EXPERIMENTAL PNEUMOCONIOSIS

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SCOPE OF THE THESIS

The introductory section of this thesis deals with the history of pneumoconiosis and silicosis, the occurrence of silica in nature, the theories of silicosis, its aetiology, pathology, diagnosis and complications, and a short account of coalworkers' pneumoconiosis and pneumoconiosis due to kaolin or china clay. The introduction to the experiments themselves points out why these studies were undertaken. A description of the various techniques used in the experiments recorded here is included for convenience. The experimental studies themselves are described in five sections and followed up with a general summary and conclusions drawn from the results of the studies.

Section I deals with the rate of elimination of pathogenic and inert dusts from the lungs of rats, and the tissue reactions produced by them. It has been shown that quartz, a fibrogenic dust, and synthetic corundum, an inert one, have nearly equal rates of elimination in the early stages. Anthracite coal has been found to remain longer in the lungs than bituminous coal, although the lesions produced by them were identical. Radio-active tantalum carbide was eliminated rapidly from the lungs and did not produce any fibrosis.

In Section II the tissue reactions produced by variable amounts and forms of tridymite, a crystalline modification of pure silica, have been studied. Earlier workers found this to be the most pathogenic of all the modifications of pure silica, and these results support that conclusion.

The pulmonary lesions produced by anthracite and bituminous coal when mixed with very small amounts of tridymite have been studied in Section III, and the results show that more fibrous reactions occur when coal and tridymite are used simultaneously, than by these substances acting alone.

The effect of cortisone on established pulmonary silicosis in rats has been investigated in Section IV. Cortisone did not have any modifying effect on the maturity of fibrous tissue in the lesions, but chemically there was less collagen in the lungs, and therefore quantitatively less fibrotic lesions.

Finally, the action of coal, mine dust and kaolin on avirulent tuberculous infection in the lungs of rats and guinea-pigs has been studied in Section V. The combination of dust and infection produced more fibrosis in both species of animals than did either factor alone, in the same period of time.

HISTORICAL SURVEY

It has been known since prehistoric times that dust can produce diseases of the lung, and conditions of this type were termed "pneumonoconioses" (from the Greek πνεύμων= lung, κόνις = dust). The term, which has since been shortened to "pneumoconiosis", is a comprehensive one and is usually used to refer to conditions arising from the inhalation of dusts which are either inert, such as iron, or fibrogenic, such as free silica. The fibrosis produced by dusts containing free silica is popularly known as silicosis.

How early in the history of mankind industrial pneumoconiosis appeared is a subject for conjecture. The works of ancient writers seldom distinguish between the various forms of respiratory disease, but merely refer to a general connection between lung affections and dust inhalation. Hippocrates (460-370 B.C.), in his Epidemics wrote about "the wan complexion and the difficulty in breathing of miners", which might well be an apt description of the pneumoconiotic miner of the present day (Sayers 1925). Perhaps one of the earliest references to the hazards of dust inhalation is the description of Pliny, the Elder (23-79 A.D.) of a protective mask for miners. Galen (131-201 A.D.) referred to occupational diseases, including that found among gypsum workers.

The dangers that confront miners and refiners were mentioned by Georgius Agricola (1494-1555) in his treatise

on mining and metallurgy, "De Re Metallica". Paracelsus (1493-1541), a Swiss alchemist and physician, described the disease of miners and smelters in a book on industrial medicine published posthumously in 1567.

The year 1649 may be regarded as a landmark in the history of silicosis, as it was then that the first section of a stone-cutter's lung was made by Diemerbrock, when he found "lung vesicles completely clogged with fine dust." He also described the shortness of breath and rigidity of the chest found among granite workers in the Carpathians, who termed their tools "widow-makers". Ramazzini (1633-1714), often referred to as the third Hippocrates and the father of Industrial Medicine, described many dust diseases and their pulmonary lesions in his book entitled "De Morbis Artificium Diatriba", published in Padua in 1700.

In 1726 and 1732, Thomas Benson of Newcastle-under-Lyme was granted a patent for the wet-grinding of flint, which up to that time was pounded dry, "the process proving very destructive to mankind" (Collis 1915; Meiklejohn 1947). "Fork-grinders who use a dry grindstone die at the ages of 28-32 years", was the statement made in 1830 by Knight, a practitioner.

The year 1832 saw the publication of a second book on Industrial Medicine - 128 years after that of Ramazzini - by Thackrah, a young physician from Leeds, who was also a keen anatomist. In his studies on the life span of workers

in various dusty trades, he came to the conclusion that all dusts did not shorten life to the same extent, e.g. sandstone quarry workers died early, while brick and limestone workers were rarely affected. In summing up the chapter on dust diseases in his book, Thackrah stated :- "Dust is the great bane of manufacture, and whether it be farina, animal or vegetable fibre, or evolved from minerals, stone, lime, coal or metal, it injures the respiratory organs in proportion to the mechanical irritation it induces on the bronchial membrane".

Gregory (1831) described the lungs of a miner as being infiltrated with black matter resembling coal, and Marshall (1834) wrote about the pathological features of coal-miners' pneumoconiosis. But it was Thomson (1837) who gave an accurate description of the condition.

It was about this time that physicians were beginning to be conscious of the distinction between fibrosis due to dust alone and the condition complicated by tuberculosis. Thus Hall (1857) stated clearly that "there is no necessary connexion between the Sheffield grinders' disease and thoracic consumption, although both affections may be present in the same individual".

Greenhow, the first state medical inspector of factories in England, studied the pathological lesions in the lungs of workers who died from dust diseases (1865). He was also the first to use polarized light to demonstrate silica particles in the lungs of metal grinders. As a result of his work, the first Rpyal Commission was appointed to

investigate the health conditions among miners (1861). Peacock, a member of this Commission, was the first to distinguish between miners' phthisis and pulmonary tuberculosis. A detailed account of the progress of dust-phthisis among stone-cutters is to be found in the works of Allison and Hugh Miller (1869).

During this period it was observed by many physicians that in those mines where miners' phthisis was prevalent, rock drillers suffered from a more rapid onset of the disease than other miners. This and other similar evidence led to the belief that the important factor in the causation of the disease was the character of the dust.

Although many writers described the effects of various dusts on the lungs and gave the resulting lesions names descriptive of the causative dusts, it was not until 1866 that the term "pneumonokoniosis" was coined by Zenker, who also described the anatomical lesions in lungs so affected. Kussmaul proved by chemical means that inhaled dust was deposited in the lungs. The term silicosis was used by Visconti, an Italian, to denote the changes produced in the lungs by the inhalation of silica.

Towards the latter part of the nineteenth century the health of miners evoked a great deal of interest throughout the world - an interest which had been slowly mounting from the time when the dangers miners were exposed to were realised. Perhaps the advancement of industries in various countries

was responsible for this concern over the health of the miner. This led to the development of the study of pneumoconiosis and attempts at its prevention, and the various stages of its evolution in different countries is particularly interesting.

