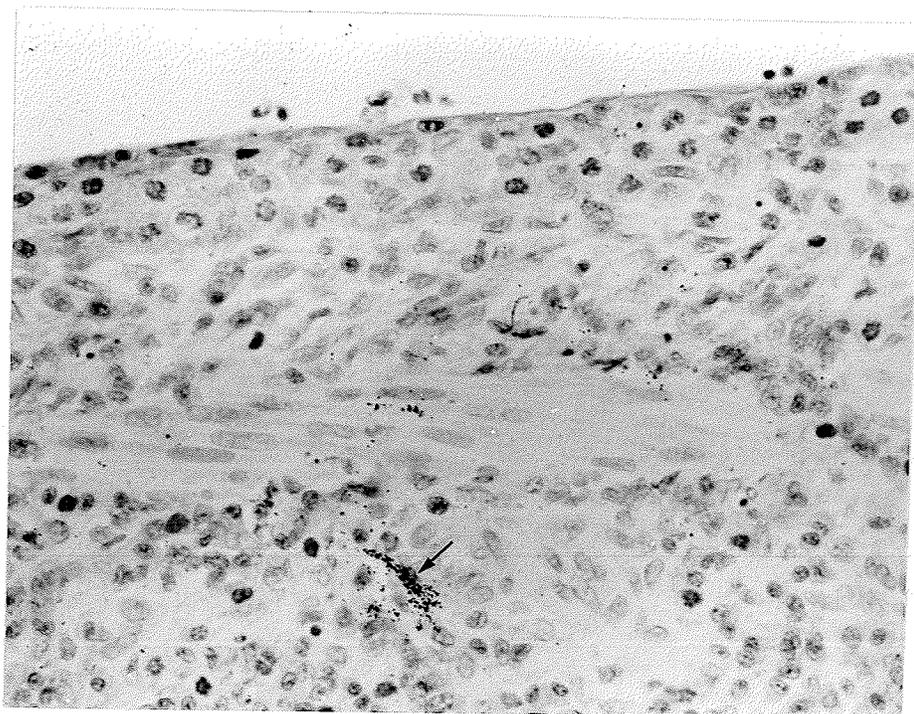
43

FIGURE 44: Metaplastic thickening of the bronchial epithelium 14 days following the intratracheal injection of labelled natural chrysotile. The grain formation at the arrow indicates abundant asbestos fibres in the peribronchiolar connective tissue. Radioautograph 100 x.

FIGURE 45: The bronchial epithelium became replaced by one of metaplastic stratified squamous type, devoid of ciliated and goblet cells. Note labelled asbestos fibres indicated by the arrow. 28 days post-administration of labelled natural chrysotile. Radioautograph 100 x.



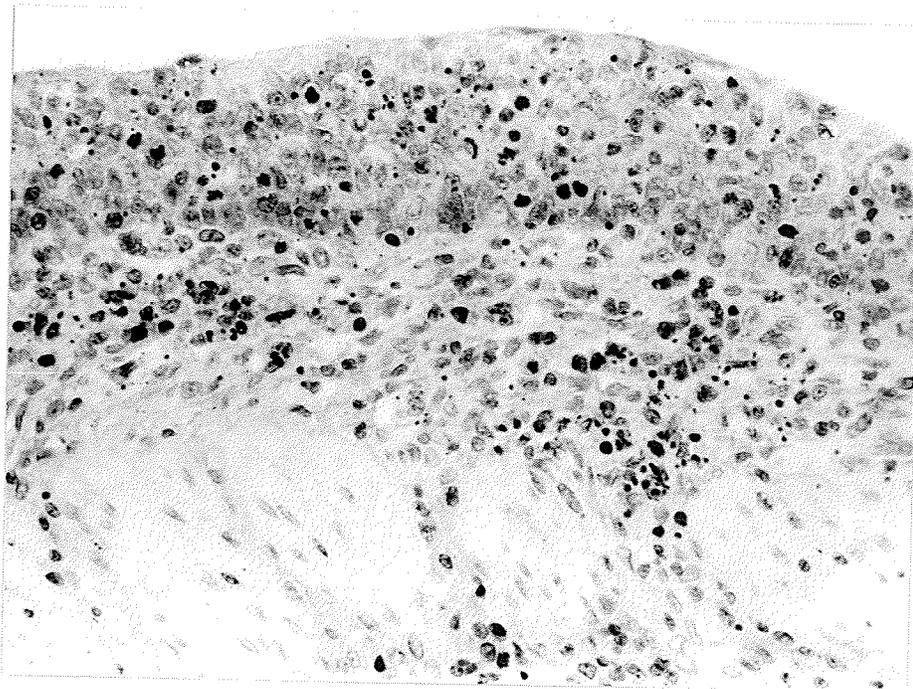
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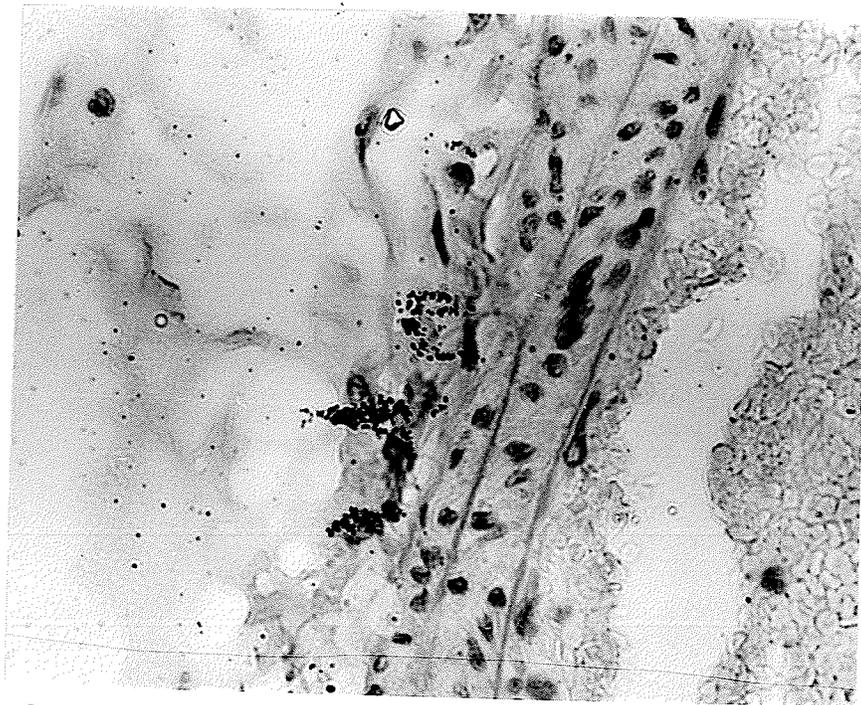
45

FIGURE 46: Bronchial epithelium 56 days after intratracheal injection of natural chrysotile. By this stage, the metaplastic epithelium became extremely thickened, and is composed of up to about 10 layers of cells. Note the numerous mitotic figures in the rapidly proliferating abnormal epithelium. Photomicrograph 100 x.

FIGURE 47: Clear localization of tritium labelled natural asbestos fibres by radioautography within periarteriolar connective tissue, merely 6 hours after treatment. Radioautograph 160 x.



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47

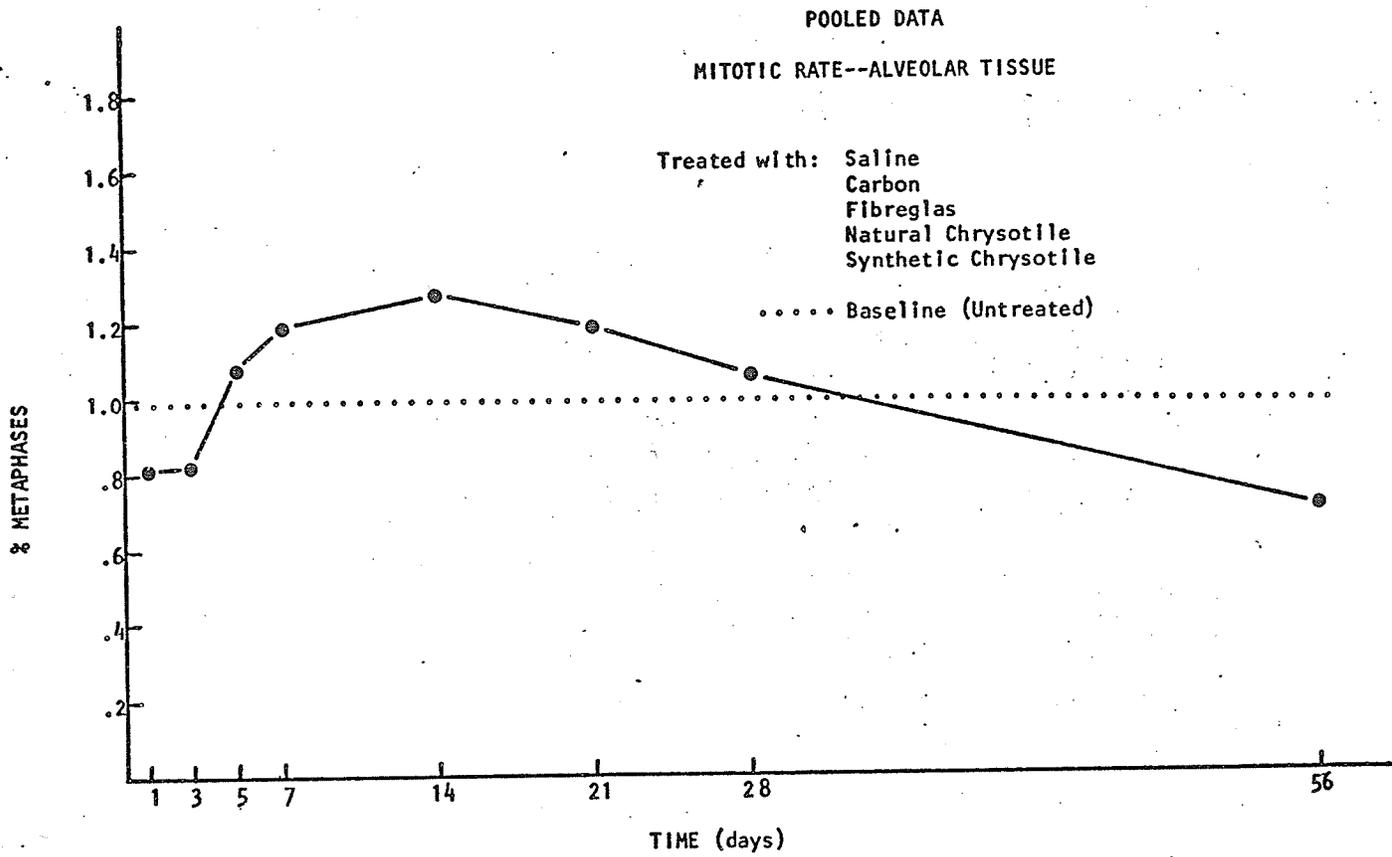
to circumscribed areas, while large portions of this tissue appeared to be relatively unaffected.

The Cytodynamic Responses of Alveolar Tissue

Overall Effect of Treatment

In order to characterize the overall effect of the various treatments on the proliferative activity of alveolar tissue, the data of all the treatments were pooled for the specific time intervals of sacrifice. It became thereby apparent that the overall effect of all the treatments, when considered in this manner, was a transient increase in the mitotic rate of the alveolar tissue (Figure 48). The earliest times (one, three days) and the latest (56 days) displayed a significantly lower mitotic rate than all other points. The general trend was a gradual increase of mitotic activity of the alveolar tissue up to seven days followed by an equally gradual return to normal levels. This signified that the general cytodynamic response of the cellular populations to the dust treatments was a transient augmentation of mitotic activity.

FIGURE 48



Saline Treated Series

The cytodynamic pattern evoked by saline treatment in the alveolar tissue was essentially similar to the overall pattern reported above. The mitotic activity of the alveolar tissue, expressed as the six hour percentage of metaphases, attained its maximum level of 1.75% by seven days after treatment. This was significantly greater than the value of 0.99% ascertained in normal untreated alveolar tissue. Subsequently, the mitotic rate declined gradually to the almost normal level of 1.05% by 28 days, and remained within the normal range until the termination of the experiment. Inasmuch as the saline treatment was common to all the treated groups, saline serving as the vehicle of the various materials administered, this group treated with saline alone served as the overall control group. The mitotic rates of the saline treated group are tabulated in Table 1.

Carbon Treated Series

A cytodynamic response of alveolar tissue very similar to that observed with saline occurred also after carbon dust administration. At seven days the percentage of metaphases reached 1.44% (Table 2), and was statistically identical to the response

TABLE 1: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER SALINE TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	88	1.10	0.5
3	4	8000	77	0.96	0.17
5	3	6000	62	1.03	0.43
7	4	8000	140	1.75	0.44
14	4	8000	124	1.55	0.19
21	4	8000	124	1.55	0.45
28	3	6000	63	1.05	0.31
56	4	8000	70	0.88	0.25
<hr/>					
Normal Untreated Animals	4	8000	79	0.99	0.22

FIGURE 49: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER INTRATRACHEAL INJECTION WITH CARBON

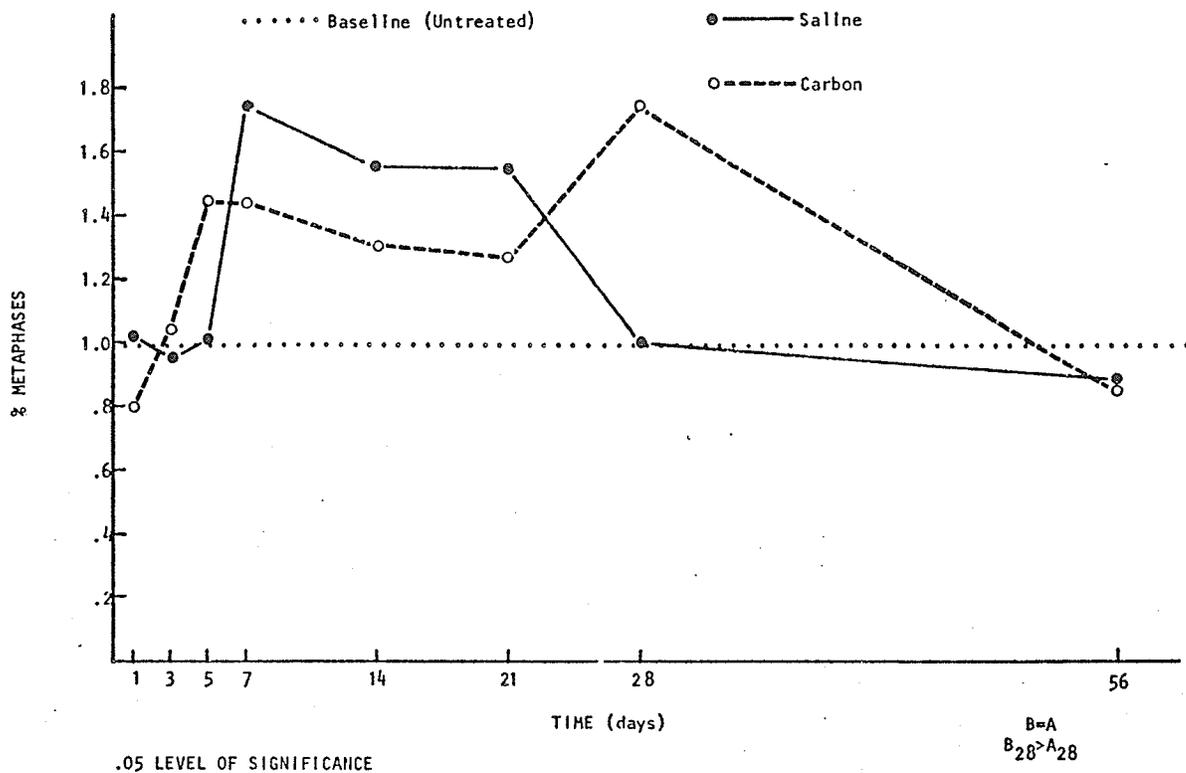


TABLE 2: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER CARBON TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	263	0.79	0.06
3	4	8000	83	1.04	0.21
5	4	8000	116	1.45	0.34
7	4	8000	115	1.43	0.78
14	4	8000	104	1.30	0.67
21	4	8000	102	1.27	0.42
28	4	8000	140	1.75	0.13
56	4	8000	68	0.85	0.17

evoked by saline alone. A difference was detected, however, at 28 days when the percentage of metaphases of the alveolar tissue of carbon treated animals attained a level of 1.75%. This was statistically different from the level of the saline treated animals at that point. A comparison of the cumulative data from these two groups revealed, however, that statistical differences did not exist between the total populations (Figure 49).

Fibreglas Treated Series

A difference between the mitotic rates of alveolar tissue was not apparent in the saline and fibreglas treated animals. The peak of mitotic rate of 1.81% in the fibreglas treated group was reached at 14 days (Table 3). By 28 days the mitotic rate had returned to normal levels where it remained until 56 days after administration, when the experiment was terminated. The response of the mitotic rate of alveolar tissue after fibreglas treatment is presented graphically in Figure 50.

Synthetic Chrysotile Treated Series

The mitotic rate of the alveolar tissue of animals treated with synthetic chrysotile asbestos was significantly lower than of

FIGURE 50: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER INTRATRACHEAL INJECTION TREATMENT WITH FIBREGLAS

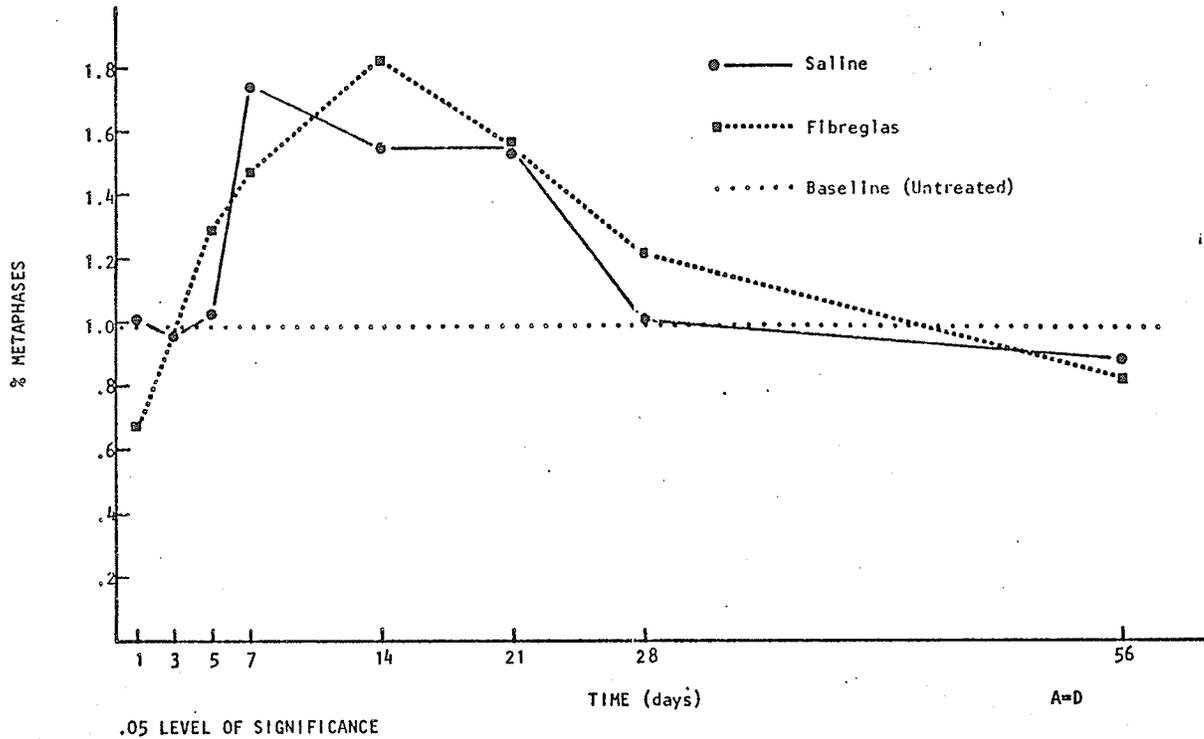


TABLE 3: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER FIBREGLAS TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	54	0.66	0.46
3	4	8000	79	0.99	0.43
5	3	6000	77	1.28	0.43
7	4	8000	118	1.46	0.25
14	4	8000	146	1.81	0.36
21	4	8000	124	1.55	0.73
28	4	8000	98	1.22	0.19
56	4	8000	66	0.81	0.40

the saline control series (Table 4). At three days after treatment, the six hour percentage of metaphases was merely 0.35. The normal level was gradually attained by seven days (0.95%), yet the mitotic rate declined again by 21 days to 0.68%. A week later the mitotic rate had returned to the normal range and was also within this range at 56 days after treatment. Figure 51 presents the mitotic response of the alveolar tissue of this group.

Natural Chrysotile Treated Series

This treatment was also effective in significantly depressing the mitotic rate of alveolar tissue below the saline control levels. One week after treatment the percentage of metaphases of alveolar tissue for the six hour period was 0.53 (Table 5). This value was significantly lower than the saline control value at that point. The mitotic rate had returned to control levels, within the normal range, by two weeks after treatment and remained so for the duration of the experiment (Figure 52).

FIGURE 51: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER INTRATRACHEAL INJECTION TREATMENT WITH SYNTHETIC CHRYSOTILE

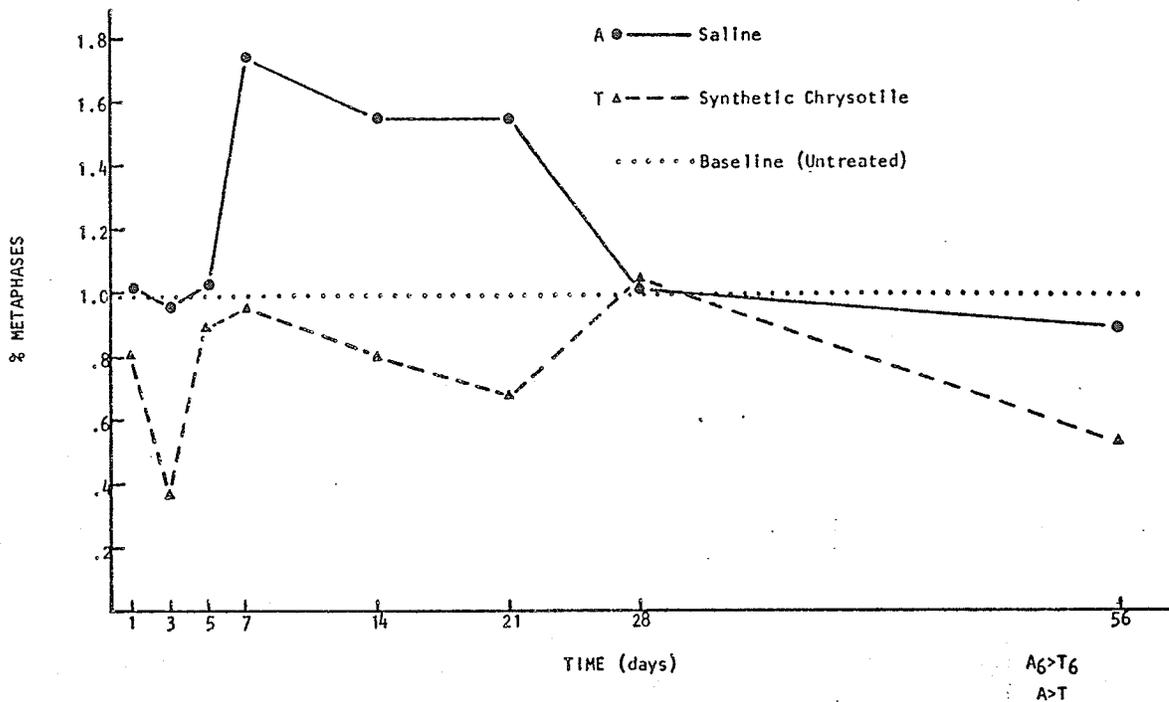


TABLE 4: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER SYNTHETIC CHRYSOTILE ASBESTOS TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	65	0.81	0.22
3	4	8000	21	0.35	0.11
5	4	8000	70	0.88	0.10
7	4	8000	76	0.95	0.37
14	4	8000	64	0.80	0.32
21	4	8000	54	0.68	0.32
28	4	8000	83	1.04	0.36
56	4	8000	42	0.53	0.24

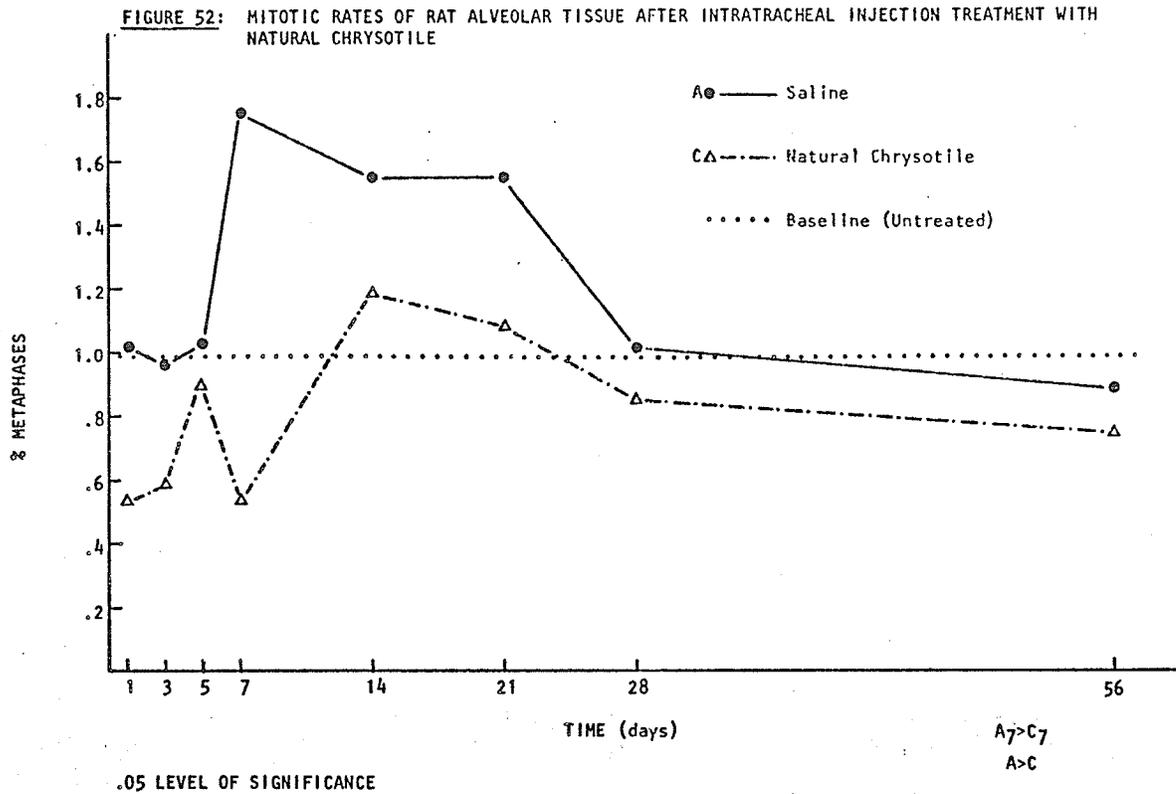


TABLE 5: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER NATURAL CHRYSOTILE ASBESTOS TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	42	0.53	0.26
3	4	8000	46	0.58	0.19
5	4	8000	72	0.90	0.22
7	4	8000	42	0.53	0.17
14	4	8000	94	1.18	0.49
21	3	6000	65	1.08	0.48
28	4	8000	68	0.86	0.39
56	4	8000	60	0.75	0.15

TABLE 5 - Addendum :

MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER LABELLED
NATURAL CHRYSOTILE ASBESTOS TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	36	0.45	0.23
3	4	8000	42	0.53	0.44
5	4	8000	64	0.79	0.69
7	4	8000	82	1.02	0.47

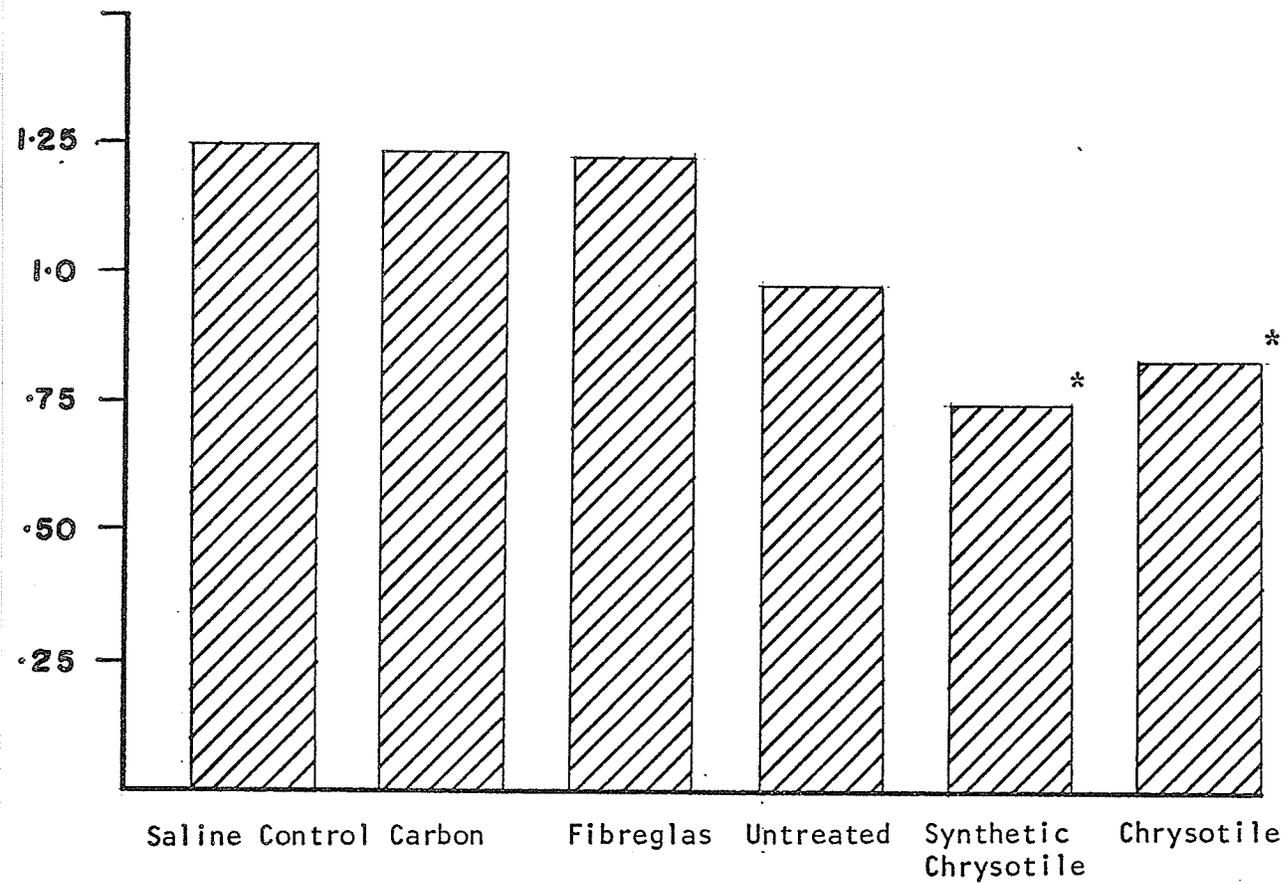
Comparison of the Treatments

Statistical comparison of the treatments with the different materials indicated that the treatments with the two types of asbestos produced mitotic responses in alveolar tissue significantly different from those of all the other treatments. In fact, differences of mitotic rate for the total population at all time intervals were not detected between the saline, fibreglas, and carbon treatments. The mean of the percentages of metaphases of those groups was 1.23% for the six hour period. In contrast, natural and synthetic chrysotile reduced the mitotic rate of alveolar tissue respectively to 0.79 and 0.75% on the average. The mitotic rates of alveolar tissue were not significantly different in the synthetic and natural asbestos treated series (Figure 53).

Weight Consideration

Only those animals treated with natural chrysotile asbestos exhibited a significant weight loss. That weight loss amounted up to 60 grams of body weight within the first five days after asbestos administration. By one week after the treatment, the animals began to regain the lost weight. Animals administered with the other types of material did not experience a significant weight loss,

FIGURE 53: MEANS OF MITOTIC RATE FOR SPECIFIC TREATMENTS



*Significant difference from control at 0.05 level.

although some of them failed to gain weight during the early phase of the experiment. The animals receiving natural asbestos recuperated their loss in weight more slowly and their body weights were comparable to that of the other groups only by 28 days after treatment had commenced. The actual data of body weights of the different experimental groups are presented in Tables 6 - 10.

TABLE 6: WEIGHT ALTERATIONS OF RATS AFTER SALINE TREATMENT

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	220	230	+ 10.0	7.1
	230	230		
	245	260		
	245	260		
3	230	250	+ 20.0	4.1
	250	270		
	230	255		
	210	225		
5	230	255	+ 18.75	14.9
	240	255		
	225	260		
	235	235		
7	230	280	+ 52.5	2.8
	225	280		
	240	290		
	210	265		
14	220	310	+ 80.0	26.7
	245	340		
	210	300		
	230	325		
21	250	350	+118.7	8.5
	230	350		
	240	370		
	225	340		
28	220	360	+153.0	11.5
	220	380		
	255	415		
56	250	490	+218.7	15.4
	230	440		
	240	445		
	220	440		

TABLE 7: WEIGHT ALTERATIONS OF RATS AFTER CARBON TREATMENT

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	250	260	+ 1.25	6.3
	240	240		
	250	250		
	240	235		
3	250	260	+ 16.25	17.5
	250	260		
	230	250		
	235	260		
5	260	280	+ 27.5	9.5
	225	245		
	250	280		
	250	290		
7	240	290	+ 37.5	18.4
	245	290		
	250	295		
	240	250		
14	260	355	+ 86.2	8.5
	235	325		
	250	335		
	245	320		
21	265	390	+126.2	6.3
	235	370		
	240	360		
	245	370		
28	250	390	+138.7	15.5
	240	370		
	245	370		
	250	410		
56	255	480	+192.5	27.2
	240	410		
	255	460		
	250	420		

TABLE 8: WEIGHT ALTERATIONS OF RATS AFTER FIBREGLAS TREATMENT

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	285 295 260 305	300 295 270 305	+ 6.3	7.5
3	285 285 310 285	290 310 320 300	+ 13.8	8.5
5	320 320 275 305	355 340 305 330	+ 25.0	4.1
7	290 275 305 180	330 270 335 315	+ 25.0	20.4
14	290 305 310 275	355 385 370 345	+ 68.8	8.5
21	295 280 275 300	380 380 350 380	+ 85.0	10.8
28	290 290 310 290	400 390 420 375	+101.3	11.8
56	270 320 300 300	435 460 400 460	+141.3	29.5

TABLE 9: WEIGHT ALTERATIONS OF RATS AFTER SYNTHETIC CHRYSOTILE ASBESTOS TREATMENT

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	260 230 250 260	275 235 250 265	+ 6.3	6.3
3	250 240 220 250	265 230 240 270	+ 11.3	14.3
5	260 255 255 250	280 280 280 275	+ 23.8	2.5
7	250 250 260 260	270 275 285 275	+ 21.3	4.8
14	250 250 245 250	310 310 285 320	+107.5	61.8
21	270 260 240 190	340 310 320 290	+ 85.0	31.1
28	250 260 255 245	350 390 370 370	+118.8	14.4
56	- Not Available -			

TABLE 10: WEIGHT ALTERATIONS OF RATS AFTER NATURAL CHRYSOTILE ASBESTOS TREATMENT

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	340 330 325 330	320 325 300 300	- 20.0	10.8
3	270 260 255 245	245 200 235 215	- 33.75	17.9
5	300 240 275 270	250 230 240 280	- 21.25	26.5
7	285 260 250 260	330 230 240 245	+ 2.5	32.7
14	280 275 275 285	310 255 225 325	0.0	42.4
21	280 275 275 285	300 370 340 295	+ 35.0	24.8
28	295 280 270 270	410 355 385 395	+107.5	22.1
56	280 265 285 270	400 410 435 420	+138.7	14.3

EXPERIMENT 2

It was conceivable that the body weight loss after the administration of natural chrysotile asbestos may have been a major factor effecting also the decline of the mitotic rate in alveolar tissue of the experimental animals. Therefore, an experimental series was conducted to examine the effect of weight loss alone on the mitotic rate of alveolar tissue. Moreover, the body weights were determined of the animals composing a second series, intratracheally injected with saline and starved, to determine the cumulative effect of both treatments. One group of rats was administered with saline and starved for two days, while the other was merely starved for two days without any other treatment. The animals of each treatment category were sacrificed in groups of four at one, three, five and seven days after the initiation of the experiments. As previously, the colchicine technique was employed to ascertain the mitotic response of the alveolar tissue.

Observations on the Living Animals

Aside from the animals being extremely restless during the

starvation period, noticeable changes were not observed in their behavior or health.

Histological Observations

The previously described inflammatory reaction noted in saline treated animals was again observed in animals treated with saline and also starved. Alterations from normal could not be detected in lung morphology in animals that were starved only.

The Cytodynamic Responses of Alveolar Tissue

Saline Treated and Starvation

The mitotic rate of the alveolar tissue of these animals administered saline in addition to starvation fell to 0.39% from the control level of 0.99% after the first day. The rate of mitosis returned gradually to normal levels by one week at which time the percentage of metaphases was 1.16 (Table 11). At this point, the level of mitotic activity was significantly higher than that after the first day. Nevertheless, statistical differences of

mitotic rate could not be detected between the starvation and saline treatment and saline alone (Experiment 1). Neither were the mitotic rates attained by this group different from those of the natural asbestos treated group at the same time intervals.

Starvation Alone

The mitotic activity of the alveolar tissue of the starved animals declined to 0.43% after the first day of food withdrawal. On the fifth day of the experiment the percentage of metaphases in alveolar tissue had reached 1.19% but then declined once more to 0.61% at seven days, the last day of the experiment (Table 12). The level of mitotic activity attained at five days was significantly greater than that observed on the first day. However, the decline at seven days yielded a mitotic rate significantly lower than that of the saline control group at that time.

Comparison Between the Treatments

A statistical comparison between the effects of the two treatments on the mitotic rate of alveolar tissue revealed that they were not significantly different. Yet the mitotic rates of the

TABLE 11: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER SALINE AND STARVATION TREATMENT

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	31	0.39	0.19
3	3	6000	24	0.40	0.30
5	4	8000	49	0.61	0.09
7	4	8000	93	1.16	0.87

TABLE 12: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER STARVATION TREATMENT (2 DAYS)

Time After Treatment (Days)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	35	0.43	0.16
3	4	8000	38	0.48	0.31
5	4	8000	94	1.19	0.82
7	4	8000	49	0.61	0.25

starved group were significantly different from the saline control group, notwithstanding the lack of such difference between the saline group and the starved and saline treated group (Figure 54).

Weight Consideration

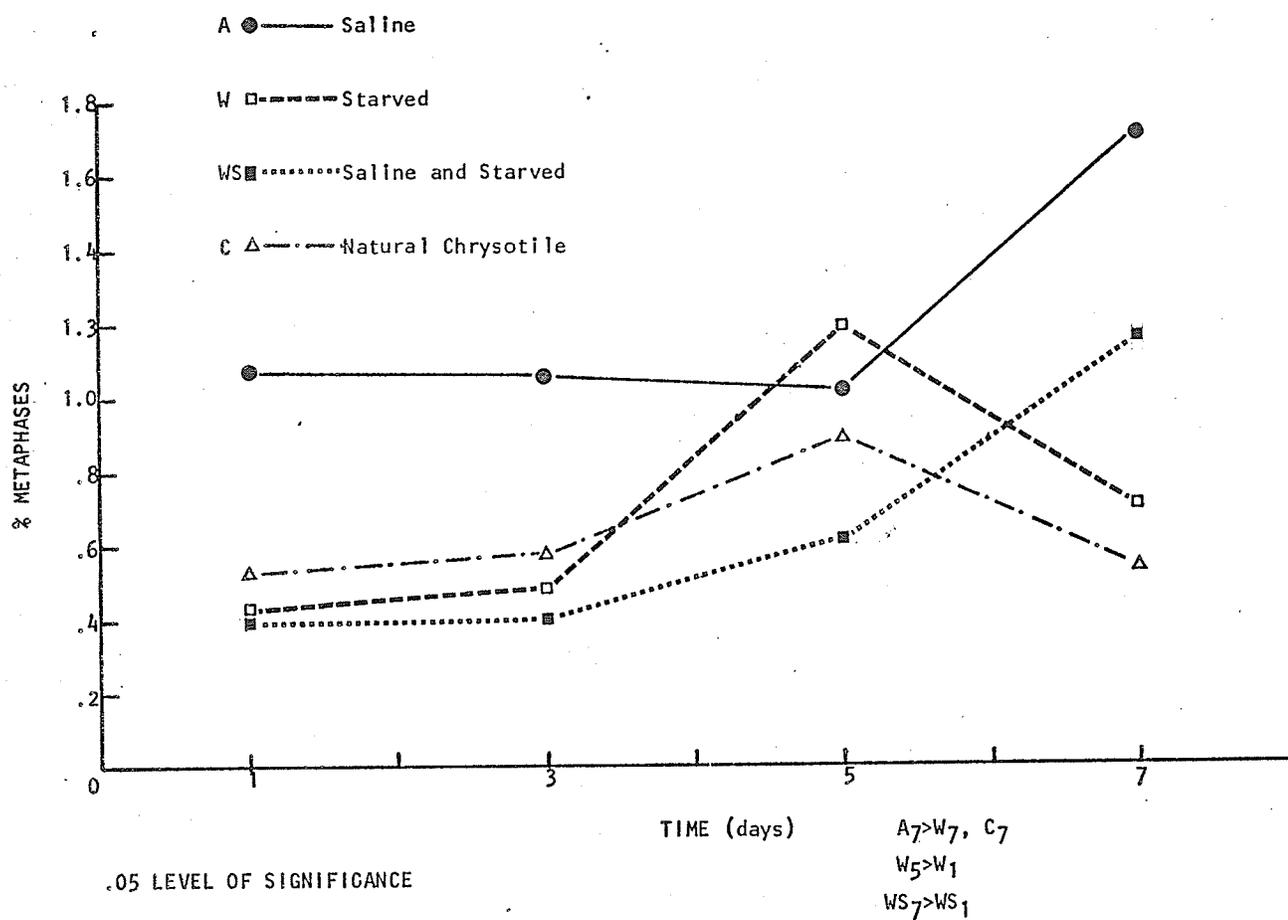
The rats of both groups lost weight during the two days of starvation. These weight losses of the two groups were quite similar (Tables 13, 14). After three days of refeeding (Day Five of the experiment), differences in regaining of weight were noted between the two groups. The animals that had been starved only gained weight above their original weight at the beginning of the experiment by five days. In contrast, those animals that had been both starved and saline treated had not even regained their original weight by that time.

Statistical Evaluation of the Effect of Weight Loss on Mitotic Rate of Alveolar Tissue

Inasmuch as the cytodynamic data or the weight data alone did not clarify the contribution of weight loss on the modification of the mitotic activity of alveolar tissue, further statistical analysis was undertaken employing the data from experiments 1 and 2.

FIGURE 54

MITOTIC RATE--ALVEOLAR TISSUE



Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	260	240	- 25.0	4.1
	260	235		
	285	260		
	270	240		
3	280	260	- 16.25	8.5
	275	270		
	290	265		
	275	260		
5	280	285	- 8.8	35.2
	280	300		
	290	230		
	280	280		
7	320	330	- 5.0	19.1
	300	290		
	270	240		
	280	290		

TABLE 14: WEIGHT ALTERATIONS OF RATS AFTER STARVATION AND SALINE INTRATRACHEAL INJECTION

Time After Treatment (Days)	Mean Weight at Time of Treatment	Mean Weight at Time of Sacrifice	Mean Weight Alteration	Standard Deviation (\pm)
1	405	395	- 10.0	4.1
	415	400		
	410	400		
	410	405		
3	385	365	- 18.8	2.5
	390	370		
	395	370		
	365	340		
5	410	415	+ 22.5	13.2
	400	430		
	410	430		
	390	425		
7	400	420	+ 12.5	8.7
	410	410		
	405	420		
	405	420		

A one way analysis of co-variance was executed utilizing weight as the independent variable and mitotic rate as the dependent variable. An F ratio of 3.320 with 27 and 80 degrees of freedom was obtained even after the adjustment of the dependent variable. It became thereby apparent that the effects on mitotic rate were independent of weight loss as an important contributory factor of the pulmonary cell populations.

EXPERIMENT 3

This experiment was merely a pilot study examining the feasibility of conducting more extensive series on the cytodynamic reactivity of the pleural mesothelium to intrathoracically injected asbestos dusts. Groups of rats were injected into the pleural cavity with either a sterile saline suspension of natural chrysotile (3.5 mgm./cc.) or else with 1 cc. of saline alone. Groups of four rats each of both series were sacrificed at seven and 14 days after the treatments.

Observations on the Living Animals

The animals responded well to the treatments. Mortalities did not occur, and the animals were without obvious distress throughout the experimental period.

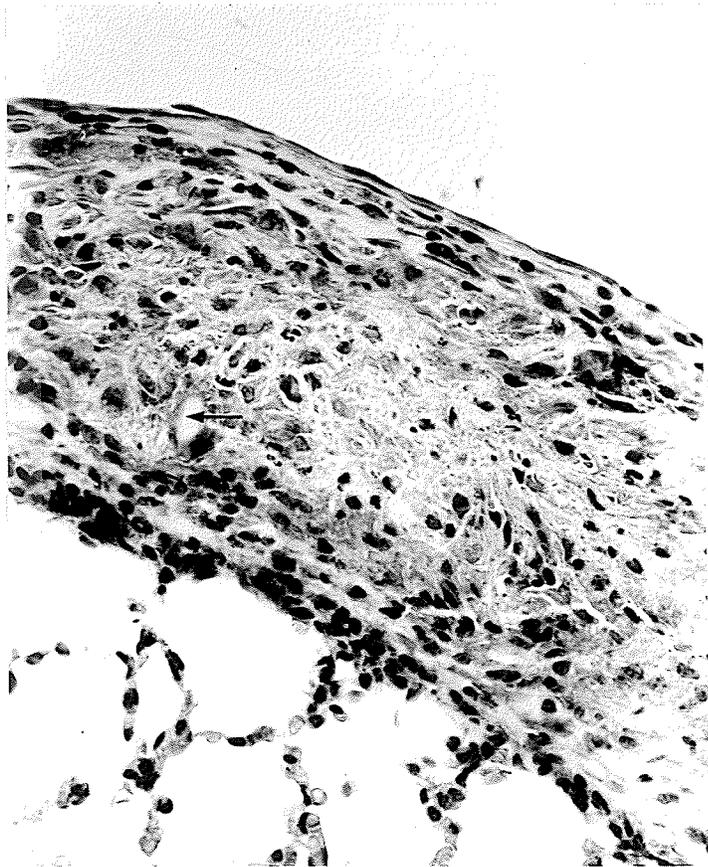
Histological Observations

The pleurae of saline treated animals, both visceral and parietal, did not exhibit any unusual morphological features; they

were indistinguishable in that regard from those of untreated animals. In contrast, the pleurae of the animals administered with natural chrysotile contained numerous plaques both in the parietal and visceral pleurae. Notwithstanding the injection of the chrysotile into the left side alone of the thorax, such plaques were observed in both the right and left visceral pleurae and to a lesser extent also on the parietal pleurae. Plaques were also observed in some of the animals on the diaphragmatic parietal pleurae. In addition, numerous adhesions between the lungs and the parietal pleurae had formed. The plaques were largely composed of increased collagenous tissue in which was embedded the asbestos fibres (Figure 55).