GREAT BRITAIN

In Great Britain, fifty years after the first Royal Commission of 1861, a second Commission was appointed to study the prevalence of disease among Cornish tin-miners (1902). Haldane (1904), a member of this Commission, thought that the condition was due to the rock dust present in the atmosphere inhaled by the miners. It was found that a definite relationship existed between the quartz content of the inhaled dust and the incidence of tuberculosis, as shown among workers in slate quarries in Wales. Thereafter, in 1906, the Workmen's Compensation Act made provision for the payment of compensation to workmen contracting occupational diseases, though only a few such diseases were included. Following on this, investigations were made in various dusty trades, e.g. slate refractories, sandstones, potteries, coal-mining, etc.

McCrae (1913) demonstrated the particle size of dusts which were respirable - "the largest particles to gain access to, and become embedded in the lungs were not more than 10 μ in diameter and 70% of all particles were less than 1 μ."

Haldane in 1914 claimed that the pathological process was essentially the same in the following words :- "I believe

the disease is the same all over the world and the end comes through tubercular infection".

Collis, in his Milroy lectures (1915), expressed the view that silica in the form of free SiO_2 alone stimulated the formation of fibrous tissue. Many investigations have been made since 1917 in various industries - pottery and granite industry (1930), metal grinding and casting, refractories industry, coalmining industry (Middleton 1931-35), iron mining (Stewart and Faulds 1934). Dust counts were made in industries where the atmosphere was known to be charged with dust, using the Owen's jet dust counter (1922), and petrographic determinations were also made. These investigations revealed the fact that only exposure to free silica was capable of producing the disease.

The chemical aspect of the problem of silicosis was studied by Gye and Kettle (1922) and Gye and Purdy (1922). The latter workers advanced the theory that finely divided silica in the lungs slowly formed a silica sol which acted as a direct cell poison. With this view Heffernan and Green (1928) were not in agreement, while King (1945) held that this theory did not always apply in practice.

Kettle believed that silica in the lungs was absorbed in a colloidal form and then acted as a tissue poison and paralysed phagocytes, lowering their powers of action. This latter fact appears to be of some importance when the relation of infection - particularly of tuberculosis - to pneumoconiosis is studied. Gye and Kettle (1922) and

Kettle (1924) proved by animal experiments that the presence of silica in the tissues enhanced the lesions produced by the tubercle bacillus.

Middleton (1931-35) showed that in South Wales and Monmouthshire coalminers the death rate from silicosis with, and without tuberculosis was very high as compared with that of workers in the rest of England and Scotland. In the South Wales coalfields the type of silicosis is "infective" (Irvine 1937) - massive fibrosis in a silicotic lung - probably due to tuberculosis, although the causative organism is not always demonstrable.

Jones (1933) put forward the hypothesis that sericite or secondary white mice - a hydrated silicate of aluminium and potassium - was the substance responsible for the development of silicosis. His conclusion was based on the finding that mineral residues of lungs examined by him showed the presence of sericite. Fallow and Banting (1934) disproved this by their discovery that intratracheal and subcutaneous injections of a suspension of sericite failed to produce any nodules, while quartz similarly injected produced classical silicotic nodules.

The study of the action of various substances in accelerating or retarding the progress of silicotic fibrosis in tissues has formed an interesting problem for many workers. Haldane (1917) held that certain rocks were antidotal to silica. He also believed that coal might inhibit the action of silica. Hart and Aslett (1942) found that coal by itself produced a type of pneumoconiosis referred to as "dust

reticulation". This condition has been described by Belt and Ferris (1942) and Gough (1940; 1947 (a) and (b)). Ray, King and Harrison (1951) showed by animal experiments that coal, far from inhibiting the action of quartz, produced more fibrosis when used with it than when quartz was used alone. Heffernan and Green (1928) found that clay, carbon and shale dust inhibited the action of silica. King (1938), Whitehouse (1938) and Bremmer (1939) proved that, in the presence of stone and shale dust, silicic acid had a lower solubility than by itself.

Kettle (1932 and 1934) showed that the depression of the solubility of quartz particles by coating them with iron oxide rendered them harmless. King, McGeorge and Roman (1938) found that metallic aluminium was very effective in depressing the solubility of quartz. Later, King, Wright, Ray and Harrison (1950) found that in dusting experiments on animals carried out by using a mixture of 2% metallic aluminium and quartz, the fibrotic action of silica was retarded by the simultaneous use of the aluminium.

U.S.A.

Peterson of New York was the first to report the autopsy findings in a case of what appeared to be silicosis in a foundry worker in America in 1887. Canedy (1887) and Betts (1900) reported similar cases in a cutlery factory and gold-crushing factory respectively. In 1915, the U.S. Bureau of Mines and U.S. Public Health Service investigated

the occurrence of silicosis among lead, zinc and hard metal miners. This was followed by investigations in many industries. Greenberg and Smith introduced a dust-sampling device - the impinger - in 1922, and Pancoast and Pendergrass described the X-ray diagnosis of silicotic lesions in their book published in 1926.

Gardner (1923) indicated that silicosis was due to exposure to quartz dust and later demonstrated that attenuated tubercle baccili and quartz dust, when injected into animals, produced fatal tuberculosis (Gardner 1929). In 1933 the U.S. Public Health Service undertook a survey of lung disease in anthracite and bituminous coalmines, which revealed a high incidence of disease often associated with tuberculosis in the anthracite-mining areas and none in the bituminous areas. The term anthraco-silicosis was given to this form of disease to denote that the cause was silica.

Many industries, such as foundry, granite, pottery, marble, cement and abrasives were looked into by the local authorities, and the conclusions reached were that the incidence and clinical features of silicosis were proportional to the duration of exposure to silica dust and infection. Work done by Gardner and his associates at Saranac Lake Laboratory supported these findings. Gardner, Dworski and Delahant (1944) found experimentally that quartz could be neutralized by coating with aluminium oxide. Vorwald, Pratt, Dworski and Delahant (1950) found that when guinea-pigs inoculated with BCG vaccine were infected with attenuated human tubercle

bacilli (R1 strain) and exposed to quartz inhalation, they succumbed to the disease, and showed no signs that the inocculation served to modify the infection.

SOUTH AFRICA

Gold mining was started in South Africa in 1886, and in 1902 the occurrence of miners' phthisis was noted among rock-drillers and investigations made. Compensation was inaugurated in 1912 and 1916. Engineering methods were introduced to lower the dust concentration in mines, and the Kotze konimeter, which was devised by Sir Robert Kotze for the estimation of the concentration of dust, was used. The safe limit of concentration was placed at 300 particles/c.c. Among the many workers in South Africa who have been responsible for the furtherance of the knowledge of silicosis, Watkins-Pitchford (1927), Watt, Irvine, Johnson and Stewart (1916), Irvine (1930 and 36), Mavrogordato (1922 and 26) and Simson (1930 and 35) deserve special mention.

CANADA

As early as 1875, Sir William Osler described the pathology of miners' dusted lungs. Silicosis has been studied in the gold fields as well as in various other industries. Cunningham and Riddell in the Industrial Hygiene Division of the Ontario Health Department worked on the clinical aspects of the disease. Belt (1929) carried out experimental work and clarified the pathology of the condition. Much work on the subject has also been done

at the Banting Institute by King, Fallon, Stantial, Irwin and others. Irwin in 1934 described a micro-incineration technique for detecting the silica in tissues in relation to fibrosis, while in 1937 and 1939, the same worker, in conjunction with Denny and Robson, showed that quartz fibrosis could be prevented by the simultaneous inhalation of metallic aluminium.

AUSTRALIA

Silicosis was found among workers in the mining and metalliferous industries in Australia. In 1902, "sewer disease" was reported among those digging a system of sewers, and the cause was believed to be dust. In 1914, a Royal Commission reported that at Broken Hill the incidence of lung infection among underground workers was very high and that dust inhalation was a predisposing factor. X-rays were first used for the diagnosis of silicosis in 1919, and in 1921 standards for diagnosing different stages of the disease were set up. Badham (1924) placed the standard permissible concentration of dust at 200 particles/c.c. Compensation for silicosis was established in 1927.

GERMANY

Although Paracelsus first described lung diseases due to dust inhalation, it was Arnold (1900) who definitely distinguished between tuberculosis and dust fibrosis. Mortality rates among workers in various trades were studied by Summerfeld (1894), Moritz and Ropke (1899), Lewin, Hirt and Wilbrahdt (1876). The X-ray appearances of the

lungs of silicotics were described in detail by Staub-Otiker (1916), the earlier lesions being described by Koelsch (1930) and Koestle (1928 and 1936). Compensation for silicosis was introduced in 1929. The experimental aspects have been studied by Lehmann (1935 and 1936), Gross (1931) and Jotten (1933, 1935 and 1936).

FRANCE

Coal mining was started in the coal fields in Northern France in 1720, and was extended gradually to the rest of the mining areas (Leclerq, 1950). Oddo (1899) was the earliest worker to correlate an X-ray picture of pneumoconiosis with the post-mortem findings. Although the occurrence of pneumoconiosis among coalminers in the Pas-de-Calais had been recognized for many years, it was not until recently that the condition was investigated from the point of view of compensation.

In 1929 the French Ministry of Labour passed a decree which made it obligatory on doctors to notify pulmonary affections caused by dusts, in order to obtain information for purposes of compensation (Feil, 1930). In 1930, a systematic study of the occurrence of pneumoconiosis in miners was made and the proportion of workers affected by silicosis was found to be 8% (Brehan and Leclerq, 1934). Courozier and Magnin (1937) reported a high incidence of tuberculosis among miners exposed to silica dust. In 1945, all miners were subjected to medical examinations and mass radiography, as a result of which the incidence of silicosis

among all miners was estimated at between 16% and 18% (Leclerq 1950).

Several workers have been engaged in the study of the problem of pneumoconiosis in France and among the many such the following names may be included :- Policard (1930 and 1931); Policard and Devuns (1930); Policard and Martin (1930); Magnin (1935); Policard (1939 and 1947); Nicod (1950); Major (1950).

ITALY

Pneumoconiosis in various industries in Italy has been studied by Davoto and Cesa Bianchi (1911), Pesenti, Mazzi (1913) and Bardi. Giglioli's work established that silica was the cause of the lung lesions produced in pneumoconiosis.

SCANDINAVIA

The first cases of silicosis were diagnosed in Norway in 1933, and since that time over 2,000 cases have been diagnosed till 1948, out of a group of about 20,000 exposed workers (Brumsgaard, 1950). These include asbestosis, and talc pneumoconiosis as well as silicosis. Under an Act of 1937, all miners must undergo a full medical examination, including X-rays, before taking up employment.

In Sweden, silicosis was recognised as an occupational disease under the Workmen's Compensation Act in 1931 (Forssmann, 1950). An investigation undertaken by Bruce revealed that about 6,500 workers are exposed to silicosis hazards in various industries. Workers exposed

to such risks must submit themselves to a medical examination before, and periodically during, employment. This practice was introduced in 1939 and enforced since 1941.

NOMENCLATURE AND ITS SIGNIFICANCE

The word "pneumonoconiosis" or "pneumoconiosis" as it is more popularly used, is of Greek derivation (πνεύμων = lung, κόνις = dust), and was introduced in 1866 by Zenker, a German pathologist, to describe pulmonary fibrosis resulting from dust inhalation. In its widest sense the word pneumoconiosis is used as a generic term to describe all forms of pulmonary reaction to inhaled dust, with no implication as to its character, severity or effect on function, a view which covers the whole field of pneumoconiosis (Gardner, 1940).

It is now generally accepted that when the dust causing pulmonary fibrosis contains a large percentage of silica, e.g. quartz, the condition be termed silicosis. Cooke (1924) and McDonald (1927) used the term asbestosis to indicate fibrosis resulting from the inhalation of asbestos dust. Various organic dusts are also known to be capable of producing fibrosis, and these conditions have a nomenclature indicative of the causative agent, e.g. byssinosis from inhalation of cotton dust, bagassosis from inhalation of cane sugar fibres.

At the present time pneumoconiosis is an accepted occupational disease in many industries all over the world. It is important from the point of view of Workmen's Compensation, and in Great Britain it is defined for this purpose in the National Insurance (Industrial Injuries) Act, Sect. 57(3) as :- "Fibrosis of the lungs due to silica dust,

asbestos dust or other dust, and includes the condition of the lungs known as dust reticulation.

NATURE OF THE DUST AND ITS RELATION TO PNEUMOCONIOSIS

Dust may be defined as particulate matter in a state of fine subdivision to be blown about in clouds by the wind, and may be organic or inorganic. Mavrogordato (1930) classified dusts entering the lungs into four groups :- (1) harmless dusts; (2) dust which is harmless but may contribute to the damage when inhaled with harmful dust, e.g. coal, iron ore; (3) harmless dusts which have a protective or antidotal value when inhaled with harmful dust; (4) harmful dusts, such as free silica and silicates - quartz and asbestos.

Dusts met with in industries may be of animal, vegetable or mineral origin (Doig, 1949). Of these, only the inorganic mineral dusts are regarded as being responsible for the production of pneumoconiosis in the strict sense of the word. Miller and Sayers (1924) divided them into two artifical groups, based on the tissue reactions evoked by them in guinea-pigs intra-peritoneally.