FIGURE 55: Plaque on the visceral pleura of rat one week after intrathoracic injection of natural chrysotile asbestos. The submesothelial connective tissue is considerably thickened and contains embedded asbestos fibres (at arrow).

Photomicrograph 100 x.



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EXPERIMENT 4

This experiment was designed in an endeavour to enable a characterization of the clearance of intratracheally instilled natural or synthetic asbestos from the lungs of the rats. The first series of rats was intratracheally injected with tritiated synthetic chrysotile (Experiment 1). In addition to the time intervals reported in experiment 1, the rats were also sacrificed in groups of four at one, three, six, 12 and 18 hours after treatment. The second series of animals was similarly treated with 0.75 cc. of a 3.5 mgm./cc. suspension of natural labelled chrysotile. These animals were sacrificed in groups of four at three, six, 12 hours and also one, three, five, seven, 14, 21, 28 and 56 days after treatment. The lungs of these animals were utilized for the radioautographical localization of natural chrysotile, as described in Experiment 1. Moreover, lung tissue of these two groups of animals was prepared for scintillation counting. The measurement of the radioactivity remaining within the lungs of the animals at various times after the administration of the dusts represented the amount of the intrapulmonary material still retained, and was thus a measure of the clearance efficiency of the lung. The data of scintillation counts are plotted graphically in Figures 56 and 57. Little radioactivity

FIGURE 56: RADIOACTIVITY REMAINING IN RAT LUNG AFTER TREATMENT WITH NATURAL CHRYSOTILE ASBESTOS

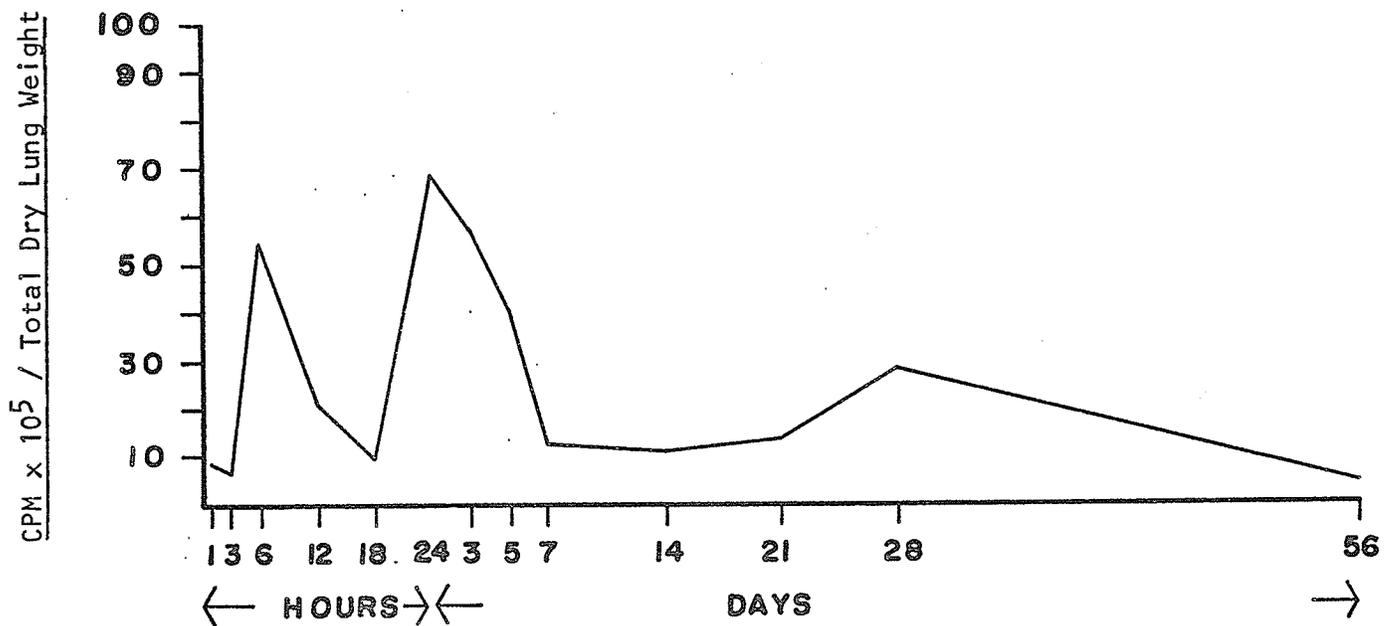
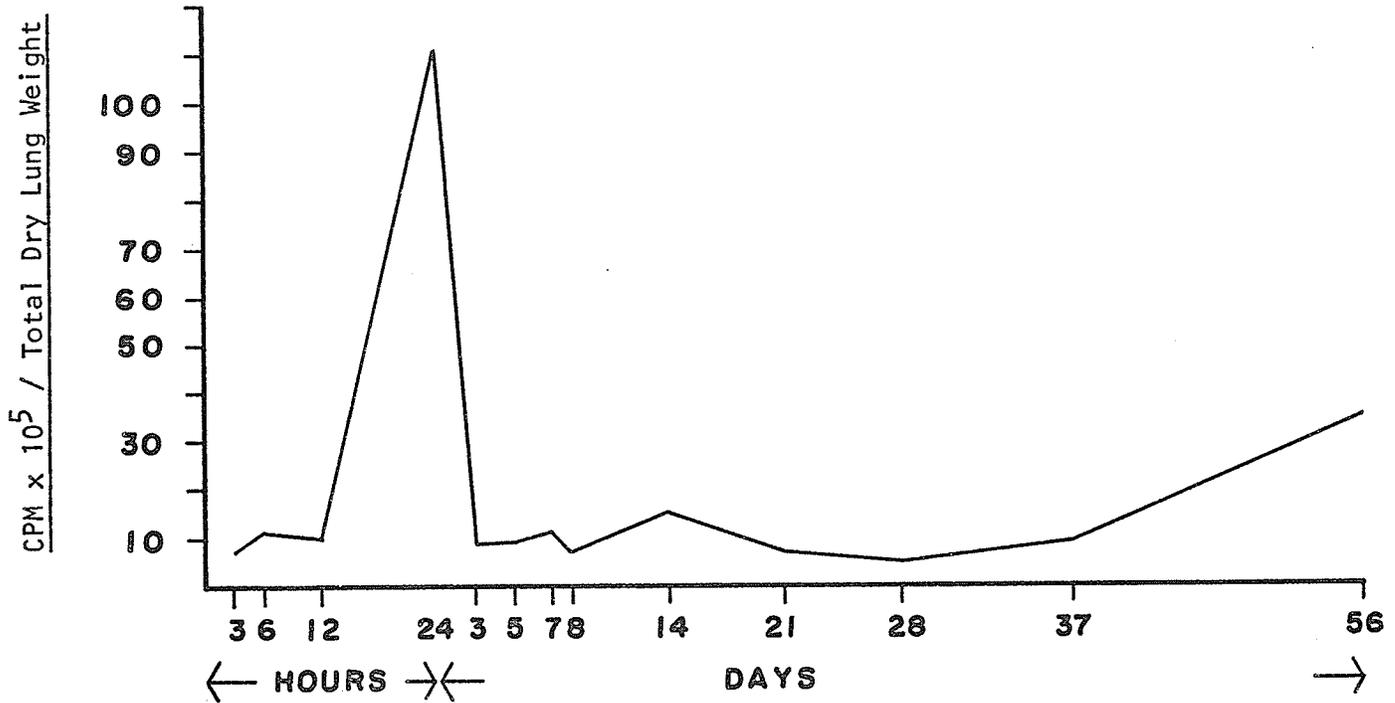


FIGURE 57: RADIOACTIVITY REMAINING IN RAT LUNG AFTER TREATMENT WITH SYNTHETIC CHRYSOTILE ASBESTOS

could be detected at very early phases in the lungs of animals administered either synthetic or natural chrysotile. In fact, with the labelled natural chrysotile the level of radioactivity was very low for the first 12 hours after its administration. By 24 hours after treatment it increased, and thereafter a high level of radioactivity of the order of 111.2×10^5 cpm./total dry lung weight was ascertained (Table 15). By three days after administration, the cpm. had greatly declined to 8.4×10^5 . This low range of radioactivity was maintained rather constant for nearly the entire duration of the experiment, yet at the final stage, 56 days after treatment, the cpm. had risen to 35.8×10^5 .

Regarding the labelled synthetic chrysotile, the level of radioactivity was likewise low during the first three hours after treatment. The peak of activity was recorded at six hours after administration, followed by a decline by 12 and 18 hours. Shortly thereafter a second peak occurring at 24 hours was observed (as in the case also of the natural chrysotile). The level of radioactivity declined steadily over the next six days and then remained at a low and constant level for nearly three weeks subsequently. At the last point examined (56 days), the level of radioactivity had declined to the very low level of 3.5×10^5 cpm. (Table 16).

TABLE 15: RADIOACTIVITY REMAINING IN RAT LUNG AFTER TREATMENT WITH
NATURAL CHRYSOTILE ASBESTOS

Time After Treatment	Number of Samples	Mean CPM/Total Dry Weight Lung ($\times 10^5$)
3 Hours	4	6.7
6 Hours	4	10.9
12 Hours	2	9.4
1 Day	3	111.2
3 Days	2	8.4
5 Days	4	9.0
7 Days	4	10.3
8 Days	1	6.3
14 Days	1	15.8
21 Days	3	6.4
28 Days	2	4.4
37 Days	1	9.7
56 Days	2	35.8

TABLE 16: RADIOACTIVITY REMAINING IN RAT LUNG AFTER TREATMENT WITH SYNTHETIC CHRYSOTILE ASBESTOS

Time After Treatment	Number of Samples	Mean CPM/Total Dry Weight Lung ($\times 10^5$)
1 Hour	3	9.8
3 Hours	4	7.7
6 Hours	3	54.1
12 Hours	3	21.1
18 Hours	4	10.0
1 Day	3	69.0
3 Days	4	57.6
5 Days	4	40.1
7 Days	4	13.1
14 Days	4	12.2
21 Days	4	14.5
28 Days	4	29.7
56 Days	3	3.5

EXPERIMENT 5

The purpose of this experiment was to examine the reactivity of lung tissue of animals exposed to dust clouds of natural chrysotile asbestos, administered in the inhalation exposure environmental chamber.

Pilot Studies

To begin with, four rats were maintained in the chamber for a week without exposing them to any dust material. They were sacrificed at the end of that time span and their alveolar⁸ tissue was examined both morphologically and also cytodynamically by the colchicine technique. The percentage of metaphases of the alveolar tissue was 0.71 (Table 17). This value was somewhat lower than that of normal controls (Experiment 1), but was still within the normal range. Subsequently a group of 12 rats was placed into the chamber and exposed to dust clouds of chrysotile four hours per day. The rats were sacrificed in groups of four at one, two and three days after the initiation of the exposure. After a single day of exposure (four hours), the mitotic activity of the alveolar tissue had

TABLE 17: MITOTIC RATE OF RAT ALVEOLAR TISSUE AFTER CHAMBER EXPOSURE TO NATURAL CHRYSOTILE ASBESTOS

Time of Exposure; Days (4 hrs/day)	Number of Animals	Total Number of Nuclei	Metaphases	Percentage of Metaphases	Standard Deviation (
1	4	8000	21	0.26	0.22
2	4	8000	32	0.40	0.36
3	4	8000	43	0.54	0.22
<hr/>					
Untreated Control (7 Days in Chamber)	4	8000	57	0.71	0.16

declined to 0.26%. It began to increase above that level, attaining 0.40% by two days, and 0.54% by three days. Nothing unusual could be observed in the lung tissue of these animals. Asbestos fibres were not detected despite intense microscopic examination of the lung tissue.

Principal Study

This experimental series served two principal objectives. Firstly, it was intended to reveal the effects on the pulmonary tissue of a relatively short term intensive exposure of rats to asbestos dust clouds. This was followed up by both morphological and cytodynamic observations of both the alveolar tissue and tracheal epithelium. Secondly, it served as a comparison between the effects of asbestos dust on these parameters of a very short exposure and a long term exposure. More than 70 rats were exposed daily for a period of four hours in the environmental chamber to asbestos dust clouds. Rats were removed in groups of four from the chamber and sacrificed after one, three, five and seven days of such exposure. At the end of the first week of exposure, half of the remaining animals were transferred from the dusty environment and no longer exposed to the asbestos dust clouds. The remaining animals in the chamber continued to be exposed to the asbestos

laden air. Subsequently, matching groups of rats, one group exposed continually, the other exposed only for seven days to the asbestos, were sacrificed at 10, 12, 14, 21, 42 and 56 days after the commencement of the experiment.

Observations of the Living Animals

The rats appeared not visibly affected by the dust clouds of asbestos, apart from their frequent coughing and huddling together, seemingly in an attempt to avoid the irritating asbestos fibres suspended in the air.

Histological Observations

There appeared to be very little morphological difference between the lungs of animals exposed merely for seven days, and those exposed for two months. Asbestos fibres could not be detected by light microscopy within the lung tissue of any of the animals of the entire experiment. Sporadic thickenings of the epithelium of the airways was on occasion observed but this was not a general phenomenon. Also the peribronchiolar adventitial connective tissue was thickened particularly where peribronchilar

lymphatic tissue encircled larger bore airways. However, such is commonly observed in untreated animals as well and was therefore not considered a phenomenon specifically attributable to asbestos exposure. Tiny, discrete fibrotic nodules were observed at the level of the terminal bronchioles in the lungs of animals that had been exposed to the dust clouds for an extended time span (Figure 58). Such nodules could extend from the respiratory bronchiole into adjacent alveolar tissue and were the most conspicuous evidence of the exposure to asbestos. These regions exhibited likewise aggregations of free alveolar macrophages (Figures 59, 60). Nevertheless, the effect of the exposure to asbestos dust clouds in the alveolar tissue and airways was quite unremarkable even at the later stages of the experiment.

Cytodynamic Observations

Alveolar Tissue

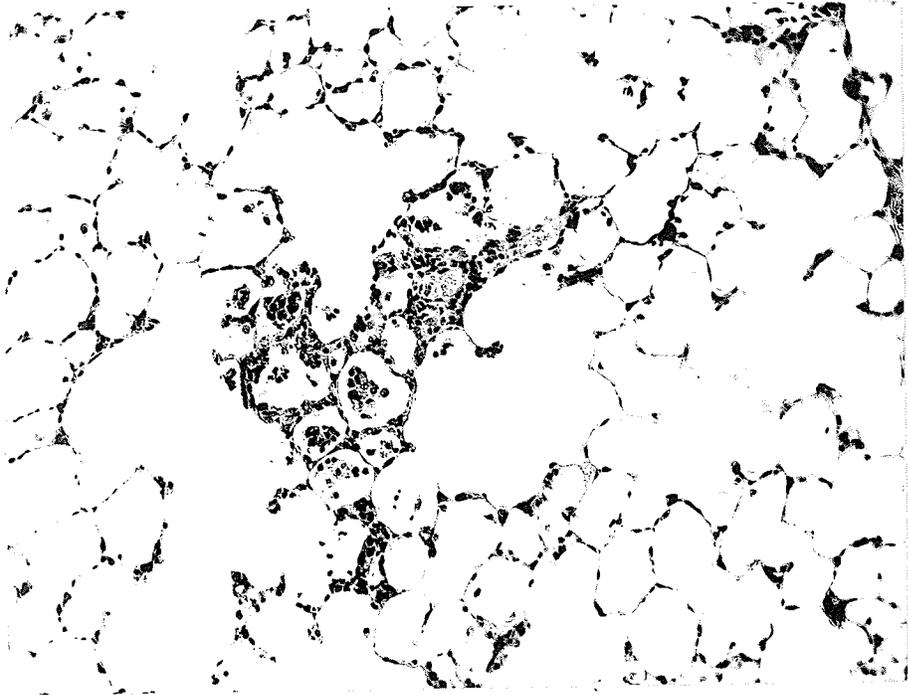
Overall Effect of the Treatments

At 10 days after the commencement of exposure, the mean six hour percentage of metaphases of the pooled data was 0.44%. This

FIGURE 58: Rat alveolar tissue of animal exposed to asbestos dust clouds in the environmental chamber for 21 days. Note small fibrotic area adjacent to the terminal bronchile at arrow. Photomicrograph 40 x.

FIGURE 59: Aggregation of alveolar macrophages in alveolar tissue. Twenty-one days of chamber exposure to asbestos. Photomicrograph 40 x.

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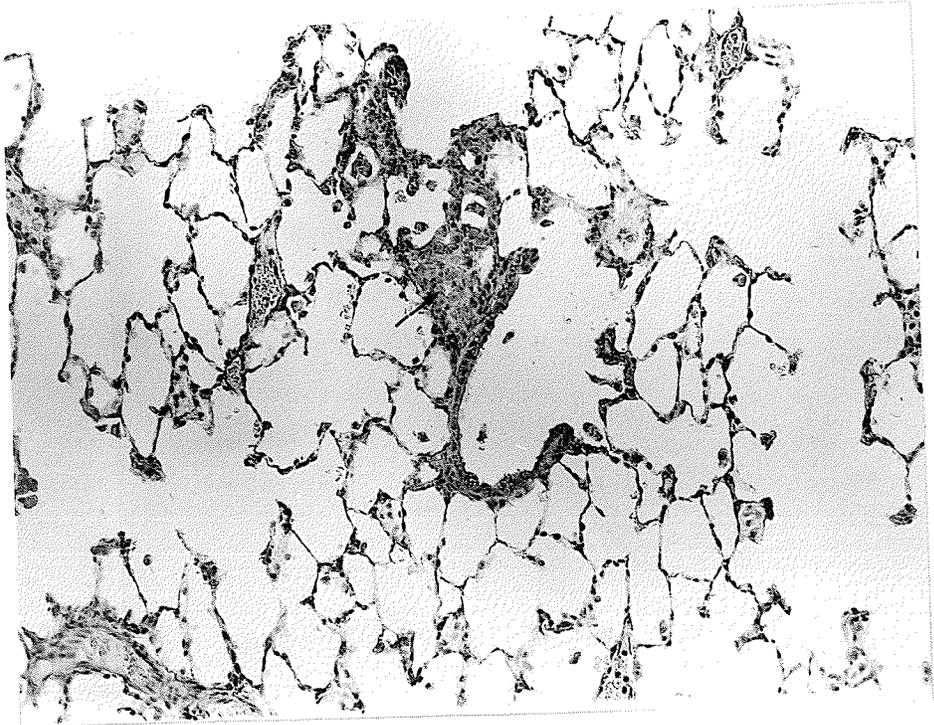
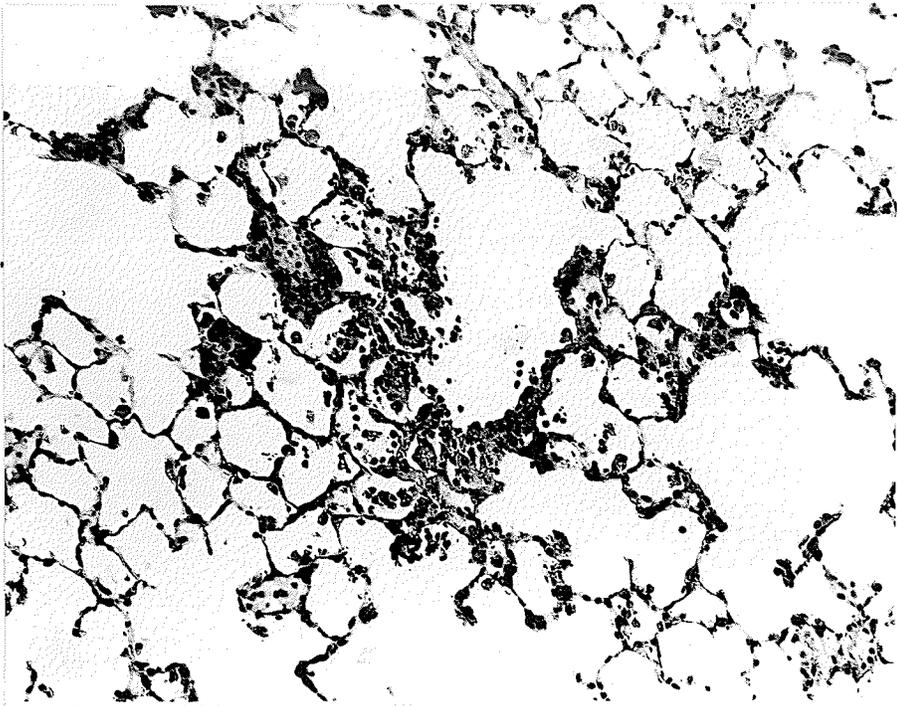


FIGURE 60: Alveolar tissue of rat after 12 days of inhalation exposure to asbestos. Some of the alveolar walls are thickened and fibrotic. Numerous macrophages are in the alveolar tissue.
Photomicrograph 40 x.



60

was considerably lower than the normal six hour mitotic rate (0.71%). This initial decline of the mitotic rate was gradually followed by an increase, achieving the maximum level at 14 days. That level of the percentage of metaphases (0.78%) was, however, also within the normal range. The six hour mitotic rate declined gradually, subsequently reaching the low value of 0.45% by 56 days. The general response was therefore an initial decline of the mitotic rate, followed by a transient increase in mitotic activity and a further decline at a later stage.

Short Term Exposure

Three days after the removal of these animals from the dust chamber, the percentage of metaphases of alveolar tissue was very low (0.39%), but it had returned to normal levels by one week (Table 18). Subsequently, the mitotic rate exhibited an aberrant course, decreasing at two weeks' exposure, rising again at three weeks and finally declining to a low value (0.27%) which was not significantly different from the rate of the long term exposed group at that point.

Long Term Exposure

The mitotic rate of the alveolar tissue of rats exposed for the longer time span was generally decreased over the experimental period, the mean value being 0.44%. At 14 days after the commencement of the exposure, however, the six hour percentage of metaphases reached 0.84. This was a transient increase inasmuch as the mitotic rate then declined gradually, the lowest value being reached at 28 days (Table 19). This trend of low mitotic activity continued up to the last point examined (56 days).

Comparison of Treatments

Statistical comparison between the two experimental groups indicated that the long term exposure series displayed a generally lower mitotic rate of the alveolar tissue than the normal control animals. The mitotic rate of the short term exposure group was not significantly different from either the normal or the long term exposure group, however, Figure 61 illustrates graphically the mitotic responses in the alveolar tissue of the two experimental groups as related to the duration of dust cloud exposure of the animals.