(A) Dusts containing free silica, silicates or silica mixed with other dusts, and capable of producing fibrosis - active or proliferative dusts.

(B) Dusts which do not contain silica in any form and therefore produce no specific action, but only a foreign-body reaction with mild reticulinosis - inert or non-proliferative dusts.

In many industries these two types of dust may co-exist and so modify the clinical and pathological features of the diseases produced by their inhalation (King and Nagelschmidt, 1945).

King (1950), classified industrial dusts according to their varying degrees of pathogenicity as follows :-

1. Pathogenic dusts which produce the maximum lung lesions, e.g. quartz, flint. They are mostly free silica dusts and have a fair solubility in body fluids.
2. Inert or diluent dusts, e.g. coal, graphite. These are usually non-siliceous, of low solubility and produce only very mild lung lesions due, perhaps, to the blocking of lymphatics.
3. Anti-dotal dusts, e.g. shales. They are mainly silicates, have a low solubility and produce hardly any pulmonary lesions. When mixed with quartz, they depress its silica solubility.

Pneumoconiosis is a serious problem in many industries where the workers are exposed to free silica, silicates, mixed stone-dusts and mixed stone and coal dust (King, 1951). The industries concerned include mining and quarrying of siliceous rocks, manufacture of abrasives, potteries, coal-mining, metal grinding, sand-blasting, etc.

Various factors play a part in the rate of incidence and the grade of intensity of the disease, the significant ones being the nature of the dust (chemical and physical), the degree of exposure to the dust, presence of

infection, and individual susceptibility.

The chemical nature of the dust and its relation to pneumoconiosis depends on the amount of free silica or silicate it contains. Dusts containing large amounts of free silica are harmful, while some silicates too are harmful.

The concentration of dust in the atmosphere inhaled, and the period of exposure to it, directly influence the incidence of the disease. Small amounts of dust when inhaled can be eliminated from the lungs without giving rise to any deleterious effects, whereas larger amounts may remain in the lung and set up pathological changes. The so-called "safe limit" of dust concentration in the atmosphere has been placed at various levels by many workers on this subject - Kotze (1916) in South Africa set it at 300 particles/c.c., Badham (1924) in Australia at 200 particles/c.c. It is now believed that concentrations below 110 particles/c.c. cause no damage, 185-370 particles/c.c. produce nodular fibrosis in some cases, and over 370 particles/c.c. are definitely dangerous.

The amount of dust in suspension in the air breathed depends on the particle size, specific gravity, agglutinability and the capacity of the dust to absorb water. Heavy and large particles settle quicker than smaller ones, while those which clump remain in suspension for a shorter period than those which do not. Particles less than 1 μ in diameter settle 1-3 ft/hr., while in the case of particles 5 μ in diameter, specific gravity exerts a greater influence -

sp. gr. of 7 falls at 60 ft/hr. and sp. gr. from 2-6 at only 25 ft/hr.

The size of dust particles - mainly silica- which produce harmful effects on the lungs, has been studied by McCrae (1913) Watkins-Pitchford and Moir (1916), Mavrogordato (1926), Giese (1931) and Policard (1933). It is agreed that most of these particles are about the same size as ordinary micro-organisms. The smaller particles have a greater surface area which is available for chemical activity. Thus they have a greater solubility. Particles below 0.5 μ may be dissolved and excreted in the urine without causing much damage. Similarly, particles over 10 μ are removed by ciliary action in the upper part of the respiratory tract. Particles between 0.5 μ and 10 μ - particularly those under 5 μ - enter the terminal bronchioles which have no ciliated epithelial lining, and can be removed only by phagocytes via the lymph channels. These are the particles that cause damage to the lungs. Gardner and Cummings (1933) showed that the most dangerous particles were those between 1 μ and 3 μ. With asbestos fibres the dangerous range is 15-50 μ, but even up to 200 μ may produce lesions.

In general, therefore, the smaller the silica particle inhaled - within the harmful range - the greater the chances of its penetrating into the most vital parts of the respiratory organs and there producing lesions.

SILICA - ITS DISTRIBUTION IN NATURE, SOLUBILITY AND METABOLISM

28% of the earth's crust consists of silica. Oxides of silicon (SiO_2) form about 60% of all siliceous rocks, the actual amount varying from 30% - 80%, depending on whether a rock is acidic or basic. Silica is also present in plants, some of which, like horsetail, have a purely siliceous skeleton.

Silica occurs in nature as free silica (SiO_2) or in combination with metals as silicates. Free silica occurs in earths, ores and stones in various states of purity. Pure silica exists in three forms :-

(1) Crystalline, e.g. quartz, tridymite, cristoballite.

(2) Cryptocrystalline, e.g. flint, chert, jespar, chalcedony.

(3) Amorphous, e.g. opal, tripoli, diatomaceous earth.

Coal, haematite, granite, graphite and slate may contain varying amounts of silica as a contaminant.

Silicates occur in two forms :-

(1) Hydrous silicates, such as mica, talc and kaolin varieties.

(2) Anhydrous silicates, e.g. felspar, pyraxene, amphibole, garnet and topaz.

Solubility of Silica

Silica is usually believed to be insoluble owing to its inert and crystalline nature. The results of earlier

workers on the solubility of silica have little to commend them, since the finely divided particles of quartz could not be easily separated from the solution. As early as 1874, Lunge and Schorer-Tscherny reported the complete solution of powdered quartz in 1% KOH and NaOH in 32 and 34 hours respectively.

In 1913 Miller found that the particle size of quartz had an effect on its solubility, the smaller particles having a greater solubility. Other workers confirmed these findings (Briscoe et al 1937, Lucas and Dolan, 1938). By the use of high speed centrifugation (King and McGeorge, 1938) and ultra-filtration (Titus, 1937) it was found that quartz particles could be removed from the suspension, and the true amount of silica in the solution estimated chemically.

Besides particle size, the presence of small amounts of impurities was found to effect silica solubility by depressing it, e.g. the metals aluminium, iron, calcium, magnesium and their oxides (Kettle, 1934, Denny et al 1937, 1939 (a) and (b)), mineral silicates such as kaolin (Gardner, 1938; Belt and King, 1945), coal (Haldane, 1917 and 1931), and clay (Hefferman and Green, 1928). Silicates have a lower silica solubility as compared to similar-sized particles of free forms of silica, dissolving only to the extent of about 1 mg. SiO_2 in 100 ml., whereas quartz dissolves between 4-14 mg. SiO_2/100 ml.

It seems, then, that the factors influencing the degree of solubility of silica are (a) the particle size,

(b) the concentration of suspended solid, (c) the presence of small amounts of impurities in the dust, (d) the method employed to separate the solid from the liquid phase.

The solubility values for silica and silicate dusts from the work of Archer (1932), Briscoe et al (1937), Denny et al (1937), Whitehouse (1937), King and McGeorge (1938) are as follows, as from King's "Silicosis: A Review " (1942).