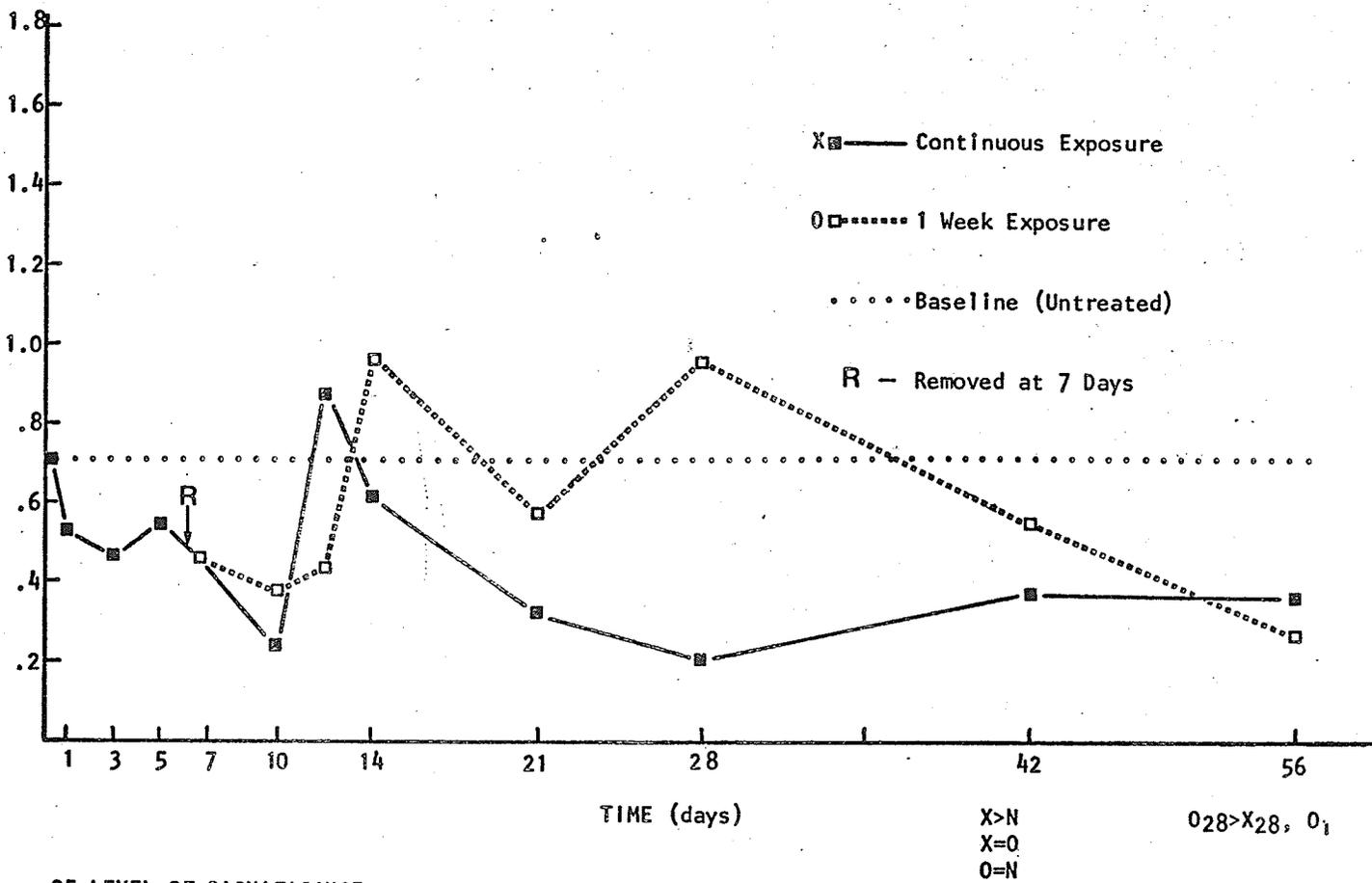
TABLE 18: MITOTIC RATE OF RAT ALVEOLAR TISSUE AFTER SHORT TERM INHALATION EXPOSURE TO NATURAL CHRYSOTILE ASBESTOS

Time After 1 wk. of Inhalation Ex- posure (4 hrs/day)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
3	4	8000	31	0.39	0.44
5	4	8000	35	0.44	0.29
7	4	8000	79	0.99	0.49
14	4	8000	44	0.55	0.36
21	4	8000	77	0.96	0.58
35	4	8000	44	0.55	0.16
49	4	8000	22	0.28	0.10

TABLE 19: MITOTIC RATE OF RAT ALVEOLAR TISSUE AFTER LONG TERM INHALATION EXPOSURE TO NATURAL CHRYSOTILE ASBESTOS

Time of Exposure; Days (4 hrs/day)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
1	4	8000	43	0.53	0.29
3	3	6000	46	0.77	0.20
5	4	8000	44	0.55	0.43
7	4	8000	37	0.46	0.47
10	4	8000	17	0.21	0.29
12	4	8000	67	0.84	0.37
14	4	8000	51	0.64	0.47
21	3	6000	27	0.45	0.56
28	4	8000	17	0.21	0.23
42	4	8000	31	0.39	0.61
56	4	8000	30	0.38	0.19

FIGURE 61: MITOTIC RATES OF RAT ALVEOLAR TISSUE AFTER CHAMBER EXPOSURE TO NATURAL CHRYSOTILE



Tracheal Epithelium

Overall Effect of the Treatments

The epithelium of the trachea revealed a mitotic response that was quite similar in pattern to that of the alveolar tissue. At 10 days after the commencement of exposure, the six hour percentage of metaphases was 0.18. This was reduced from the untreated control value of 0.29%, but still not significantly different. The highest level of mitotic activity of the tracheal epithelium occurred at 14 days (0.96%). The mitotic rate subsequently declined, and at 21 days was 0.21%. It remained low, but within the normal range throughout the remainder of the experiment.

Short Term Exposure

Three days after the removal of the animals from the dusty environment, their tracheal epithelium exhibited merely a mitotic rate of 0.14%. The mitotic rate of this epithelium gradually increased, until at seven days after removal a value of 1.59% was attained, this being significantly greater than that of the

untreated control. A declining trend became apparent thereafter at 14 days post-removal (0.17%). This low trend was maintained for the duration of the experiment but was not significantly different from the untreated control values (Table 20).

Long Term Exposure

The group exposed to asbestos dust clouds for two months displayed essentially the same mitotic response as those animals subjected to the dust for merely a brief period. At seven days from the commencement of treatment the mitotic rate was 0.13%. A level of 1.0% of metaphases was then reached by 14 days. As in the former group, after that time the mitotic rate assumed a declining trend (Table 21).

Comparison of the Treatments

As is represented graphically in Figure 62, the mitotic responses of the two groups of animals exposed to dust clouds of chrysotile were very similar. In fact, statistical analysis revealed that significant differences did not exist between the mean mitotic rates of the tracheal epithelium of these two groups.

TABLE 20: MITOTIC RATE OF RAT TRACHEAL EPITHELIUM AFTER SHORT TERM INHALATION EXPOSURE TO NATURAL CHRYSOTILE ASBESTOS

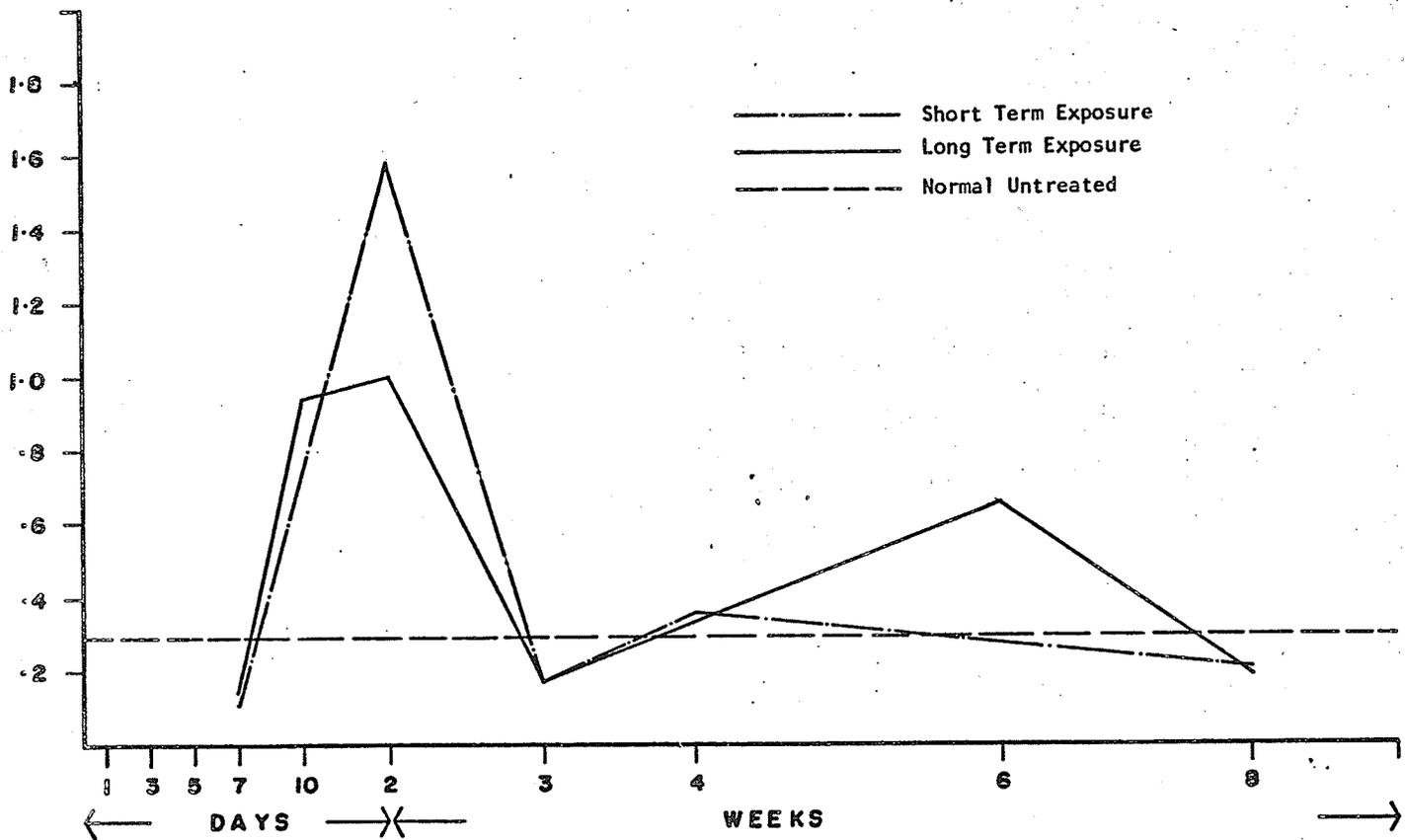
Time After 1 wk. of Inhalation Ex- posure (4 hrs/day)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
3	4	8000	11	0.14	0.05
5	4	8000	59	0.74	1.05
7	4	8000	127	1.59	0.67
14	3	6000	10	0.17	0.10
21	4	8000	28	0.35	0.28
35	4	8000	22	0.28	0.12
49	4	8000	16	0.20	0.27

TABLE 21: MITOTIC RATE OF RAT TRACHEAL EPITHELIUM AFTER LONG TERM INHALATION EXPOSURE TO NATURAL CHRYSOTILE ASBESTOS

Time of Exposure; Days (4 hrs/day)	Number of Animals	Total Number of Nuclei	Number of Metaphases	Percentage of Metaphases	Standard Deviation (\pm)
10	3	6000	8	0.13	0.15
12	4	8000	75	0.94	1.24
14	3	6000	60	1.00	0.68
21	4	8000	14	0.18	0.16
28	3	6000	20	0.33	0.41
42	4	8000	52	0.65	0.66
56	3	6000	11	0.18	0.06

Normal Untreated Animals	4	8000	23	0.29	0.18
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FIGURE 62: MITOTIC RATES OF RAT TRACHEAL EPITHELIUM AFTER CHAMBER EXPOSURE TO NATURAL CHRYSOTILE



Weight Consideration

A significant loss of weight of the experimentally treated animals was not observed during the course of the experiment. The body weights of the animals during the experiment are listed in Tables 22 and 23.

TABLE 22: WEIGHT ALTERATIONS OF RATS EXPOSED FOR SHORT TERM (7 DAYS)
TO ASBESTOS DUST CLOUDS

<u>Time After Exposure (Days)</u>	<u>Body Weight of Animals</u>	<u>Mean Body Weight</u>
3	260 290 295 290	283
5	270 285 275 275	276
7	290 320 315 300	306
14	310 335 345 320	327
21	340 350 345 330	341
35	375 375 430	393
49	400 360 390 410	390

TABLE 23: WEIGHT ALTERATION OF RATS EXPOSED FOR LONG TERM TO
CHRYSTILE ASBESTOS DUST CLOUDS

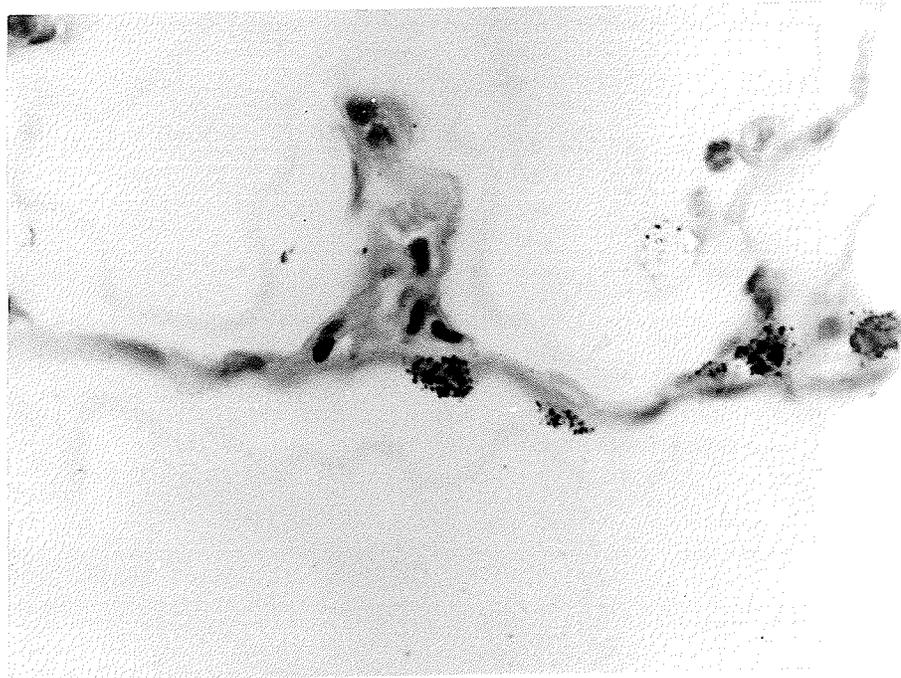
Time of Exposure (Days)	Body Weight of Animals	Mean Body Weight
10	240	253
	265	
	280	
	230	
12	275	267
	280	
	275	
	240	
14	320	302
	310	
	290	
	290	
21	340	315
	320	
	310	
	290	
28	330	335
	325	
	340	
	345	
42	360	378
	395	
	380	
	380	
56	370	400
	410	
	400	
	420	

EXPERIMENT 6

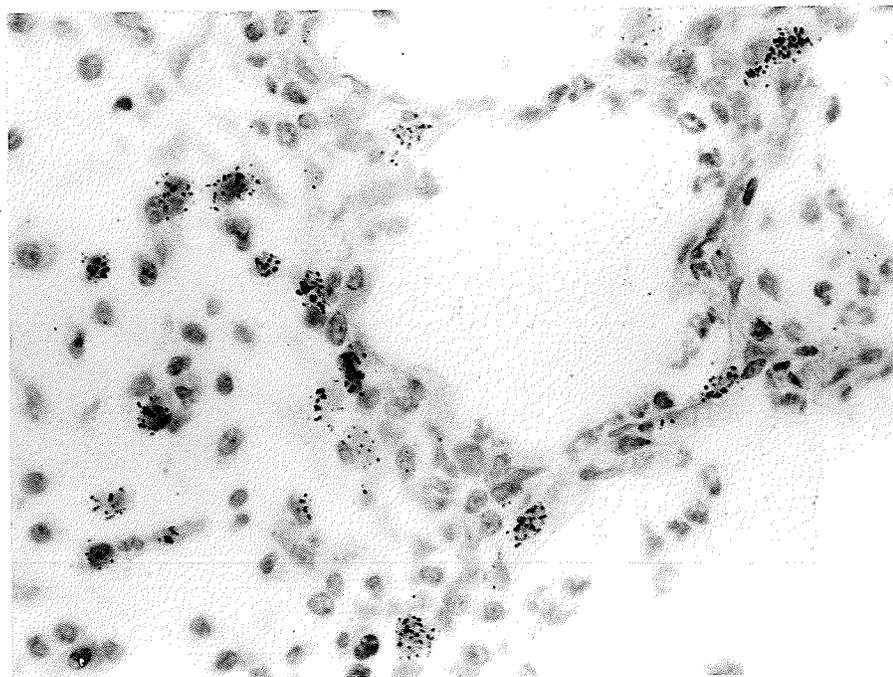
The purpose of this experiment was to examine the cytodynamic behavior of the alveolar tissue and pleural mesothelium of rat lung following intratracheally imposed chrysotile asbestos both natural and synthetic. In this series, the tritiated thymidine technique was employed for accumulating cytodynamic data. The animals were treated as previously described; three separate groups receiving intratracheal injections of saline, synthetic, or natural chrysotile asbestos. The only deviation from Experiment 1 was that 0.75 ml. of pure saline or a saline suspension of the dusts (3.5 mgm./cc.) was administered. The experimental series were divided into two sections. Firstly, groups of treated animals were given a pulse label of tritiated thymidine and sacrificed one hour after the administration of the tracer. The times of sacrifice were five and seven days after the treatment with the various substances. Secondly, groups of similarly treated animals were administered an identical dose of tritiated thymidine concurrent with the administration of the saline and saline suspensions of asbestos but were allowed to live for seven and 14 days. The morphological observations noted in the lungs of the two groups of animals were essentially identical to those reported in Experiment 1. Examples of the thymidine labelling of the pleural mesothelium and the alveolar tissue are shown in Figures 63 and 64.

FIGURE 63: Pleural mesothelium of rat lung seven days after intratracheal injection of natural chrysotile. Note the labelled mesothelial cells. Radioautograph 160 x.

FIGURE 64: Alveolar tissue of rat lung seven days after intratracheal administration with natural chrysotile. Numerous labelled alveolar macrophages are visible. Note the large number of labelled cells in the connective tissue. Radioautograph 160 x.



63



64

Pulse Labelled Series

Alveolar Tissue

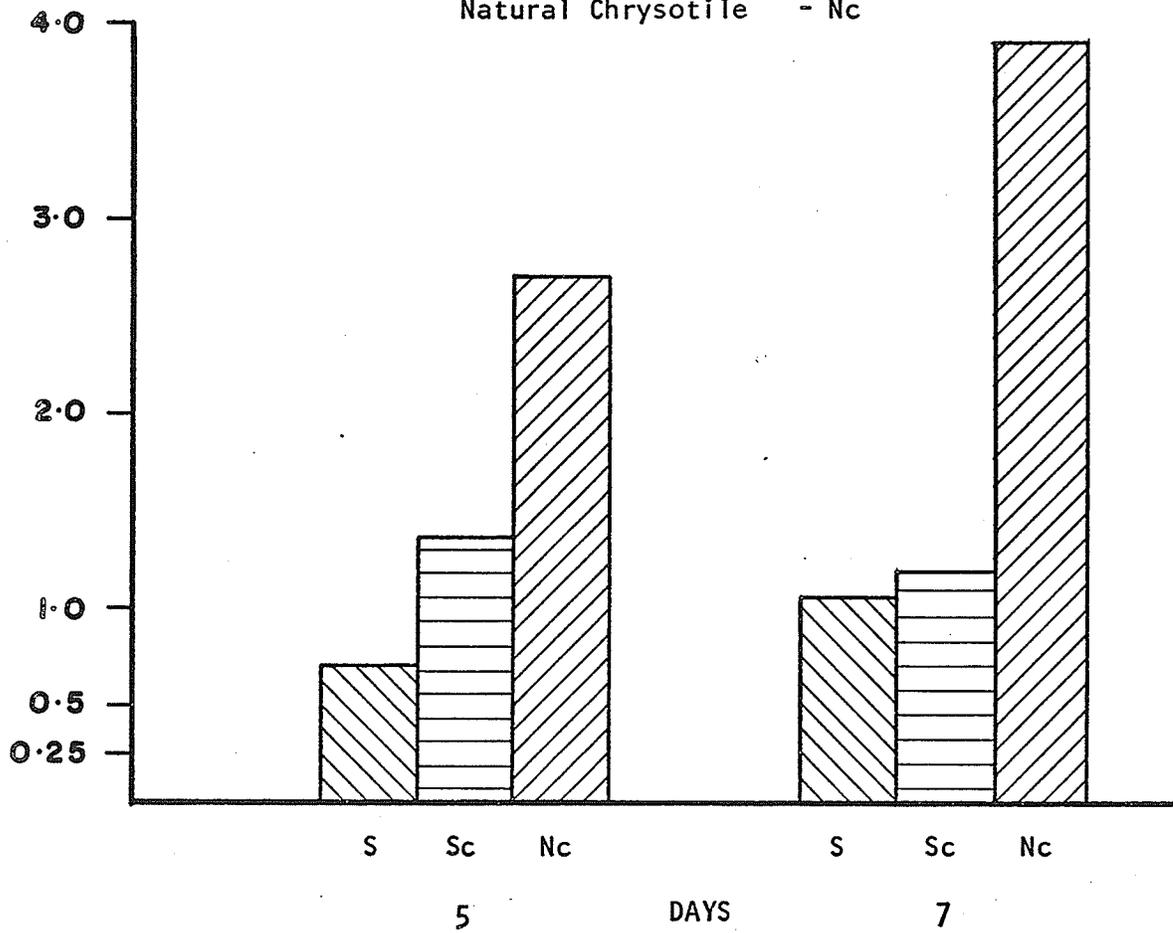
At five days after the intratracheal injection of the respective materials, it was evident that the labelling indices of alveolar tissue of those animals treated with the two forms of chrysotile were significantly higher than those in the saline group (Table 24). Further, the group treated with natural chrysotile displayed a significantly higher labelling index (2.7%) in alveolar tissue than did the animals injected with the synthetic chrysotile (1.33%). By seven days, the labelling index of the natural chrysotile treated group had increased to 3.5%, a level that was significantly greater than that after five days. As at five days, the natural chrysotile group exhibited a statistically greater labelling index than the other two groups. Although the labelling indices of the alveolar tissue of the saline and synthetic chrysotile groups became augmented at seven days, the values attained were not statistically different from those at five days. At that time (seven days) the synthetic chrysotile treated group did not exhibit a significantly higher labelling index in alveolar tissue than the saline treated animals. The cytodynamic observations ascertained in this portion of the experiment are presented in Figure 65.

TABLE 24: THYMIDINE LABELLING OF RAT ALVEOLAR TISSUE ONE HOUR AFTER PULSE LABEL OF TRITIATED THYMIDINE

Treatment	Time After Treatment (Days)	Number of Animals	Number of Cells	Number Labelled	Percentage Labelled	Standard Deviation (\pm)
Saline	5	4	4000	28	0.70	0.141
	7	4	4000	41	1.03	0.189
Synthetic Chrysotile	5	4	4000	53	1.33	0.40
	7	4	4000	47	1.18	0.74
Natural Chrysotile	5	3	3000	81	2.70	1.30
	7	4	4000	156	3.90	0.96

FIGURE 65: MEAN LABELLING INDICES OF RAT ALVEOLAR TISSUE AFTER INTRATRACHEAL INJECTION TREATMENT WITH

Saline - S
Synthetic Chrysotile - Sc
Natural Chrysotile - Nc



Pleural Mesothelium

The mesothelium of the visceral pleura of natural chrysotile treated animals displayed a labelling index of 2.0% at five days after the intratracheal injections. This labelling index was significantly higher than both those of that epithelium in the saline and synthetic chrysotile treated groups. The labelling indices of the pleural mesothelium of the latter two groups were not significantly different from one another at that time (Figure 66). As well, on the seventh day of the experiment, significant differences in labelling indices did not exist between these two groups. In contrast, the natural chrysotile treated group exhibited an even higher labelling index of the pleural mesothelium at that time; as before, it was significantly higher than that of the former groups. That labelling index of 3.18% was significantly greater than that attained also by the natural chrysotile group at five days. The values of labelling index reached by the other two groups at this time did not differ significantly from those attained by five days (Table 25).