Solubility values for Silica and Silicate Dusts

Dust	mg. SiO_2/100 c.c.	Liquid
Amorphous silica	10.0	Water
" "	14.0	Pleural fluid
" "	9.0	Blood serum
" "	9.1	Ascitic fluid
Quartz	4.3	Water
"	3.8	"
"	2.7	"
"	19.0	1% $NaHCO_3$
"	9.1	Ascitic fluid
"	8.9	Blood serum
Flint	14.7	Water
"	6.5	Ascitic fluid
Granite	0.7	Water
"	5.0	1% $NaHCO_3$
Asbestos	11.2	Water
"	2.1	"
Fuller's earth	1.4	Ascitic fluid
Kaolin	0.7	Water

Dust	mg. SiO_2/100 c.c.	Liquid
Kaolin	0.4	Ascitic fluid
Sericite	0.7	Water
"	0.8	Ascitic fluid
Talc	0.8	Ascitic fluid

Metabolism of silica

Silica is a normal metabolite of the body (King and Stantial 1933), and is found in small amounts in nearly all tissues. When excessive amounts of silicic acid are absorbed from the intestines of animals excretion takes place in the urine without an appreciable rise in the blood silicic acid level, which is normally 1-2 mg/100 ml. The accumulation of large quantities of silica in the body is thus prevented. An entirely different state of affairs ensues when solid silica is introduced into the tissues. The particles are not easily removed and remain indefinitely in the tissues releasing silicic acid in high concentrations, consequently giving rise to silicotic nodules.

THEORIES OF SILICOSIS

It was a common belief among the earlier workers in this field that the lung fibrosis in pneumoconiosis was due to the mechanical irritation caused by the sharp edges of inhaled dust particles (Thackrah 1832; Holland 1843; Allison and Hugh Miller 1869). This was known as the mechanical theory and for some time it was accepted. However, Gardner (1923) showed that carborundum particles, which have very sharp edges, when introduced into the lungs of animals did not produce any fibrosis. On this piece of work, the mechanical theory could not be upheld without question, and in fact, has long since been discarded.

Collis was probably the first to suggest that the chemical nature of the dust was responsible for the production of fibrosis. The chemical theory received support from many workers. Gye and Kettle (1922), and Gye and Purdy (1922 and 1924) showed experimentally that fibrosis was due to the toxic action of silica on tissues. Conclusive proof of the chemical theory was produced by Kettle in 1932, when he demonstrated that quartz particles, which stimulated fibrous tissue formation when introduced into the lungs of rats, failed to do so if they were coated with a layer of iron oxide, which in no way altered their angular nature. He suggested that a toxic substance - silicic acid - was released from the dust particles and this irritated the surrounding tissues, giving rise to fibrosis. Many workers,

including Mavrogordato (1926), Policard (1933), Fallon (1937), King and Belt (1938) and Gardner (1940), agreed with Kettle's views.

King and Belt (1938) stated that the solubility of any stone dust was directly related to its pathogenicity. This is so in the majority of cases but it does not always appear to work in practice (King, 1945). Several such dusts are mentioned by King (1947) where the pathogenicity and solubility are not in direct proportion to each other, e.g. a powdered sandstone of low solubility was found to be very pathogenic, while 20 angstrom silica produced no fibrosis, although its solubility was high, and olivine which had an intermediate solubility produced very little reaction in the lungs of animals.

In the study of the solubility theory of silicosis, the exchange of bases between the mineral particles and the surrounding tissues is of importance. According to Bragg, silica minerals are classified into two groups, depending on whether their crystal structure is octahedral or tetrahedral. When molecular exchange takes place between the mineral and its environment, elements such as calcium, potassium, sodium, are replaced by water or hydroxyl ions which enter the molecular lattice of the mineral. This produces hydration of the mineral and alkalinisation of the medium. The latter occurrence has been found to increase the solubility of silica in <u>in vitro</u> experiments. Policard (1933) showed that alkalinisation also has an effect on the cell reaction -

cells enlarge, nuclei hypertrophy and vacuolation of the cytoplasm occurs.

Freshly-split particles of dust were believed to have greater biological activity than old particles (Heffernan and Green, 1928), since the fractured surfaces possess at numerous points free and unsatisfied valency forces whereby water is attracted and held in the hydrated particle. This hydration can liberate bases held in the crystal lattice. This theory has not received much support, while another put forward by Jones (1933 and 1934) that the pathogenicity of a dust was due to the presence in it of the fibrous mineral silicate sericite, could not be substantiated by the results of animal experiments.

AETIOLOGY

It was realised from the earliest times that lung affections of workers in certain occupations were due to the inhalation of dust. The predominant feature of the disease was lung involvement, usually associated with tuberculosis, and the condition was spoken of as phthisis. Some confusion existed as to the relationship between this condition and tuberculosis itself. Hall (1857) pointed out that there was no connection between Sheffield Grinders' disease and thoracic consumption, and Koch's discovery of the tubercle bacillus in 1882 finally settled the question.

Dust was considered to be the harmful factor in these occupational diseases by various workers through the ages. Gregory (1831) described miners' lungs as being infiltrated with black material like coal. Thackrah (1832) believed that any kind of dust - animal, vegetable or mineral - produced injury to the respiratory organs, depending on the degree of mechanical irritation of the bronchial mucous membranes. Calvert Holland (1843) gave an account of the structural changes in the lungs, brought about by dust inhalation.

In 1902, an English Department Committee which included Dr. Haldane among its members, reported that while the primary lesion in the lungs was due to dust inhalation, it predisposed to pulmonary tuberculosis, so that many miners ultimately died of the latter disease.

Collis in 1915 came to the conclusion that silica in the form of free silicon dioxide was the sole dust capable of stimulating the formation of fibrous tissue. This was based on analytical studies, from which he also concluded that non-siliceous dusts were far less harmful than siliceous ones. McCrae (1913) demonstrated that the majority of dust particles gaining access to the lungs were less than 3 μ in diameter, and the largest ones were not more than 10 μ. Gye and Purdy (1922) and Gye and Kettle (1922) stated that crystalline silica dissolved in the body to form colloidal silica which acted as a cell poison.

Up to this time free silica was unanimously held to be the sole causative agent of pneumoconiosis. More recently, however, it has been found that dusts other than those containing free silica were capable of producing pulmonary fibrosis. Of these, silicates or compounds of silica are well known. Out of the many mineral silicates, asbestos - a silicate of iron, magnesium and aluminium - has been proved to produce fibrosis in the lungs of experimental animals. Talc, french chalk, kaolin and a variety of other silicates have also been found to produce a mild form of pneumoconiosis. King (1947) is inclined to the view that silicates are less toxic than free silica-containing dusts, owing to the lower solubilities of the former.