FIGURE 66: MEAN LABELLING INDICES OF RAT PLEURAL MESOTHELIUM AFTER INTRATRACHEAL INJECTION TREATMENT WITH

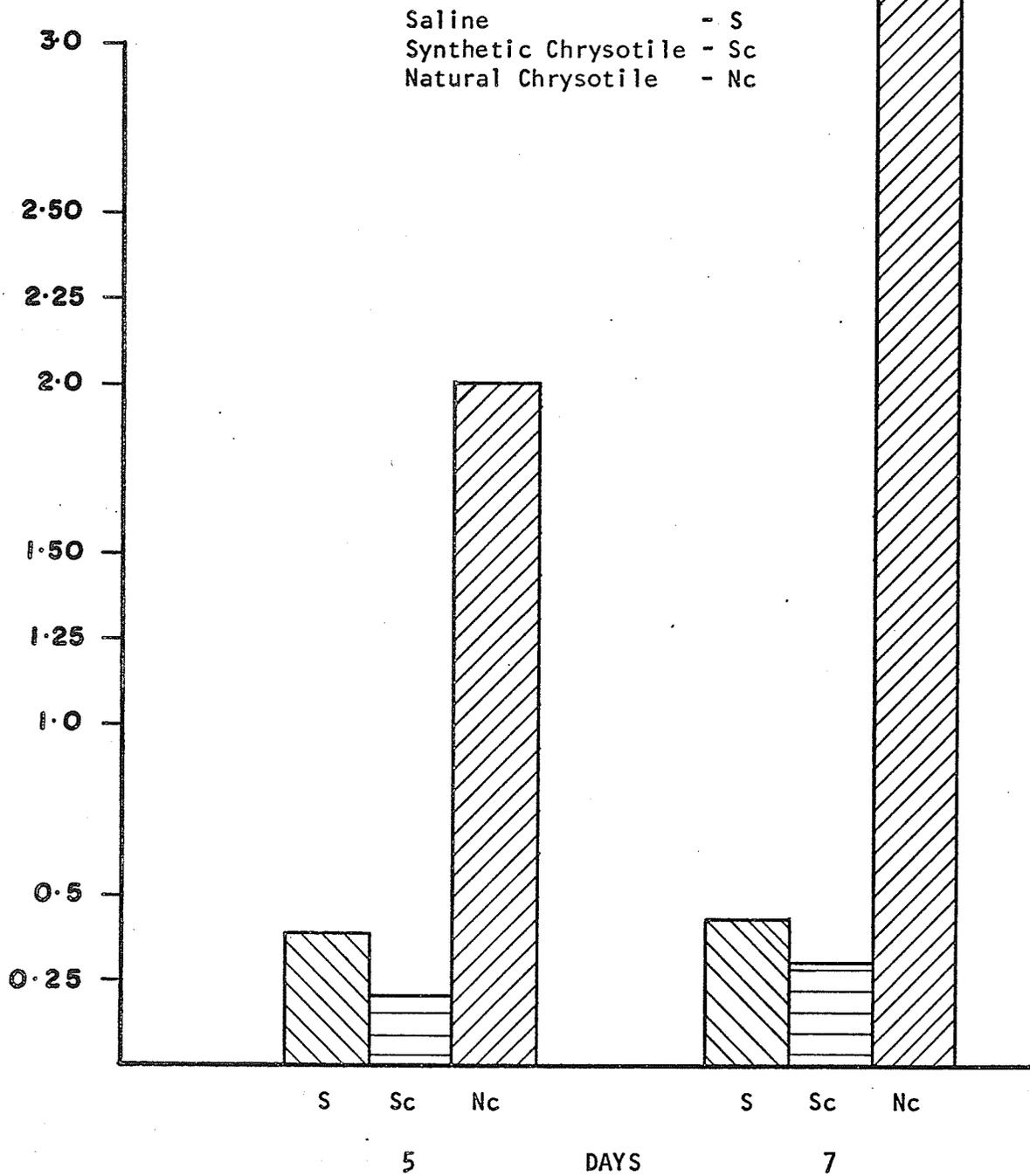


TABLE 25: THYMIDINE LABELLING OF RAT PLEURAL MESOTHELIUM ONE HOUR AFTER PULSE LABEL OF TRITIATED THYMIDINE

Treatment	Time After Treatment (Days)	Number of Animals	Number of Cells	Number Labelled	Percentage Labelled	Standard Deviation (\pm)
Saline	5	4	4000	15	0.38	0.28
	7	4	4000	17	0.43	0.25
Synthetic Chrysotile	5	4	4000	8	0.20	0.08
	7	4	4000	12	0.30	0.21
Natural Chrysotile	5	4	4000	80	2.00	0.88
	7	4	4000	127	3.18	1.15

Series Labelled for One and Two Weeks

Alveolar Tissue

Seven days after an injection of the tritiated thymidine, significant differences between the labelling indices of the alveolar tissue could be demonstrated only between the saline and synthetic chrysotile administered groups (Figure 67). A difference of statistical significance did not exist between the natural chrysotile treated group and the other groups at that time. However, at 14 days after administration, the labelling index in alveolar tissue of the saline treated group was significantly greater than the indices reached by the other groups (Table 26). These latter groups did not differ significantly in this parameter at that time. Therefore, the general trend signified that the saline treated group exhibited a higher labelling index than the other two treated groups.

Pleural Mesothelium

A situation similar to that displayed by alveolar tissue was

FIGURE 67: MEAN LABELLING INDICES OF RAT ALVEOLAR TISSUE 7 AND 14 DAYS INJECTION OF TRITIATED THYMIDINE

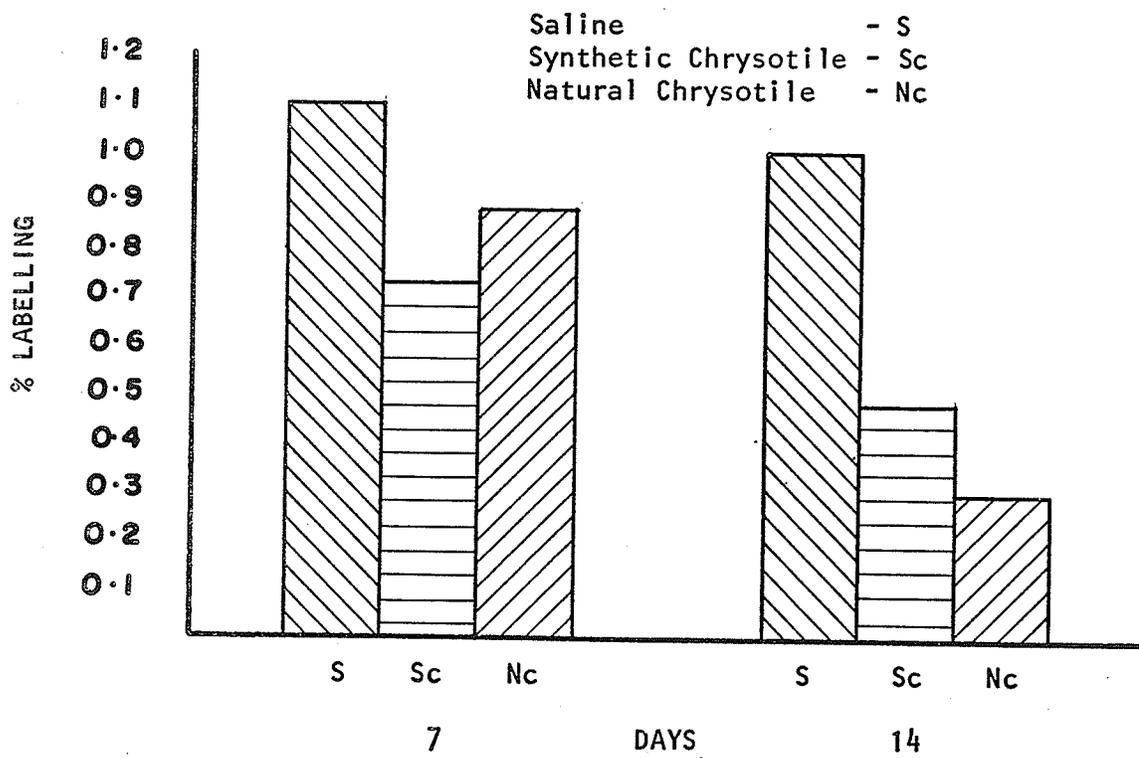


TABLE 26: LABELLING INDICES OF RAT ALVEOLAR TISSUE 7 AND 14 DAYS AFTER INJECTION OF TRITIATED THYMIDINE

Treatment	Time After Treatment (Days)	Number of Animals	Number of Cells	Number Labelled	Percentage Labelled	Standard Deviation (\pm)
Saline	7	3	3000	33	1.10	0.20
	14	4	4000	40	1.00	0.67
Synthetic Chrysotile	7	4	4000	29	0.73	0.22
	14	4	4000	19	0.48	0.32
Natural Chrysotile	7	4	4000	35	0.88	0.38
	14	4	4000	12	0.30	0.36

revealed by the visceral pleural mesothelium in this series. Although the labelling index of the mesothelium of the natural chrysotile treated animals was slightly higher at seven days than those shown by the other treated groups, this difference was not of statistical significance. Thus, after one week, the labelling indices of the three groups did not differ statistically. At 14 days after treatment, however, the saline treated group exhibited a significantly higher labelling index of the mesothelium (1.0%) than the other groups. These latter groups did not differ significantly at that time. As is shown in Table 27, the difference between the saline group and the two chrysotile groups was dependent largely on a decrease of the labelling indices of the latter groups at 14 days from higher values at seven days. In contrast, the labelling index of the mesothelium in the saline treated group increased during the same time interval, although not significantly. The findings of this series are shown graphically in Figure 68.

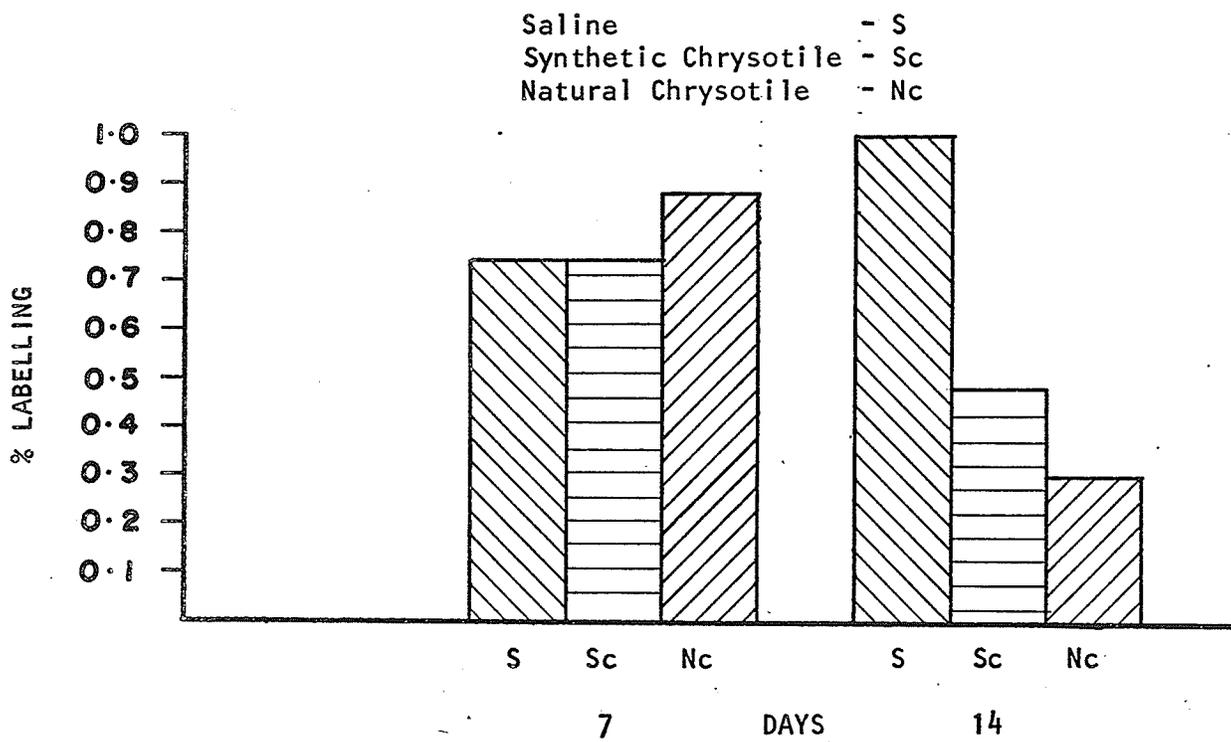
Weight Consideration

As with the previous experimental series, the weights of the animals were routinely recorded. The natural chrysotile treated animals exhibited also in this series the most severe losses of body weight during the course of the experiment.

TABLE 27: LABELLING INDICES OF RAT PLEURAL MESOTHELIUM 7 AND 14 DAYS AFTER INJECTION OF TRITIATED THYMIDINE

Treatment	Time After Treatment (Days)	Number of Animals	Number of Cells	Number Labelled	Percentage Labelled	Standard Deviation (\pm)
Saline	7	4	4000	29	0.73	0.57
	14	4	4000	40	1.00	0.67
Synthetic Chrysotile	7	4	4000	29	0.73	0.22
	14	4	4000	19	0.48	0.32
Natural Chrysotile	7	4	4000	35	0.88	0.38
	14	4	4000	12	0.30	0.36

FIGURE 68: MEAN LABELLING INDICES OF RAT PLEURAL MESOTHELIUM 7 AND 14 DAYS AFTER INJECTION OF TRITIATED THYMIDINE



ELECTRON MICROSCOPE OBSERVATIONS

Representative sections of alveolar tissue from the lungs of rats exposed to natural chrysotile administered either by inhalation or intratracheal instillation, were examined with the electron microscope. Alveolar tissue of animals treated with other dusts was not studied thusly.

Intratracheal Injection Series

Numerous alveolar macrophages, both free in the alveolar spaces and also located superficially on the alveolar wall, were observed in the alveolar tissue of rats that had been intratracheally injected with natural chrysotile. This was true in the alveolar tissue both in the early and also the later stages after treatment. It was evident at 28 days after treatment (the latest time interval from which tissues were examined) that numerous alveolar macrophages laden with asbestos were present in the alveolar tissue. The alveolar macrophages often appeared to have been engaged in phagocytosing asbestos fibres at the time of fixation inasmuch as pseudopods were frequently discerned surrounding the fibres of chrysotile (Figure 69). In addition to the numerous asbestos fibres within the macrophages,

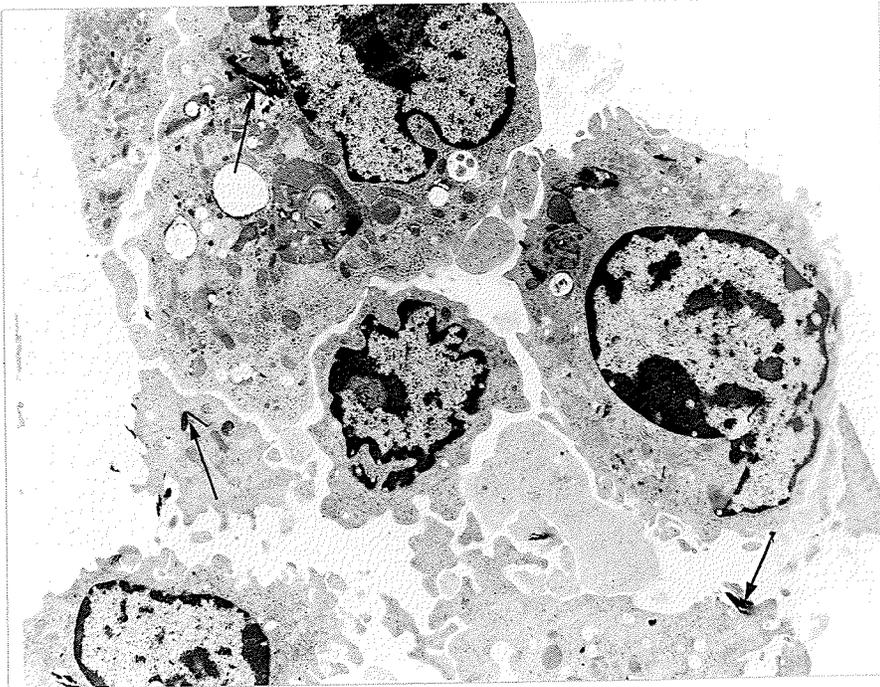
osmiophilic lamellated bodies were also observed within these cells (Figures 70, 71). Moreover, groups of macrophages apparently in the process of congregation were frequently observed (Figures 69, 72). Such congregation was more than a simple collection of cells inasmuch as the occasional desmosome was even noted between adjacent macrophages (Figure 72).

On occasion, an area of the alveolar tissue was visualized in which numerous alveolar macrophages, containing ingested asbestos fibres were situated, yet it was difficult to clearly discern the structure of this area. A great deal of cellular debris was noted in which the macrophages and asbestos fibres were embedded (Figure 73). In general, however, the alveolar tissue observed retained an essentially normal appearance. Metaphases arrested by colchicine were only rarely encountered. An example of a septal cell arrested metaphase is shown in Figure 74. Asbestos fibres were sometimes observed within cells located in the alveolar walls, present in the cytoplasm of surface epithelial or septal cells (Figures 75, 76). Such observations were rare, however.

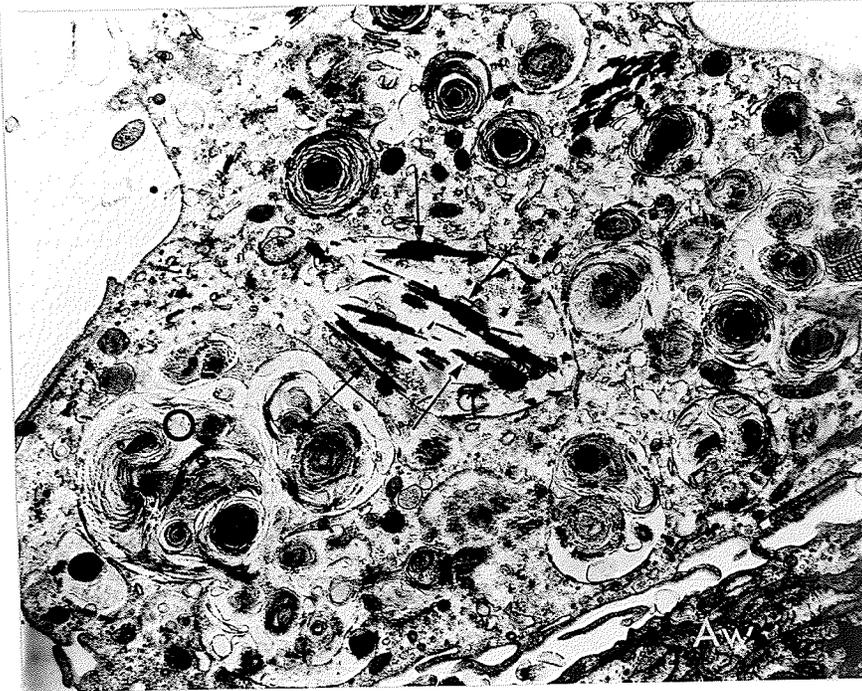
The fibres of chrysotile within free alveolar macrophages varied greatly in length; all were of ultramicroscopic dimensions, however. They were usually present as small fibre bundles composed of as few as two or so many fibres as to be practically innumerable.

FIGURE 69: Groups of free alveolar macrophages within the alveolar space. Note the numerous fibres of chrysotile at arrows. The phagocytosis of fibres can be seen at dark arrows. The macrophages appear to be congregating. Rat alveolar tissue seven days after intratracheal injection of chrysotile.
Electron micrograph 3,876 x.

FIGURE 70: Alveolar macrophage on portion of alveolar wall (AW). Note numerous osmiophilic lamellated bodies (Os) within phagosomes. In addition many chrysotile fibres can be seen at arrows.
Rat alveolar tissue five days after intratracheal injection of natural chrysotile.
Electron micrograph 6,780 x.



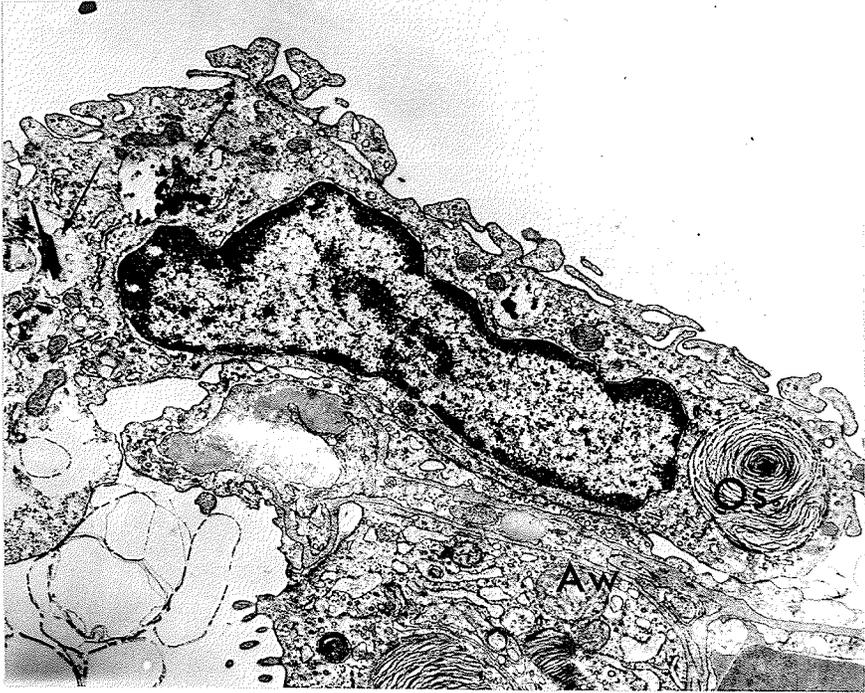
69



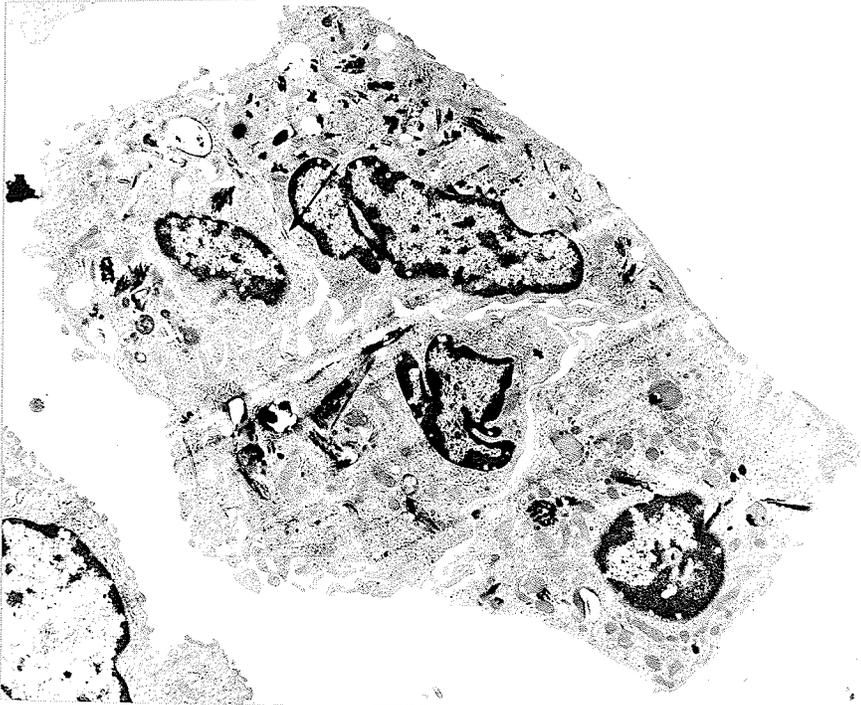
70

FIGURE 71: Alveolar macrophage in movement along alveolar wall (AW).
Note phagosome of osmiophilic lamellated body at (Os).
Also chrysotile fibres in phagosomes at arrows.
Fibres at extreme left of cell are in process of being
phagocytosed.
Seven days after intratracheal injection of natural
chrysotile.
Electron micrograph 6,840 x.