Various mixed dusts and some non-siliceous mineral dusts too are capable of producing pulmonary lesions. Coal, graphite, granite and haematite produce a mild degree of

fibrosis, the so-called "dust reticulation". Aluminium, according to some workers, is a harmful dust, producing lung disease in those who inhale it, while others believe that it has an inhibitory action on quartz, preventing the occurrence of silicosis to some extent. King and his associates (1950) found that,in experimental animals, aluminium had an inhibitory effect on the silicosis-producing action of quartz.

Predisposing Causes

While all races are susceptible to dust disease, the incidence has been found to be higher among those previously exposed to dust and to respiratory infection. Some individuals among those exposed to dust seem to show a greater predisposition than others, although the reason for this individual susceptibility is still beyond explanation.

PATHOLOGY OF SILICOSIS

In order to have a clear concept of the pathology of pneumoconiosis and silicosis, it is necessary to understand the mechanisms provided for the elimination of particulate matter from the lungs. These can be considered under the following processes :- the ciliary action of the cells of the mucous membrane lining the respiratory passages; alveolar phagocytosis; and the pulmonary lymphatic system.

Under normal circumstances the cells of the lining membranes of the bronchi are ciliated and capable of removing inhaled particulate matter. This protective mechanism was first demonstrated by Leonard Hill (1928), when he found that a suspension of lamp black in Ringer's solution was carried along the mucous membrane by cilia at the rate of 3 cm/min. Both fine and coarse particles were carried in that way. If a small area of ciliated epithelium is damaged, e.g. by repeated attacks of infection or injury, the cells are replaced by squamous epithelium which is non-ciliated and permits the accumulation in the lungs of inhaled dust particles. Any factor which causes inhibition of the normal ciliary action, therefore, prevents the elimination of inhaled dust from the lungs.

When dust particles enter the alveoli through failure of the protective mechanisms, the formation of "dust cells" occurs. These cells are formed by hypertrophy and hyperplasia of the septal cells of the alveoli but their exact origin - whether epithelial or mesenchymal - is still

in doubt. Policard believes that they originate from mesenchymal elements and histiocytes. They appear in large numbers, acquire phagocytic powers which they do not normally possess, and engulf dust particles they come in contact with (Fenn 1921). These macrophages or phagocytes, or "dust cells" as they are called, accumulate in the alveoli and pass through their walls to enter lymphatic vessels. Some cells may enter fine bronchioles and be removed in the sputum by coughing up.

Miller (1947) divided the lymphatics of the lung into closed and open types. In man they are of the closed type and do not communicate with the pleural cavity or the connective tissue spaces by means of stomata. The lymphatics of the lung are arranged in a superficial set in the pleura and a deep set accompanying the bronchi, bronchial and pulmonary vessels and in the connective tissue septa between the secondary lobes. A communication exists, at the pleura and the hilum, between the two sets. At the points of communication between the lymphatic trunks within the lung are small collections of lymphoid tissue - lymph follicles - which act as filters and gradually increase in size as the hilum is approached. The lymphatics discharge most of their contents into the tracheo-bronchial lymph nodes.

Drinker and Field (1933) stated that once dust particles entered the alveoli they were carried into the lymphatics either by means of dust cells or as free particles. The first sites of deposition are the intra-pulmonary lymph

follicles and then the tracheo-bronchial lymph nodes (Simson 1930).

Histogenesis of the silicotic nodule

Dust particles ingested by cells exert a specific influence on them, depending on the type of dust ingested. Inert dusts like coal and soot do not affect the protoplasm of the cell in any harmful way and are excreted (Gardner 1923). Silica particles, on the other hand, are toxic to cell protoplasm and inhibit cell motility. Mavrogordato (1922) and Policard (1933) have stated that the cell is killed and undergoes a process of "mummification", when acted on by silicic acid which is formed by the slow disintegration of silica particles. Some cells may even undergo destruction, leaving behind insoluble particles of silica which may be phagocytosed anew and so the process continues. Gye and Purdy (1922) and Kettle (1932) believed that the resulting changes in the tissues were due to the toxic action of colloidal silicic acid formed by the disintegration of silica particles. Fallon (1937) was of the opinion that the formation of the silicotic nodule was due to the action of phospholipids released by the macrophage cells containing the irritant particles. Whatever the cause, the dissolution of silica stimulates the proliferation of fibroblastic tissue in its vicinity, with the formation of a nodule in consequence. This nodule is composed of connective tissue fibres - reticulin in the early stages and collagen later - characteristically arranged in the form of a wicker-basket. With increased

fibrous tissue formation the cells disappear and leave free silica particles within the nodule.

The initial phase in the formation of the silicotic nodule is the accumulation of dust-laden phagocytes in the intrapulmonary lymph follicles, which according to Simson (1930) and Strachan (1930) are the primary sites of involvement. The lesions, which are characterised at first by the occurrence of a network of reticulin fibres among the dust cells, progress with the reticulin becoming thicker and more compact, till collagenisation follows. Localisation of the lesions are in the root zones and the upper lobes in the beginning, but spread to all parts of the lung later. Macroscopically the early lesions may not be visible.

The next stage - discrete nodulation of classical silicosis - follows if the inhalation of silica dust goes on for a considerable time. The silicotic nodules increase in size and further accumulation of dust cells takes place. These cells are prevented from entering the lymphatics owing to back pressure and obstruction to the lymph flow. Aggregations of dust cells form on the alveolar walls and give rise to intra-alveolar nodules, which may vary in size up to about 5 mm. and are composed entirely of hyalinised collagen fibres arranged concentrically. A mild degree of emphysema may be found in the lung tissues between the nodules. An accompanying patchy pleurisy with adhesions to the chest wall is a common occurrence. The tracheo-bronchial lymph nodes, at this stage, are extremely fibrous, hard and small.

As the disease progresses to the stage of massive confluent fibrosis, neighbouring nodules coalesce to form large irregular fibrotic masses. Confluent fibrosis may involve any part of the lung and results in the replacement of normal lung tissue by massive areas of collagenous fibrous tissue, which may even be the size of a cricket ball. Blood vessels in the vicinity exhibit varying degrees of endarteritis obliterans and bronchi may show chronic inflammatory changes. The lungs are bulky, firm and gritty, with pleural adhesions and very often a layer of fibrin over the pleura indicating recent inflammation.

The picture of simple silicosis, then, is one of characteristic nodule-formation, which may be very cellular with little fibrous tissue in the early stages, completely fibrous and acellular later, with coalescence of nodules still later.