FIGURE 72: Four alveolar macrophages in congregation.
Note numerous chrysotile fibres. Note also desmosome
at arrow.
Fourteen days after intratracheal injection of
natural chrysotile.
Electron micrograph 3,876 x.



71



72

FIGURE 73: Area of lung tissue largely consolidated. Note the many chrysotile fibres.

Twenty-eight days after intratracheal injection of natural chrysotile.

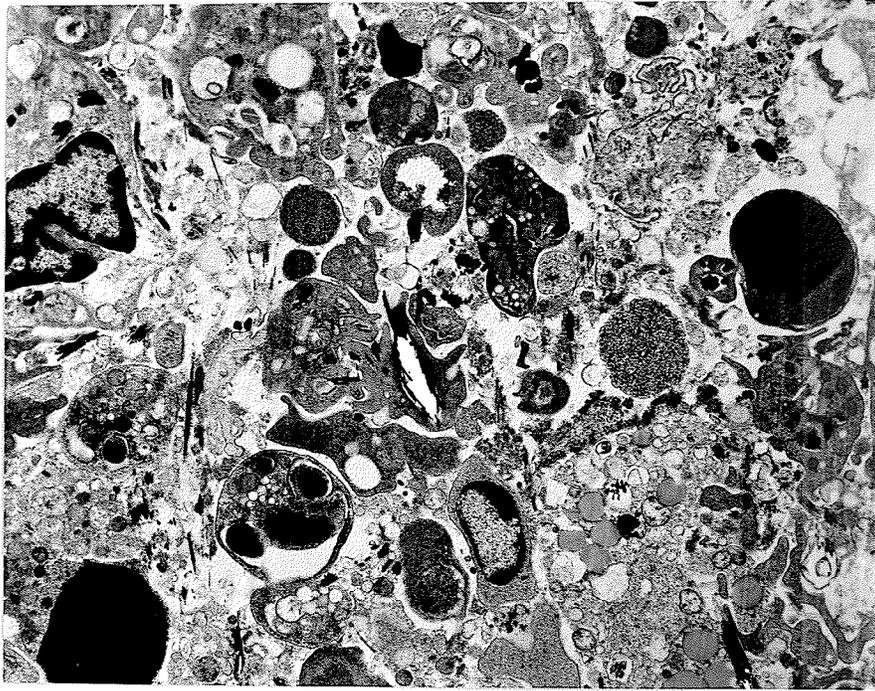
Electron micrograph - Magnification unavailable.

FIGURE 74: Colchicine arrested metaphase of septal cell.

Note traces of osmiophilic lamellated bodies (Os) largely dissolved by fixative.

Twenty-one days after intratracheal injection of natural chrysotile.

Electron micrograph 7,100 x.



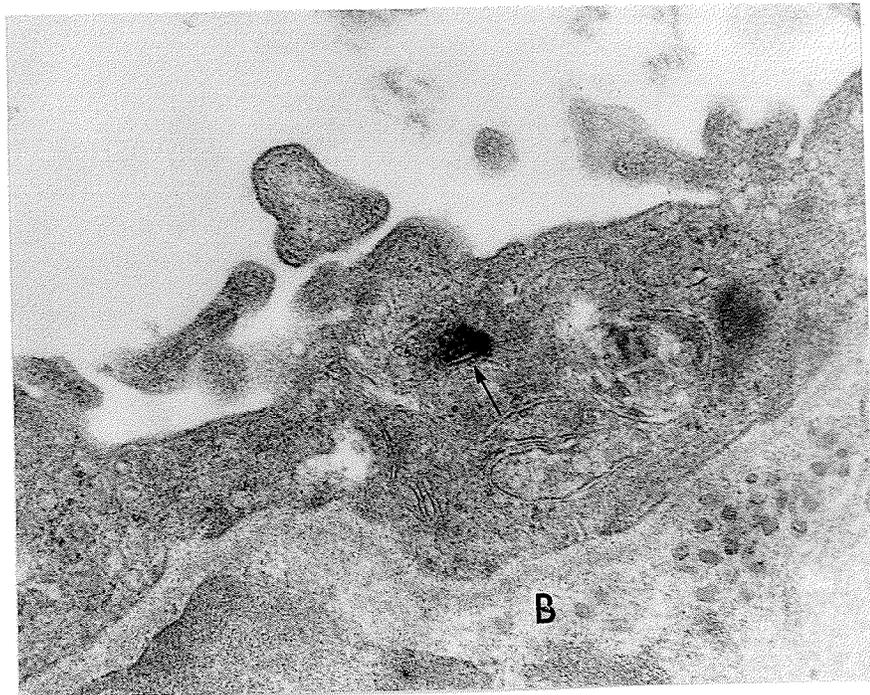
73



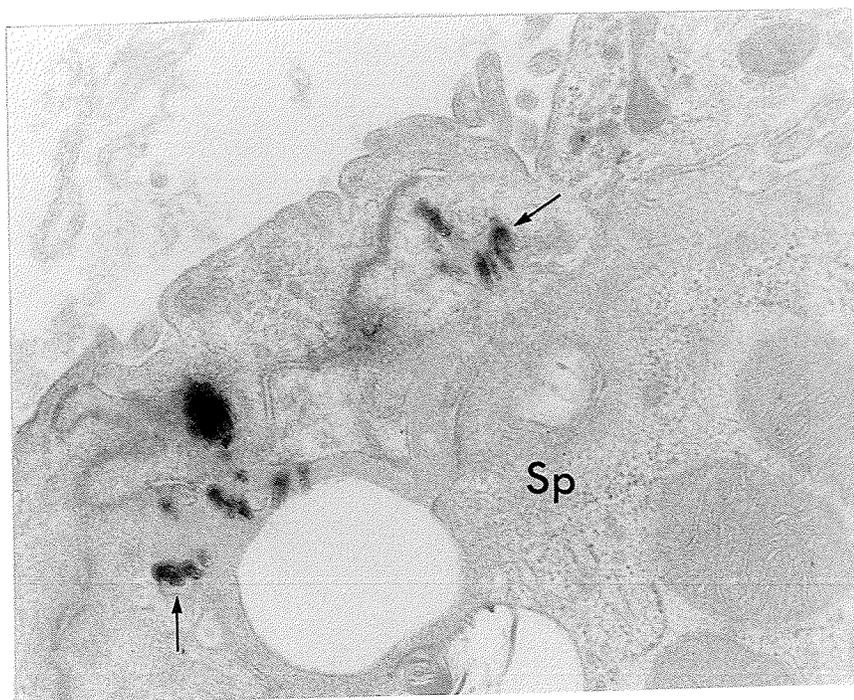
74

FIGURE 75: Asbestos fibres within pulmonary surface epithelium (PSE) at arrow. Note basement membrane at B. Seven days after intratracheal injection of natural chrysotile. Electron micrograph 25,080 x.

FIGURE 76: Asbestos fibres within septal cell (Sp) and PSE cell. Seven days after intratracheal injection of natural chrysotile. Electron micrograph 20,520 x.



75



76

Single fibres of chrysotile were observed infrequently. A portion of an alveolar macrophage containing both a single fibre and also a fibre bundle of chrysotile is illustrated in Figure 77. The chrysotile fibres within alveolar macrophages were located both free in the cytoplasm or contained within lysosomes (Figures 78, 79). In the former instance, lysosomes were often situated in close proximity to the fibres (Figure 80). The cytoplasm of the macrophages appeared especially electron dense in those regions surrounding and containing the ingested fibres of chrysotile (Figure 81). In addition, tiny blebs of unidentifiable material occurred on the surfaces and ends of the ingested fibres (Figures 77, 82). Lysosomes within the same alveolar macrophage were sometimes noted to contain either asbestos fibres or osmiophilic lamellated bodies (Figure 83).

The peribronchiolar connective tissue was likewise examined. Figure 84 illustrates a portion of peribronchiolar connective tissue of a terminal bronchiole. An alveolar macrophage virtually engorged with asbestos fibres is evident. None of the fibres or fibre bundles in that cell are contained within lysosomes. The cell exhibits numerous large vacuoles and appears to be in a catabolic condition.

The structure of the typical asbestotic nodules was also studied electron microscopically. In the centre of such nodules were usually numerous alveolar macrophages containing large amounts of

FIGURE 77: Portion of alveolar macrophage.

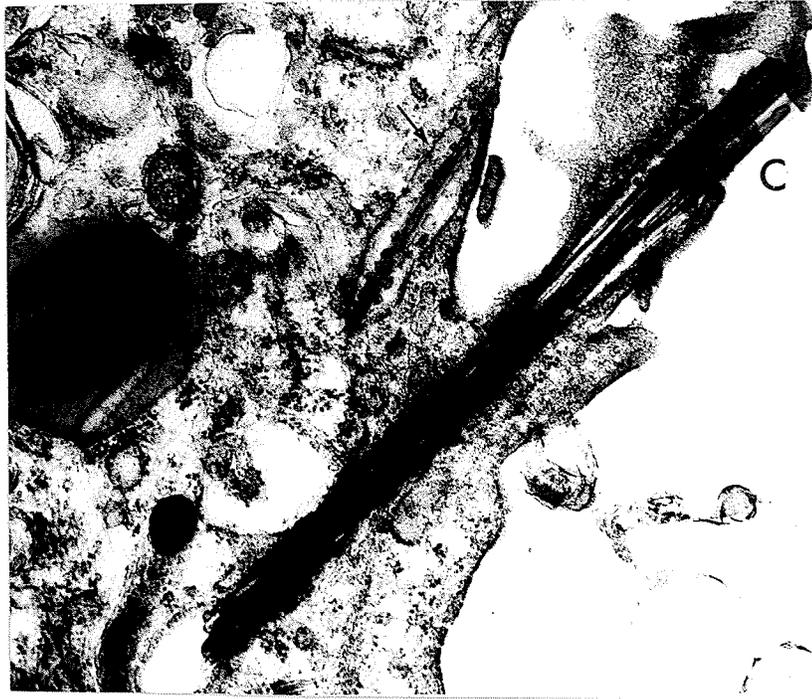
Partially phagocytosed bundle of chrysotile asbestos fibres at C. Note also single fibre of chrysotile at arrow, with small blebs of material along the fibre. Rat alveolar tissue three days after intratracheal injection of chrysotile.

Electron micrograph 20,520 x.

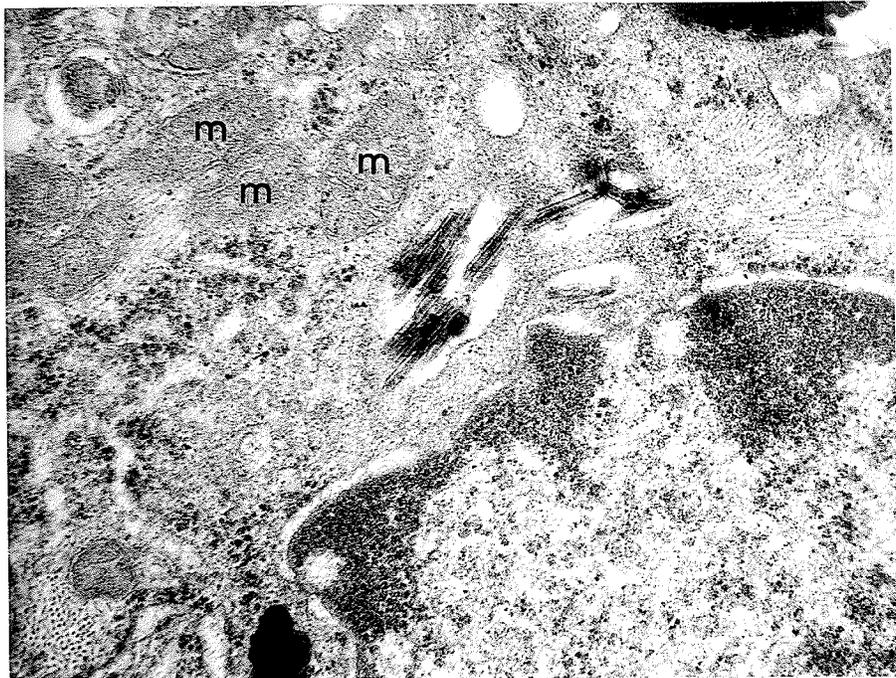
FIGURE 78: Fibres of chrysotile within phagosomes of alveolar macrophage. Note numerous mitochondria (m).

Rat alveolar tissue five days after treatment.

Electron micrograph - Magnification unavailable.



77



78

FIGURE 79: Asbestos fibres within lysosomal structure.

Rat alveolar tissue 21 days after intratracheal
injection of chrysotile.

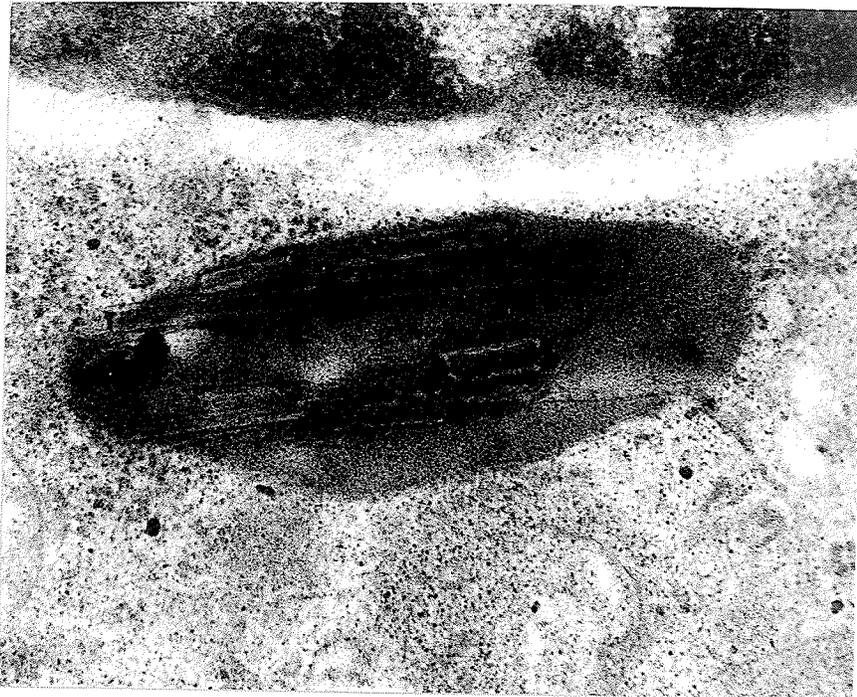
Electron micrograph 31,920 x.

FIGURE 80: Asbestos fibres within cytoplasm of alveolar macrophage.

Note lysosomal structure at L.

Rat alveolar tissue 28 days after intratracheal
injection of chrysotile.

Electron micrograph 39,900 x.



79



80

FIGURE 81: Asbestos fibres in the cytoplasm of alveolar macrophage.

Note osmiophilic lamellated body at Os.

Blebs of unknown material (X) are located at the end of the fibres.

Rat alveolar tissue three days after intratracheal injection of chrysotile.

Electron micrograph 25,080 x.

FIGURE 82: Alveolar macrophage within centre of fibrotic nodule in

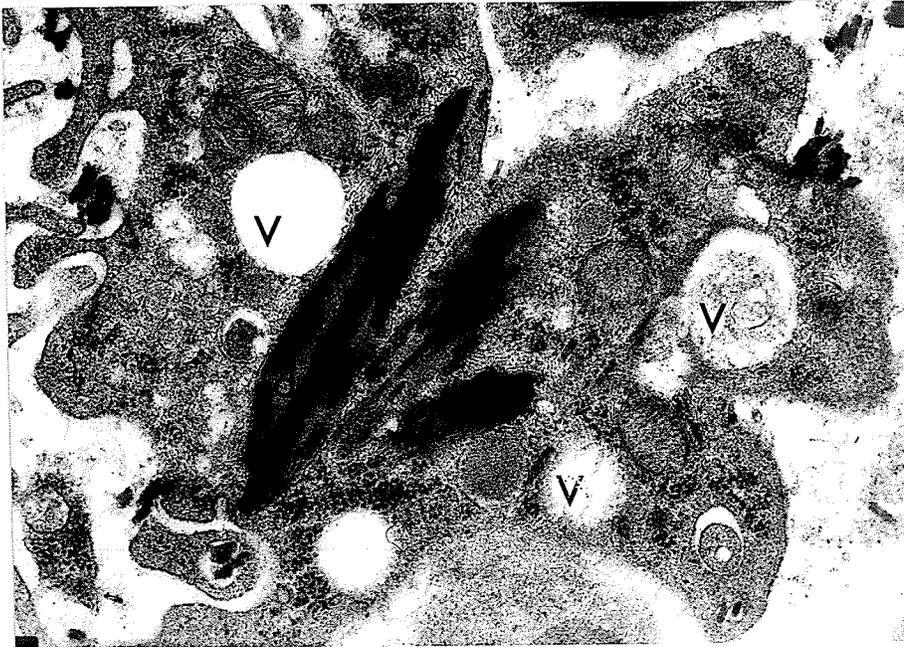
alveolar tissue. Note dense material between fibres at (d). In addition many vacuolar structures can be seen at (V).

Rat alveolar tissue 14 days after intratracheal injection of chrysotile.

Electron micrograph - Magnification unavailable.



81



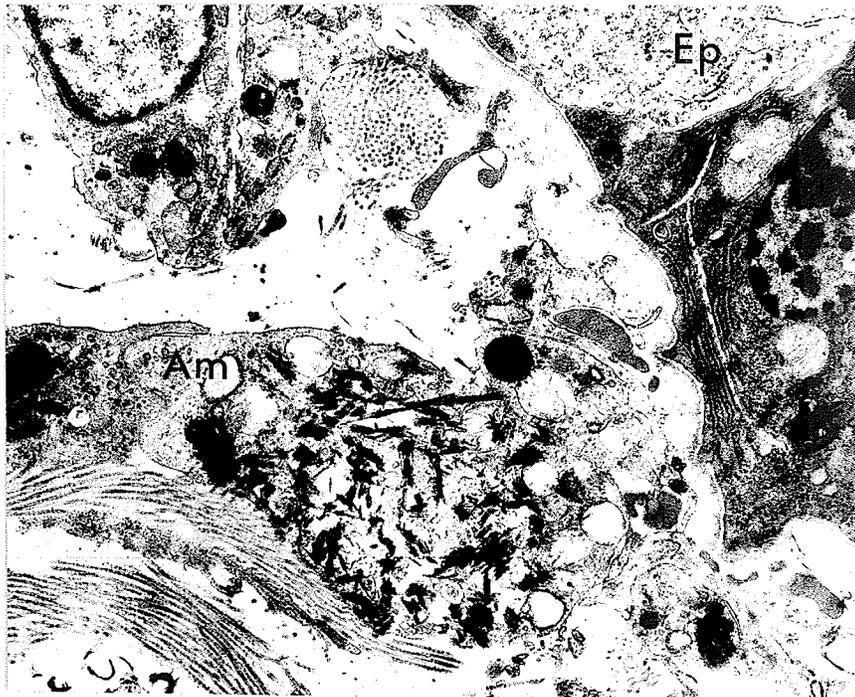
82

FIGURE 83: Lysosomal structures within alveolar macrophage.
Note osmiophilic lamellated bodies at Os and asbestos
fibres at arrow.
Rat alveolar tissue five days after intratracheal
injection of chrysotile.
Electron micrograph 20,520 x.

FIGURE 84: Alveolar macrophage (Am) laden with chrysotile
fibres within peribronchiolar connective tissue.
Epithelium of bronchiole at Ep.
Rat lung tissue seven days after intratracheal
injection of chrysotile.
Electron micrograph 6,840 x.



83



84

ingested asbestos fibres (Figures 85, 86). The asbestos fibres within the alveolar macrophages were not always arranged in true fibre bundles (Figure 87). Free asbestos fibres were observed embedded in the cellular debris of the nodules. Tiny fibrils could be seen radiating from such free fibres (Figure 88). The periphery of the nodules was composed of numerous collagenous fibrils, and between them were situated fusiform-shaped cells. Some of these cells exhibited a prominent granular endoplasmic reticulum and golgi apparatus. Other cells within the periphery of the nodules adhered to one another by means of desmosomes and appeared to be capable of phagocytosis (Figures 89, 90).

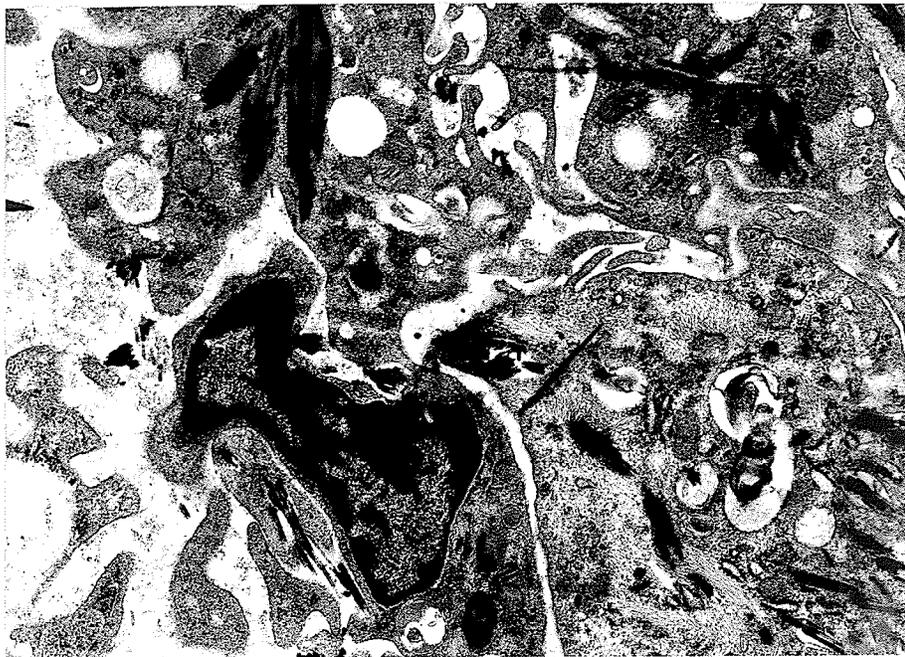
The septal cells were most notably altered by the chrysotile treatment. The osmiophilic lamellated bodies within such cells increased in number and occupied a region close to the surface of the cells. Numerous such bodies were also observed on the free surface of the cells (Figure 91).