Mixed forms of silicosis are known to exist where the essential silicotic process is complicated by infection, the commonest of these being tuberculosis. According to Gloyne, the nodules in tuberculo-silicosis may assume one of several appearances, e.g.(1)they may possess a grey tuberculous centre with a pigmented silicotic periphery; (2) a pigmented silicotic centre with a tuberculous periphery; (3) a ring of small tubercles surrounding a pigmented silicotic centre; (4) tubercles and silicotic nodules together. Histologically the nodules of tuberculo-silicosis are irregular in shape, composed partly of collagen fibres and partly of caseous

material, with a few giant cells seen outside the nodule. Tubercle bacilli may or may not be present. The appearances are so variable that Kettle and Archer (1933) stated the difficulties involved in diagnosing the condition histologically. Chemical examination of the lungs for silica, together with histological studies, may be of assistance. Rapidly Developing Silicosis is a condition characterised by the development of lung lesions leading to a fatal termination, following on a short exposure to the inhalation of siliceous dust. Earlier workers studied the pathology of the condition and its relation to infection and references to it are found under the term "acute silicosis" (McDonald, 1932; Chapman, 1932; Kettle, 1932; Gardner, 1933; Irvine, 1938). King (1938) held that it was merely a rapidly developing form of the disease.

The sequence of events is probably a breaking down of the normal defence mechanisms of the lung and inability to deal with the excessive amounts of dust inhaled, which become deposited in the lungs. This leads to the release of a large amount of silicic acid with the result that a peculiar reaction develops - alveoli contain numerous dust cells, the connective tissues throughout the lungs proliferate and produce thickening of alveolar walls with the formation of minute acellular nodules with hyaline centres. Focal areas of necrosis offer a favourable medium for the growth of tubercle bacilli. Irvine (1938) gave the term "dust pneumonia" to this condition.

The picture of classical silicosis is often modified by the mixtures of dusts inhaled - free silica may exist in a mixture with other silicates and mineral dusts. In such cases, the other dust may be found deposited round the silicotic nodule. The fibrous tissue in the nodule does not go on to the stage of full collagenisation. Nodules may coalesce to form confluent areas but they are not as massive as in cases of pure silicosis.

COMPLICATIONS AND SEQUELAE

The complications of silicosis fall into three main groups :- (1) Pulmonary infection, (2) Cardiovascular changes, (3) Malignancy.

(1) Pulmonary Infection - By far the most important complication is pulmonary tuberculosis - 21% in the series of cases studied by Burton Wood and Gloyne (1934). Silicosis appears to predispose to the development of tuberculosis and the latter aggravates the course of silicosis. Other forms of infection due to various organisms, such as streptococci and pneumococci, also occur, probably as a result of the break-down in the defensive mechanisms in the lung. The occurrence of fungal infection has been noted (Fawcett, 1936). Due to the fibrosis, bronchiectasis is a common complication. Compensatory emphysema develops round the nodules and bullae may be found along the lung margins. Rupture of an emphysematous bulla gives rise, not infrequently, to spontaneous pneumothorax.

(2) Cardiovascular changes - The development of emphysema and pulmonary hypertension in silicotics results in right ventricular hypertrophy (cor pulmonale) and ultimately in right heart failure. Gloyne places importance on the occurrence of flabby myocardium in cases of silicosis, while fibrous myocarditis following on mediastinal lymphatic stasis is also found.

(3) Malignancy - The relationship between carcinoma of the lung and silicosis is not clearly established. Statistics show that there is no true relationship between the

two conditions, except in the Schneeberg and Joachimstaal mines where uranium and arsenical ores have been thought to be the carcinogenic agents.

DIAGNOSIS

The diagnosis of silicosis rests on the occupational history of the patient, the clinical examination and X-ray examination of the chest.

The occupational history is of importance in assessing the intensity of the risk which the worker is exposed to.

The clinical examination is not of much value as the symptoms develop very slowly in the early stages and very little information is obtained by chest examination at this stage. Cough and dyspnoea are the usual symptoms and bronchitis and primary fibroid tuberculosis should be considered in the differential diagnosis of the condition.

Biochemical examination of blood and urine do not yield any useful information in the diagnosis of the disease.

X-ray examination of the chest is perhaps the most helpful factor in diagnosis, particularly in the early stages, and according to Meiklejohn (1948), is the most reliable single piece of evidence.

The importance of radiological examination in the diagnosis of the various stages of silicosis has led to different forms of classification based on X-rays, being put forward by many radiologists. The simplest of these is the three-stage classification which describes the opacities as

pre-nodular, nodular and massive fibrotic stages, and is used in most countries, such as Germany, Switzerland, Holland, U.S.S.R., Italy, Japan. Modified forms of this classification are used in Australia, South Africa and America. In the U.K. a classification distinguishing between 'simple' and 'complicated' pneumoconiosis, with the former subdivided into stages, is used (Fletcher et al 1949, Davies and Mann, 1949). France and Belgium have adopted a similar classification.

More recently the I.L.O. (1950) has put forward the following classification :- (1) Radiographs within normal limits (Category 0); (2) Pneumoconiosis with discrete opacities including three different categories, depending on the increasing degrees of abnormality; (3) Pneumoconiosis with coalescent or massive shadows, including three subdivisions, depending on the extent and density of the shadows.

Cochrane, Davies and Fletcher (1951), in association with French workers, suggested the following "Anglo-French" classification:- (a) Normal appearances, or those not necessarily pneumoconiotic - O and X respectively; (b) Simple pneumoconiosis - 1, 2 and 3 - indicating the profusion of the opacities, with the letters P (pinhead), M (micro-nodular) and N (nodular) added to 2 and 3 to denote the dominant histological type; (c) Progressive Massive Fibrosis with further division into groups - A (coalescent), B, C (Massive) and D (massive with abnormal pulmonary

architecture). This classification is being tried in the U.K., France and Belgium.

Post-Mortem findings - The autopsy of silicosis cases yields interesting information both to confirm the diagnosis and to assess the extent of the lesion. The characteristic macroscopic and microscopic lesions (already described earlier) leave no doubts as to the aetiology.

COALWORKERS' PNEUMOCONIOSIS

The existence of pulmonary disease among workers in coal mines has been recognized for many years. Pearson (1813) was the first to draw attention to the black material present in the lungs of city-dwellers. Gregory (1831), Marshall (1834) and Thomson (1836) described the pathological aspects of the disease occurring among Scottish coalminers. Stratton (1838) coined the term "anthracosis" for this condition.

In 1848 the first Miners' Act was passed and the conditions in the mines improved to such an extent that many people believed coalmining was no longer an occupation associated with ill-health (Arlidge, 1892). In fact, it was thought that coal dust inhalation, far from producing lung disease, acted as a protective mechanism against the development of tuberculosis (Oliver 1902 and 1908, Shufflebotham, 1914, Haldane, 1923). The true nature of the condition brought about by the inhalation of coal dust was recognised only after radiological examination was introduced.