Inhalation Exposure Series

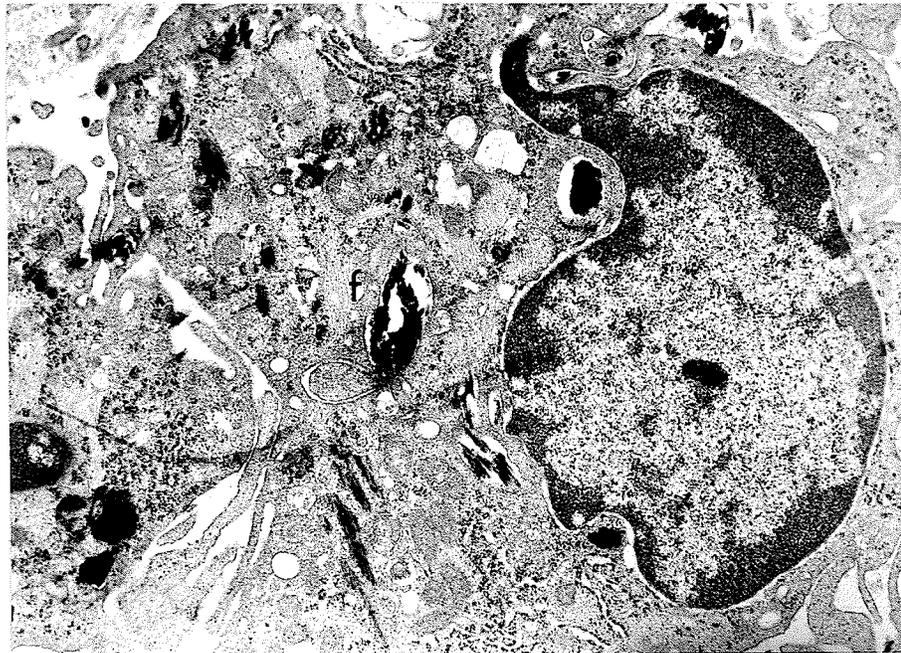
Few changes were discerned in the alveolar tissue of rats exposed to chrysotile asbestos dust clouds. Alveolar macrophages containing large quantities of asbestos were not observed as they

FIGURE 85: Centre of asbestotic nodule. Large numbers of chrysotile fibres are visible within the alveolar macrophages. Fibres are visible within the cytoplasm, in phagosomes, and also in the process of being ingested. Rat lung tissue 28 days after intratracheal injection of chrysotile.
Electron micrograph - magnification unavailable.

FIGURE 86: Centre of asbestotic nodule. Similar to Figure 85. Note the fibrillar (f) material radiating from the fibres of chrysotile. Rat lung tissue 28 days after intratracheal injection of chrysotile.
Electron micrograph - magnification unavailable.



85



86

FIGURE 87: Centre of asbestotic nodule within alveolar tissue.

Alveolar macrophage contains many fibres of chrysotile.

Note aggregation of asbestos fibres in a long structure at arrows.

Rat alveolar tissue seven days after intratracheal injection of chrysotile.

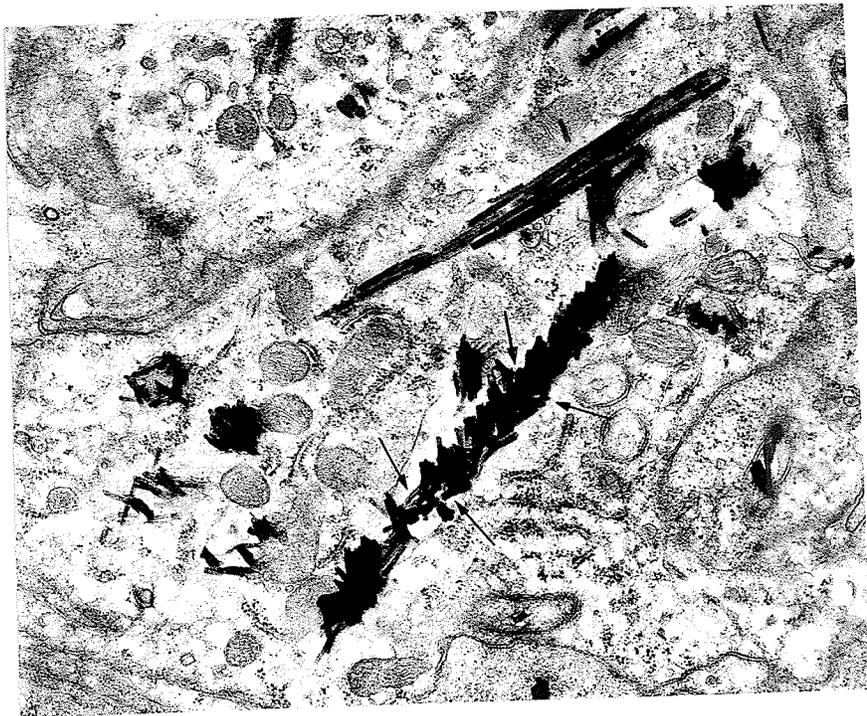
Electron micrograph 12,540 x.

FIGURE 88: Centre of asbestotic nodule.

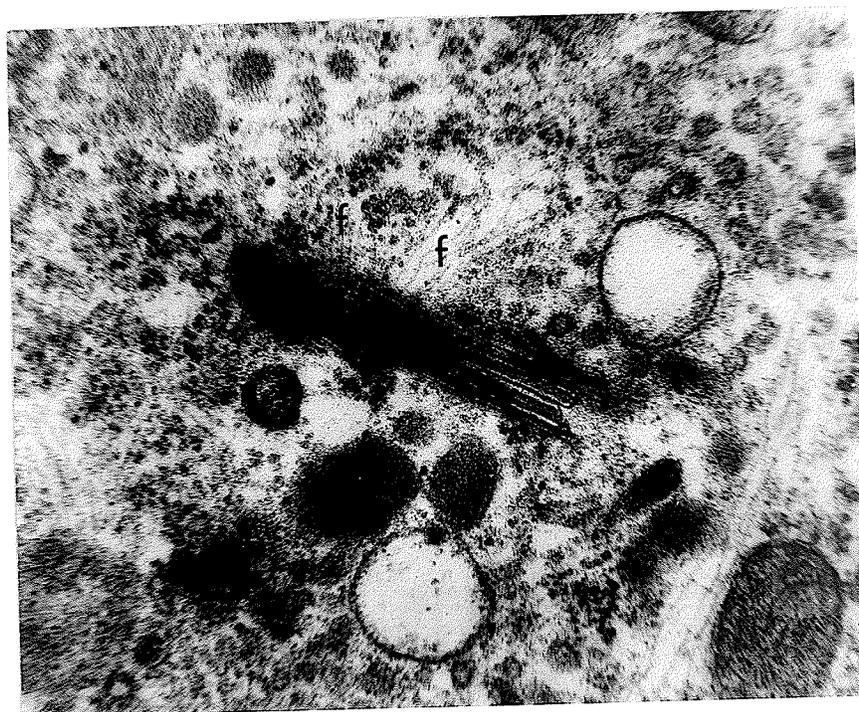
Note fibrils radiating from the asbestos fibre bundle at (f).

Rat alveolar tissue seven days after intratracheal injection of chrysotile.

Electron micrograph 25,080 x.



87



88

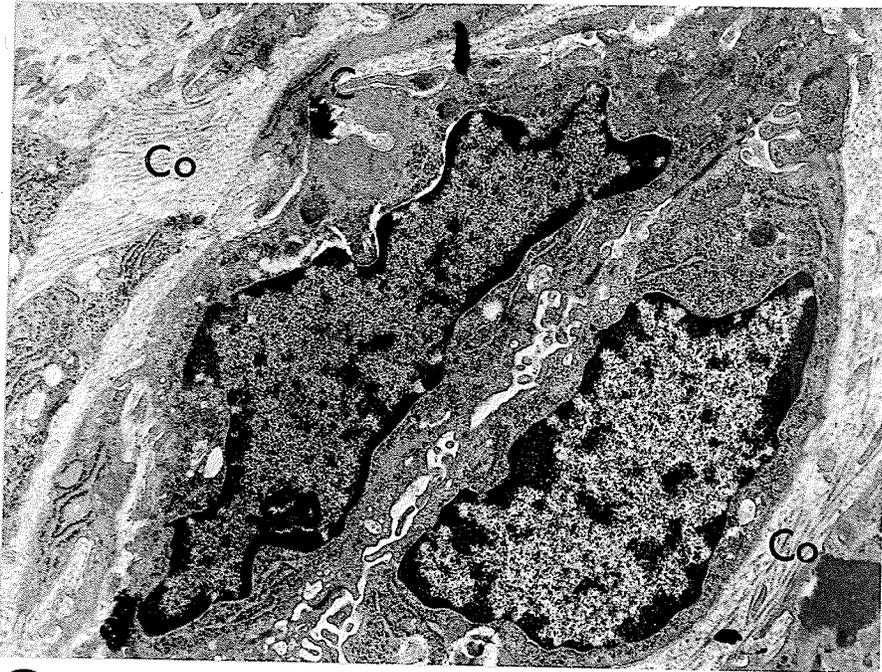
FIGURE 89: Periphery of asbestotic nodule in alveolar tissue.

Fusiform-shaped cells joined by desmosomes at
arrows. At (C), asbestos fibres are being phagocytosed.
Collagen fibrils (Co) can be seen surrounding the cells.
Rat alveolar tissue 28 days after treatment.

Electron micrograph -

FIGURE 90: Higher magnification of a portion of Figure 89.

Electron micrograph -



89



90

occurred in the intratracheal injection series. Occasionally, alveolar macrophages were encountered containing limited quantities of ingested asbestos (Figure 91); but in general few fibres were observed within the cells. The fibres that were seen within alveolar macrophages were uniformly of small size. In contrast, the alveolar macrophages contained large numbers of osmiophilic lamellated bodies either free within the cytoplasm or else contained within lysosomes (Figures 93, 94). In some areas of the alveolar tissue of rats that had been exposed to the asbestos dust clouds, numerous septal cells occurred in close proximity to one another. The number of osmiophilic lamellated bodies within these cells had become pronouncedly increased. That phenomenon is shown in Figure 92.

FIGURE 91: Septal alveolar cell.

The typical osmiophilic lamellated bodies can be seen at the apical portion of the cell.

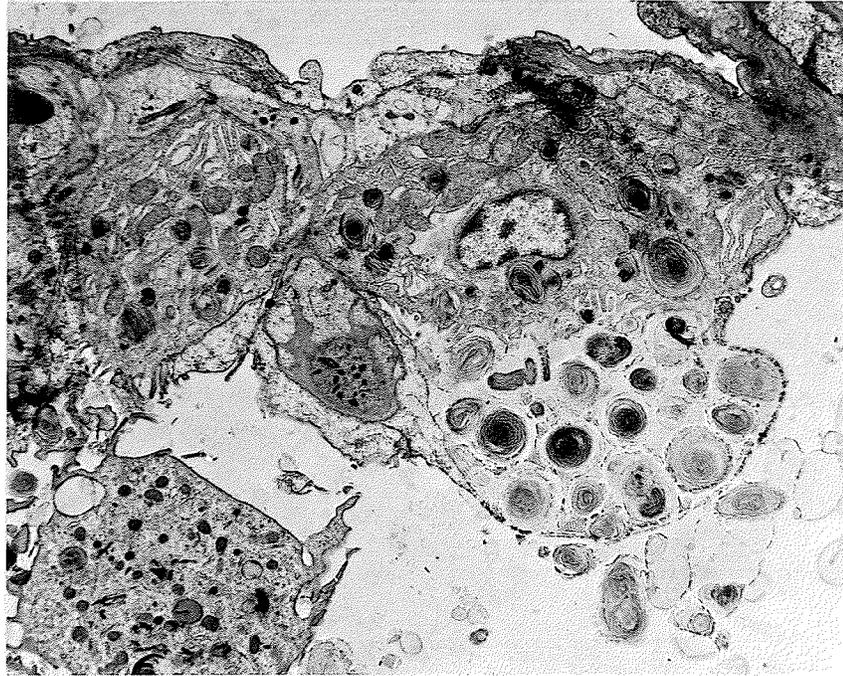
Rat alveolar tissue three days after intratracheal injection treatment.

Electron micrograph 3,192 x.

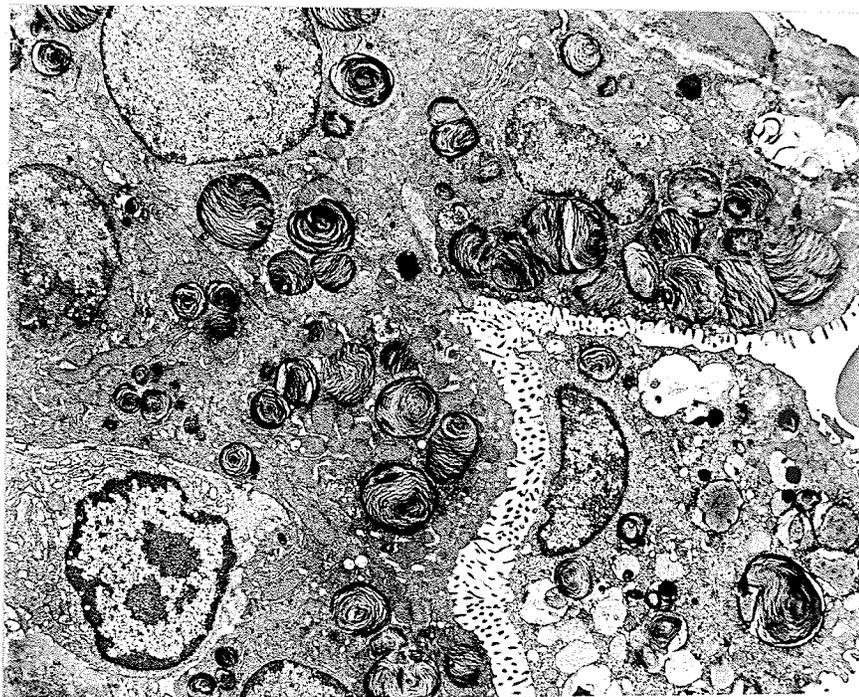
FIGURE 92: Four septal alveolar cells containing numerous lamellated bodies.

Rat alveolar tissue 14 days after chamber exposure to natural chrysotile.

Electron micrograph 3,192 x.



91



92

FIGURE 93: Alveolar macrophage containing numerous osmiophilic lamellated bodies.

Rat alveolar tissue 14 days after chamber exposure to natural chrysotile asbestos.

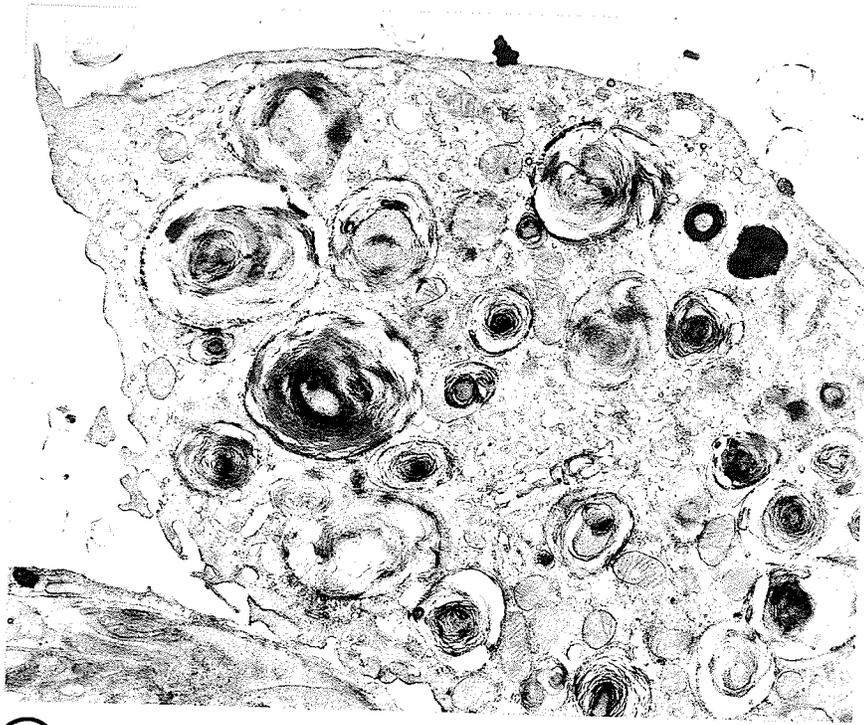
Electron micrograph 6,840 x.

FIGURE 94: Portion of alveolar macrophage. Osmiophilic lamellated bodies in cytoplasm and within lysosomes

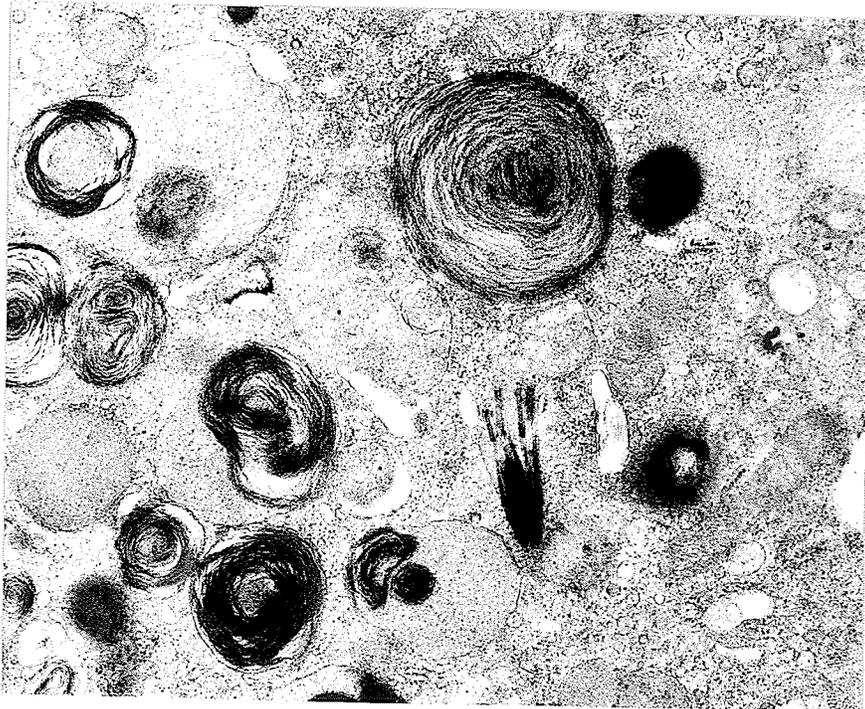
Note asbestos fibres in the cytoplasm of the cell.

Rat alveolar tissue 14 days after chamber exposure to natural chrysotile asbestos.

Electron micrograph 12,540 x.



93



94

DISCUSSION

The purpose of the present study was to examine closely the biological effects of chrysotile asbestos and to compare its action with that of control dusts in order to ascertain the specific properties of this mineral responsible for bringing about its drastic effects on the respiratory system. The cytodynamic proliferative responses of various portions of the respiratory system under the action of chrysotile were characterized in particular to enable a better understanding of the etiology of asbestosis, and also of the long range development of malignancies within portions of the respiratory tissue.

The asbestos minerals exert a most severe insult on the tissue of the respiratory system. Notwithstanding the many investigations of recent years on the biological effects of these minerals, the specific manner by which they exert their pathological effects remains to be elucidated. For instance, the relative contributing importance of such factors as chemical and physical properties, fibre size of the mineral, and the duration and type of exposure are still not clearly defined. The evidence implicating the asbestos minerals as causative agents of carcinogenesis in man is extensive and becomes augmented continually (McEwen et al., 1970; Stumphius, 1971; Whitwell et al., 1971). Nevertheless, a precise understanding of these disease processes is lacking. In the present series some

of the factors concerning the etiology of asbestos-related disease of the respiratory system were examined. For instance, in Experiment 1 the effects of two types of chrysotile asbestos on rat lung tissue were compared to those of certain control particulate materials. It was established that of the materials tested, the natural chrysotile asbestos alone exerted drastic effects on the pulmonary tissue of rats. Certain conclusions could be drawn from this observation. Thus, if the pathological effects of chrysotile were strictly dependent on the chemical nature of the mineral only, then it would be expected that the synthetic mineral with an identical chemical make-up would exert virtually identical effects. Inasmuch as this was not the case it was concluded that the pathological effects of chrysotile on rat lung tissue were not strictly dependent on the chemical nature of the mineral. In addition, it became obvious from this series that the fibrous nature of chrysotile was not the sole contributing factor to its pathological effects, inasmuch as a non-asbestiform fibrous material (fibreglas) did not produce severe pathological alterations in rat lung tissue. Furthermore, since the fibre size of the fibreglas and natural chrysotile were of the same magnitude, a mere simple physical mode of irritation, largely dependent on the size of particles, could also be excluded as comprising the major factor in producing changes of lung tissue. This assumption is further confirmed by the epidemiological study of Bjure et al. (1965) who observed that workers exposed industrially to fibreglas

did not show significant radiologically visible alterations of the lung as compared to a group of workers exposed to asbestos dusts under similar conditions. In addition, Gross et al. (1970) conducted animal experiments quite similar to those described in the present study; they reported likewise that neither fibreglas nor synthetic chrysotile asbestos produced significant pathological abnormalities in the lungs of rats, as they developed under the effects of natural chrysotile. It was therefore established by the work of the present study as by other series that neither chemical, physical properties, nor fibre size alone were responsible for the severe pathology appearing in the lungs of rats after the intratracheal injection of asbestos. It seemed likely therefore that a specific combination of all these factors was inducing cumulatively the severe lesions.

Inasmuch as the only difference between the two varieties of chrysotile was the size of the fibres, it is apparent that fibre size is an important contributing influence, but not, as demonstrated, the sole or major contributing factor to the development of asbestos-related disease.

The cytodynamic observations of Experiment 1 suggested that chrysotile of both types exerted some chemical action on the cellular populations of the alveolar tissue. Inasmuch as many workers reported previously an enhanced mitotic activity in alveolar tissue after the imposition of irritating materials, it was anticipated at the onset

that chrysotile would bring about a similar augmentation of cell proliferation. For example, Jorundson (1967) working in this laboratory, reported an enhancement of the mitotic activity of rat alveolar tissue in response to intratracheally instilled dust loads of asbestos. Yet the type of asbestos employed and the fibre size range of the mineral were not reported. Further, Boisjoli (1968) observed that the mitotic rate of rat alveolar tissue was markedly increased after intratracheal injection of the crocidolite variety of asbestos into rats, whereas it was decreased in response to a dust load of natural chrysotile asbestos. This latter finding was essentially confirmed by the present investigation. Yet, the exact reason for the inhibition of the mitotic activity of the alveolar tissue by chrysotile was obscure. The most pronounced pathological change in the respiratory system was a blockage of the lower air passages which may have prevented the extrusion of alveolar cells through the tracheobronchial tree at a normal rate. This factor may have contributed to an apparent decline in the mitotic activity of the alveolar tissue, inasmuch as the total population of alveolar cells was increased by the addition of those cells that normally would have been extruded. Presumably such cells did no longer divide, and contributed, by their presence, an apparent decline in the overall mitotic rate of the alveolar cell population. However, because the lungs treated with synthetic chrysotile asbestos exhibited a similar decline in mitotic activity although devoid of any of the pronounced

changes, as airway obstruction, the afore-mentioned hypothesis was unlikely. Another approach in attempting to explain the declining mitotic activity of the alveolar tissue was to ascertain whether deficient nutrition may have played a part. Recording of the body weights of the experimental groups revealed that only those animals treated with natural chrysotile experienced a severe loss of body weight. These extreme weight losses of the animals may have been concurrent with a deficiency of nutrients not only of the animals in general, but also of the cells that normally provide alveolar cells by proliferation. In fact, Deo and Ramalingaswami (1969) reported that a protein deficiency in rats resulted in a build-up of cells in mitosis that were incapable of completing their division. Although a similar severe loss of body weight was not apparent in animals treated with synthetic chrysotile, the effect of weight loss on the mitotic activity of the alveolar tissue was nevertheless examined in more detail. Accordingly, weight losses equivalent to those experienced by animals treated with natural chrysotile were duplicated in Experiment 2. Rats were subjected to either starvation alone for two days, or were injected intratracheally with saline in addition to the two days of starvation. Inasmuch as it had been ascertained previously that the injection of saline caused itself an increase of mitotic activity of the alveolar tissue, it was important to determine in particular the combined effects of starvation and saline administration. On the basis of the previous observations it was anticipated that the combined

treatment of the animals with starvation and saline injection would result in a proliferative response intermediate to that caused by each treatment separately. As was shown graphically in Figure 54 such a trend was in fact apparent. Yet, statistically, the saline and starved groups were not different from the series treated with saline injection alone. Thus, Experiment 2 failed to provide clear evidence characterizing the causes for the decline in mitotic activity of the alveolar tissue of natural asbestos treated animals. Statistical analysis of the data on mitotic rate and weight loss from both these experiments revealed that the decline in the mitotic activity of rat alveolar tissue experienced by the two asbestos treated groups was greater than could be explained on the basis of weight loss alone. A factor other than the nutritional deficiency responsible for the weight loss must therefore have been responsible for part of the decline of the proliferative activity of the alveolar tissue. Certain particulars became apparent from the literature, however, that may have a bearing on this problem. For instance, Pernis and Vigliani (1968) and others (Parazzi et al., 1968) reported that chrysotile exerted a cytotoxic effect on alveolar macrophages in vitro. It seems thus plausible that the decline in the proliferative activity of alveolar tissue in the present series might have been the result of such an effect exerted by the two types of chrysotile on the alveolar macrophage population. The distribution of both types of chrysotile within alveolar tissue as localized by

radioautographs, lent further support to this possibility. Large numbers of dust-laden macrophages were observed by this means. Also, the electron microscope examination of alveolar tissue from animals treated with natural chrysotile revealed numerous dust-laden macrophages virtually engorged with fibres of chrysotile. On the basis of such observations, the decreased proliferative activity of the alveolar tissue being effected by a cytotoxic action of chrysotile on alveolar macrophages appeared likely. It was hoped that additional investigation of the proliferative behavior of alveolar tissue under these conditions would contribute to the clarification of the specific causes of the apparent mitotic inhibition. Therefore, the tritiated thymidine technique was employed in a series of experiments in which rats were subjected to treatment similar to that of Experiment 1. Various kinds of data were thereby derived which could be compared to those of the colchicine series of Experiment 1. It was thus established that the labelling index of alveolar tissue following the administration of either of the two asbestos dusts was significantly higher than that attained by saline treatment alone. Specifically, at five days after asbestos administration the alveolar tissue displayed a higher labelling index than that of the saline treated control group. However, by seven days only the group treated with natural chrysotile exhibited a significantly increased labelling index of the alveolar tissue when compared to the control group. That former group, in

addition, displayed also a significantly higher labelling index than the synthetic chrysotile treated animals. These observations signified that despite the low mitotic activity of the asbestos treated alveolar tissue as determined by the colchicine technique, the incorporation of tritiated thymidine was nevertheless high. It follows from this that a fair proportion of alveolar macrophages, or their progenitor cells, did in fact undergo DNA synthesis but were subsequently prevented by the action of asbestos from continuing the cell cycle and from entering mitosis. They were thus not recognized by the colchicine technique. At five days after treatment with either of the asbestos dusts it was further noted that although both groups were significantly greater than the control in their labelling indices of alveolar tissue, yet the group treated with natural chrysotile displayed at the same time a significantly greater labelling index of alveolar tissue than the group treated with synthetic chrysotile. It appeared therefore that synthetic chrysotile exerted an intermediate effect between that of the natural chrysotile and saline treated groups. The intermediate nature of the effect of synthetic chrysotile on the labelling of the alveolar tissue became more apparent at seven days after treatment. At that time the labelling index of the group treated with natural chrysotile became augmented further to a level significantly greater than that demonstrated by the other treatments. In contrast, the labelling index of alveolar tissue of the group treated with synthetic

chrysotile was not significantly different from that reached by the saline control. It was hypothesized from these observations that the natural chrysotile treatment stimulated larger numbers of cells to enter DNA synthesis, however without avail, as the cells were subsequently unable to divide, presumably because of the cytotoxic activity of the natural mineral.

The second part of this experiment approached the problem differently. Rats were administered tritiated thymidine at the time of intratracheal injection treatment and sacrificed seven and 14 days later. In this manner, still another type of data characterizing the cytodynamic behavior of these cellular populations would be derived. It was ascertained that at seven days after the intratracheal injection of the two asbestos dusts and saline, and the concurrent injection of tritiated thymidine, that the labelling indices of the saline and natural chrysotile treated groups were not significantly different. In contrast, the lungs treated with synthetic chrysotile exhibited a significantly lower labelling index than the latter two groups at this time. However, by 14 days, the labelling index of the saline treated group had remained at the same level whereas those of the two chrysotile treated groups became reduced significantly. It was initially anticipated that the labelling indices of all groups would decline with time as the labelled cells became extruded from the lungs. Therefore, the

persistence of the labelling of the alveolar tissue of the saline treated group at a steady level between seven and 14 days was entirely unexpected. This observation can be explained, however. If the precursor cells of the alveolar macrophages within the connective tissue of the lung (but outside of the alveolar tissue proper) took up the tritiated thymidine at the time of the injection of the pulse label, then at some later time these cells would appear in the alveolar tissue as macrophages. These cells would not be included in the counts at the early times after treatment (seven days), but later they would be included inasmuch as they then would be in the alveolar tissue. In the case of the asbestos treated groups, various possibilities of explanation exist. Firstly, since the alveolar tissue of these groups was known to have a low mitotic rate it is unlikely that the reduction in labelling indices at 14 days was caused by the dilution of the label by frequent mitosis. It is possible, however, that the cells were rapidly extruded and therefore the label was removed from the lung. Inasmuch as the normal extrusion of such cells from the lung was markedly inhibited in those animals treated with natural chrysotile, as evidenced by the severe pathological alterations of the lower airways, this occurrence may not have taken place with great efficiency in that group. Therefore, the cytotoxicity of natural chrysotile on alveolar macrophages is a more likely explanation for the reduction in labelling of the alveolar tissue.

The cytodynamic investigations suffered from a number of technical difficulties. In the present series it was unfeasible to characterize precisely the proliferative behavior of each individual cell population of the alveolar tissue. Bertalanffy (1964) undertook a specific characterization of the cell population in the alveolar tissue of normal rats. He was able to provide specific cytodynamic data on each of the cellular populations by performing meticulous differential counts of those cellular populations that were distinguishable with the light microscope. This approach was not applicable in the present investigation because of the disruption of the normal frequency distribution of different cells. Inasmuch as the frequency of different cell forms may have varied at different time intervals during the experiments, such counts were not undertaken. In addition, the high incidence of morphologically indistinguishable cell types displaying characteristics common to more than one cell added further to the difficulty of this problem. Most authors (Bertalanffy, 1964, 1967; Bowden et al., 1968) held the view that only the two types of alveolar cells became rapidly renewed normally in the alveolar tissue. Recently, however, others (Evans et al., 1968) asserted that the endothelial cells of the pulmonary capillaries likewise comprised a rapidly renewing cell population with a normal turnover time of 10 days. In contrast, the studies by Bowden and his associates did not lend support to the assumption that endothelial

cells were capable of rapid turnover. These contradictions will be largely resolved in the near future as more workers begin to apply some of the techniques of tissue preparation conventionally used in electron microscopy to light microscopical studies as well. A much better characterization of the various cellular elements of the alveolar tissue is possible by the use of plastic sections ($1 - \frac{1}{2}$ micron) cut on the ultramicrotome. This is exemplified by Figure 28. In the present study it was infeasible to carry out specific cyto-dynamic considerations of the individual cell populations of the alveolar tissue for the reasons afore-mentioned. Nevertheless, it is likely that the data of metaphase arrest and thymidine labelling can be ascribed largely to the alveolar macrophage population. Furthermore, inasmuch as the macrophage is the cell primarily affected by the asbestos by virtue of its phagocytic function, it is probable that any changes in the cytodynamic parameters observed after asbestos treatment are brought about by the reaction of the macrophages ingesting the asbestos fibres.

A major aspect of the present investigation was the preparation of the radioactively labelled asbestos dusts that provided a most useful investigative tool. It could thus be ascertained readily that asbestos fibres, once they attained the respiratory tissue, were rapidly ingested by alveolar macrophages. Within a week after asbestos

administration, free asbestos fibres were indeed scarce. A large proportion of intratracheally injected labelled natural chrysotile asbestos became lodged in the air passages, surrounded by connective tissue and covered by respiratory epithelium. Within a week after treatment the conspicuous polypoid nodules of bronchiolitis obliterans were observable in some of the bronchi and proximal bronchioles. Large quantities of the asbestos were lodged within similar nodules even in the terminal and respiratory bronchioles. This material was observed by radioautography also in fibrotic regions within the alveolar tissue; such areas being frequently contiguous with nodules in the distal bronchioles. Formation of the nodules at all levels was strictly dependent on the presence of the asbestos. Labelled natural chrysotile dust was also prevalent in the peribronchiolar and periarteriolar connective tissue. Conceivably, it was transported to these sites by alveolar macrophages via the lymphatic channels. Synthetic chrysotile was detected in similar sites but the overall reaction of the lung tissue toward that mineral was much less severe. Morphological comparison between lungs of animals treated with either of the two types of asbestos dusts revealed that the synthetic chrysotile dust was evidently cleared more rapidly and efficiently than the natural mineral. A quantitative characterization of the rate of pulmonary clearance of these two types of chrysotile was carried out by scintillation counting of the remaining radioactivity in the lungs of the animals

thus treated. Although some technical difficulties were encountered, nevertheless a characteristic pattern of clearance after intratracheally injected dust loads were imposed on the lungs of animals could be ascertained. The measurement of radioactivity remaining in the lungs of those animals sacrificed at very early times after treatment presented unexpected anomalous results. At these times, the level of radioactivity in the lungs of treated animals was unusually low. Slightly later a higher level of radioactivity was measured in the lungs. It is conceivable that soon after the intratracheal instillation of the radioactive dusts most of the labelled mineral had not as yet penetrated deeply into the alveolar tissue but remained still largely lodged in the tracheobronchial tree. After the sacrifice of these animals the extrapulmonary part of the air conducting system containing a large proportion of the labelled mineral was removed, while that present in the lung may have been partly washed away by the fixing agent. At later stages the dust had penetrated more deeply into the alveolar tissue, had been phagocytosed, and fixed within the nodules; therefore it was not as easily dislodged from the lung at the time of fixation. At the later times after the initial anomalous period, a rapid phase of clearance ensued. Following this rapid rate of clearance, a slower rate of clearance became evident. This slower phase of clearance was maintained throughout the duration of the experiment, dust still remaining in the lungs of the animals at the final time

considered. It is believed that the initial rapid clearance phase (after the earliest anomalous times) is largely ascribable to the action of the epithelium of the airways removing the asbestos that settles thereupon by aid of the muco-ciliary escalator. Once the dust was expelled from the air passages by this means, clearance of the material more permanently lodged in nodules and within the respiratory tissue itself, by the alveolar macrophages, was a much slower process. By 56 days after the dust administration, a high level of radioactivity still remained in the lungs of animals treated with natural chrysotile. It was somewhat lower in those exposed to the synthetic mineral. Yet, because of some of the technical difficulties encountered, the difference between the amount of radioactivity remaining in the lungs of the two groups cannot be presented as a significant statistic. It is clear nevertheless that the synthetic chrysotile was removed much more efficiently from the lungs of the animals than was the natural mineral. Further investigations of this nature utilizing the labelled asbestos dusts will characterize the relative clearance of these two minerals very accurately.

A most significant observation in the labelled asbestos dust series was the presence of chrysotile fibres in close vicinity to or even lodged within the pleural connective tissue. This phenomenon was especially pronounced with the labelled natural chrysotile.

Inasmuch as the pleural mesothelium has been shown to be one of the principal sites at which asbestos exerts its carcinogenic effects in man, the actual demonstration of the mineral fibres at these sites was particularly significant. In addition, the observations made in Experiment 3 revealed a particular sensitivity of the pleural mesothelium to asbestos fibres, and numerous plaques of fibrous tissue were observed on the surface of the lung. They were formed in response to the irritating fibres that became encapsulated within the connective tissue in these plaques. Because of the role of the pleural mesothelium in asbestos induced neoplasia, it was attempted to characterize its cytodynamic reactivity in the series of Experiment 1. The attempt proved futile, however, for it was exceedingly difficult to distinguish the arrested metaphases of the mesothelial cells. The tritiated thymidine technique as employed in Experiment 6, however, was quite facile in enabling a clear cytodynamic characterization of the pleural mesothelium. Most unexpectedly, an extremely high level of labelling was observed of the pleural mesothelium following intratracheal injection of the rats with natural chrysotile asbestos. Five days after treatment, the labelling index of the pleural mesothelium was 2.0%, compared to 0.38% and 0.20% respectively of the mesothelium of rats treated with saline or synthetic chrysotile. The mesothelial labelling index became further augmented to 3.18% seven days after the administration of the natural chrysotile. A remarkable reactivity of the pleural

mesothelial cells to natural chrysotile was thus apparent. In the second part of this experimental series, a pulse label of tritiated thymidine was administered to the animals at the time of intratracheal injection. At seven days after treatment, the labelling indices of the rat mesothelial cells of animals treated with natural asbestos were not significantly different from that of the saline treated control group. Yet by 14 days the labelling index of the pleural mesothelium exposed to either variety of asbestos became significantly decreased, and was lower than the level of the saline group at the same time. Conceivably, the increase of labelling at 14 days in the saline group was brought about by a halving of label during subsequent mitotic divisions of the mesothelial cells. In contrast, the decreased labelling of the mesothelium of the asbestos groups may have resulted from a faster rate of cell loss by desquamation from the mesothelium under the influence of the irritating asbestos fibres. The findings of this experiment are quite significant in view of the special action of asbestos on the pleura in the initiation of pleural mesothelioma.

Inasmuch as the intratracheal injection technique, although widely employed, constitutes a highly traumatic means of administering asbestos to animals, another series was exposed to the dust in the environmental chamber, acquiring the asbestos by the more ordinary route of inhalation. Yet, even a two-month exposure of the animals to concentrated dust clouds of natural chrysotile asbestos,

four hours each day, did not induce any pronounced pathological alterations in their lungs. There was little morphological difference between the lungs of animals exposed for seven days and those exposed eight times longer, for a period of 56 days. Still, cytodynamic observations suggested interesting possibilities. During the initial phase of exposure to chrysotile, the mitotic rate of the alveolar tissue declined gradually, falling to its lowest level (0.21%) at 10 days after the commencement of the exposure. After two weeks of exposure the rate had returned to the normal level of untreated tissue. This was followed by a significant decline reaching again a low level of mitotic activity by 28 days. The mitotic activity remained at that low level during the remaining interval of the experiment. Consequently, the mitotic rate of alveolar tissue of animals exposed to natural chrysotile for 56 days was significantly lower than that of the untreated animals. This suggested again a cytotoxic action of chrysotile, similar to the intratracheal injection series, exerted on the cell proliferating in the alveolar tissue. Even the group of animals exposed to chrysotile for merely seven days, that had been removed from the dusty environment and subsequently stored in an asbestos-free environment, exhibited a very similar response. In fact, the mitotic rates of the alveolar tissue of the two exposed groups (seven days or 56 days) differed significantly only at 28 days after the commencement of exposure. But considering the overall data, a significant difference did not occur between the

mitotic rates of the alveolar tissue of these two groups. In addition, the group exposed to asbestos dust clouds for only seven days did not differ significantly overall from the untreated controls. The observations were therefore only suggestive of an inhibitory effect of chrysotile asbestos on the mitotic activity of the alveolar tissue. Similar observations were deduced in tracheal epithelium exposed to the asbestos dust clouds. Although a fleeting transient increase of the mitotic activity of tracheal epithelium seemed apparent after two weeks of daily exposure, followed by a decline to low levels maintained until termination of the experiments, nevertheless statistical differences were not detected between the mitotic rates of the two exposed groups or between these groups and the untreated control. A wide range of the mitotic rates of tracheal epithelium presented an obstacle in the precise evaluation of the data in this series.

The electron microscope studies of alveolar tissue of rats exposed to chrysotile by both means of treatment strengthened the hypothesis of cytotoxicity of chrysotile on alveolar macrophages. The most common observation made in this series was the high frequency of ingested asbestos fibres in alveolar macrophages from the intratracheal injection series. Such macrophages were found free in the alveolar spaces, on the surface of the alveolar wall, within the peribronchiolar connective tissue, and also within the typical

asbestotic nodules. It became apparent from these observations that the macrophages were incapable of dealing with the large quantities of chrysotile. Although the cells attempted to digest the fibres by their incorporation into phagosomes into which lysosomes emptied their enzymes, yet the ultimate fate of such asbestos-laden macrophages was death. Such cells in close association by means of desmosomes were observed within the fibrous connective tissue of the nodules. Although the radioautographical preparations revealed many asbestos fibres along the walls of the alveolus, yet this observation was not made with the electron microscope because of the limited number of sections that were examined. In addition, since there was much unaffected alveolar tissue, the probability of coming upon a representative portion of severely afflicted alveolar tissue was not high. It was noted also that the septal cells appeared to be in a high state of activity. Numerous osmiophilic lamellated bodies could be visualized both within the cells and on the apical portions of the septal cells. Such osmiophilic lamellated bodies were observed regularly also in the alveolar macrophages. The macrophages in fact contained both ingested asbestos fibres and also the osmiophilic lamellated bodies. This observation was also made in the alveolar tissue of the inhalation exposure series. Although asbestos fibres were not frequently observed in the macrophages of that series, yet on occasion such fibres were seen within the cells along with numerous osmiophilic lamellated bodies. Other macrophages not

containing asbestos fibres often were filled with the osmiophilic lamellated bodies. It was first believed that such bodies might represent merely the effects of the intratracheal injection treatment. Because the alveolar tissue of the control groups was not examined with the electron microscope, the significance of these osmiophilic bodies could not be theorized. Yet, the presence of these bodies also in the macrophages of animals treated with inhalation exposure hinted that their occurrence might be independent of the intratracheal injection treatment. In fact the reports of Kapanci et al. (1969) and Yuen and Sherwin (1971) suggest strongly that this phenomenon is an indicator of severe alveolar damage apart from the intratracheal injection technique.

It became evident from the present series of experiments that neither chemical nor physical properties, nor fibrous structure or fibre size alone were deciding the specific pathogenic action of chrysotile asbestos in lung tissue. The relative contributions of these various properties can be surmised at least partly, however. The role of fibre size in the pathogenicity of chrysotile is a highly significant factor once the fibres have gained access to the depths of the lung. The probability of longer fibres of chrysotile entering the respiratory tissue is small under normal circumstances as such fibres are efficiently halted by the clearance mechanisms of the upper air conducting system. In cases of severe respiratory disease

affecting the epithelium of the airways. For instance, such influencing factors as cigarette smoke, or noxious gases (NO_2 for example) may destroy the normal structure of the airway epithelium thus facilitating the entrance of longer fibres of asbestos. In such circumstances, fibre length of chrysotile may likely determine the severity of asbestos-related disease of the respiratory system. In addition, an extremely heavy exposure to asbestos dust may constitute an insult of such magnitude that the normal healthy airway epithelium cannot cope and therefore the longer fibres will gain access to the respiratory tissue. Although the fibrous structure of chrysotile fibres per se does not appear to exert a significant influence on the pathogenicity of asbestos, yet the specific behavior of the fibres ascribable to their chemical composition and physical characteristics are of major significance. For instance, the surface characteristics of chrysotile enable the fibre bundles to swell after adsorbing various solutions. Also, the ultramicroscopic size of the individual fibres facilitates a complex intermeshing of fibres and the formation of fibre bundles. Such aggregations are immensely more difficult for the macrophages to remove than individual particles. In addition, the harshness of chrysotile may bring about a physical damage to the delicate structure of the alveolar macrophages. The action of chrysotile on the surface-active element of the alveolar wall is unclear and has not been reported in the literature but it is quite within reason to suppose that the peculiar surface properties of chrysotile may exert a drastic influence on the normal surface

activity of the alveolar tissue. This may perhaps explain the apparent increase in the osmiophilic lamellated bodies both within alveolar macrophages and the septal cells. A combination of these various factors may be responsible for the failure of the pulmonary clearance mechanisms to successfully remove the asbestos fibres that attain the respiratory tissue. With synthetic chrysotile which is of an ultramicroscopic fibre size, the clearance mechanisms may be able to cope more effectively because the small unit fibres never combined to form longer fibres, fibre bundles, or aggregations of fibres. Therefore, despite the cytotoxic effect exerted on the macrophage population also by the synthetic mineral, the fibres could nevertheless be cleared effectively by the alveolar macrophages. The disease "asbestosis" can be considered to be in the first instant a failure of pulmonary clearance in response to the asbestos minerals. Such failure of clearance may be artificial in the case of the intratracheal injection series, or alternatively it may be caused by other factors such as concurrent disease of the airways or very heavy exposure. The primary effects of chrysotile appear to be exerted on the population of alveolar macrophages. These effects may result in a failure of the lower respiratory tract clearance. Subsequently, the fibrosis of the respiratory tissue ensues.

In recent years, investigators have begun to employ a number of substances in an attempt to prevent the effects of inhaled minerals

on biological systems. For instance, Schlipkoter (1970) was able to forestall the development of extensive silicosis in rats by previous intratracheal injection of polyvinyl N pyrrolidone into the experimental animals. Klosterkotter (1968) was unsuccessful by the same means to prevent asbestosis in rats. Aside from the plain and simple logic of stringent dust control measures to prevent the severe exposure of man to the asbestos minerals, the approach of assisting the clearance mechanisms of the lung by nullifying some of the effects of chrysotile in vivo as by the application of substances preventing the cytotoxic effects of the mineral appears to be a most plausible prophylactic measure, and hopefully will eventually lead to a significant decline in the incidence of asbestos-related disease of the respiratory system.

C O N C L U S I O N S

- (1.) The specific biological effects of chrysotile asbestos did not appear to be solely dependent on a single factor, as the fibrous structure, chemical or physical properties, or fibre size of the mineral. Rather, a combination of some, if not all of these properties were responsible for the specific biological effects of chrysotile and asbestosis. Therefore, neither the chemical nor the physical theory of the pathogenesis of asbestosis alone is true.

- (2.) Chrysotile asbestos, whether in a natural or synthetic form, appears to exert pronounced cytotoxic effects on the cellular populations of the alveolar tissue. The result is a decline of the mitotic rate of the cells in the respiratory tissue. Conceivably, the population of macrophages is the most severely affected. Although severe weight losses are suffered by animals treated by intratracheal injection with natural chrysotile, the factor of nutrition was not deciding in the total decline of the mitotic activity of the alveolar tissue.

- (3.) Notwithstanding the decrease in mitotic activity of the alveolar tissue exposed to chrysotile asbestos, the cellular populations seemed capable of DNA synthesis to a remarkable degree. Yet, they appeared incapable to pass on in the cell cycle, apparently prevented in the presence of chrysotile from continuing into mitosis. Conceivably, a chemical effect of the mineral was responsible, and cell division did not ensue. This in turn affected the entire clearance mechanism of the respiratory tissue, represented primarily by the macrophage cell population.
- (4.) The pleural mesothelium exhibited a high cytodynamic reactivity in response to intratracheally injected natural chrysotile asbestos.
- (5.) Significantly different effects were not noted in the respiratory tissue or trachea between a short term exposure (seven days, four hours per day) administered to rats in the form of dust clouds of chrysotile asbestos and a long term exposure (56 days, four hours per day).

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