Late in the last century, and in the early part of this one, various mechanical processes came to be used in mines - pneumatic drills, coal-cutting machines, screening plants - and these seemed to intensify the occurrence of disease among workers in them. Radiography in the diagnosis of chest disease among miners was introduced in 1925, and appearances similar to those of silicosis were found in lungs

(Collis and Gilchrist, 1928; Harper, 1934). In 1934 the Compensation Scheme was opened to all men working in coal and this was followed by a greater increase in certified cases in anthracite and steam-coal mines (hard types) than in bituminous.

The view that the lung lesions in coalminers were due to the presence of infection and not primarily to the inhalation of dust, was held by Fisher (1935) and Haldane (1935). As a result of these controversial views, the Medical Research Council undertook to investigate the problem of chronic pulmonary disease among coalminers in South Wales in 1937, and published their report in 1942.

The primary cause of the lesions was found to be the dust inhaled in the mines, particularly in those where a high rank of coal (coal with a high proportion of carbon), such as anthracite, was mined (Hart and Aslett, 1942). Radiologically, the lesions progressed through the stages of dust reticulation, nodulation, coalescent nodulation and massive fibrosis. Hart and Aslett suggested that the term "pneumoconiosis of coal-workers" should be used to include all the pulmonary manifestations due to inhalation of coal dust, and for purposes of compensation the radiological standard for diagnosis should include "dust reticulation".

Reports of pulmonary fibrosis among coalminers in other parts of the world appeared during this time - from South Africa in 1922 (Mavrogordato), from Germany in

1925 (Boehme), from Sweden in 1926 (Edling), from Australia in 1933 (Badham and Taylor), from France in 1935 (Magnin) and 1939 (Policard), from America in 1935 (Bloomfield et al), 1938 (Sayers), and 1942 (Jones).

Incidence: According to McVittie (1949) the disease requires 15-20 years' exposure for its development, and it occurs among colliers, firemen and shotsmen, hauliers, repairers, hard ground men, underground workers and surface screen workers.

Aetiology and Pathology: Although at one time several workers were of the opinion that coalminers' pneumoconiosis was due to causes other than the inhalation of dust - one among them being the sudden chilling of workmen when they came out of the pits - it is now accepted that it is the inhalation of dust which is the primary cause (Medical Research Council Special Reports 1942, 1943, and 1945). Furthermore, the occurrence of the disease seems to be proportional to the rank of coal. However, it has not been established yet whether the pathogenic element is related to the quantity or quality of the dust inhaled. Various suggestions have been put forward as to what it might be - the presence of excessive amounts of an inert dust, or the presence of small amounts of silica in the coal, or the fact that the organic matter in the coal may in some way stimulate fibrosis. More recently, Evans (1951) stated that certain types of coal contained 'vitricin', or V-coal particles with antibiotic properties. According to him a particular dust hazard was related to the amount of V-coal particles in respirable dust,

the hazard being greater when the amount was low and vice versa. Fletcher (1952) has refuted this theory.

Belt and Ferris (1942) described the pathology of pneumoconiosis as a fine reticulin fibrosis developing in the foci of accumulated dust, which may or may not go on to collagenous fibrosis. Gough (1940, 1944, 1947(a) and (b) (1949) and Heppleston (1947), described three forms of the disease - classical silicosis, simple pneumoconiosis and infective pneumoconiosis.

Classical silicosis only occurs among a few coal-miners engaged in cutting rock, the majority of workers developing one of the other forms.

Simple pneumoconiosis is due to the inhalation of dust alone and presents a picture of 'coal foci' distributed throughout the lungs. These foci are aggregations of dust-laden phagocytes and are black in colour, with crenated or scalloped edges and processes extending from the edges into the surrounding lung tissues - the so-called "medusae head" appearance. A delicate network of reticulin fibres develops within these foci, but there is no concentric arrangement of fibrous tissue as in the silicotic nodule. The reticulin fibres may progress to the stage of collagenous fibrosis. The alveoli around these foci are dilated and give a honey-comb appearance characteristic of the disease and termed "focal emphysema" by Gough (1947(a)). He attributed this to a mechanical disturbance within the secondary lobules of the lung, since it appeared first around the smaller bromchioles.

The emphysema is focal and no bullae are found along the margins of the lung. The degree of emphysema is not proportional to the amount of fibrosis.

Infective pneumoconiosis is held to be due to the combined action of dust and infection, very often tubercular. It is characterised by massive fibrosis, generally in the upper parts of the lungs. These areas may be firm in consistency. Areas of necrosis appear within these fibrotic masses, due to endarteritis obliterans of vessels, and this results in the formation of cavities. The tubercle bacillus may be demonstrated in the sputum in 30% of cases (Fletcher 1952). The progress of massive fibrosis in many cases is slow, with the formation of a large amount of fibrous tissue resulting ultimately in right ventricular failure. In a few cases, rapid progress occurs with the development of clinical tuberculosis.

Gough (1953), in summarizing the difference between coalworkers' pneumoconiosis and silicosis, places emphasis on the severe focal emphysema and massive fibrosis, giving rise to heart failure in the majority of cases of the former, with tuberculosis as a less common complication; in the latter, the usual terminal event is open tuberculosis, with heart failure occurring in but a few cases.

KAOLIN OR CHINA-CLAY

The main constituent of china-clay is the mineral 'kaolinite' which is a hydrated silicate of aluminium, and one of the most important exports from the United Kingdom at present. China-clays are used in many industries - pottery, china-ware, ceramics, soap, paper and cement being some of the commoner ones. The largest production of china-clay in the world is in Cornwall, while Czechoslovakia, Germany and Japan also produce it.

Lemaistre (1894) and Hlava (1897) were two of the first workers to report cases of pneumoconiosis due to kaolin. Recently others have made references to the condition (Koelsch, 1930; Pancoast and Pendergrass, 1931; Hartman, 1935; Langelez, 1935; Koestle, 1936). The lesions produced in the lungs are not similar to those produced by quartz (Middleton, 1936). Radiographs of the lungs of some workers in the Cornish china-clay industry were found to show well-marked reticulation (Hale, 1946). Thomas (1952) records evidence of silicosis in the necropsy findings on two workers engaged in the ball clay industry and in grinding china stone, a granite containing china-clay.

Experimentally, Kettle (1934) introduced kaolin into the lungs of guinea-pigs and found that only a catarrhal reaction resulted, while the same dust, when introduced with dead tubercle bacilli, produced definite fibrosis. Belt and King (1945) were able to produce only very minor changes in the lungs of animals exposed to dust from South Wales coal-

mines containing a large amount of kaolin. King, Gilchrist and Rae (1947) found that acid treatment increased the pathogenicity of kaolin.

King, Harrison and Nagelschmidt (1948) carried out further investigations on two types of kaolin occurring in the United Kingdom. They found that only a mild reticulinosis was produced by kaolin in the lungs of experimental animals, while ignited kaolin containing amorphous silica produced more reticulin but no definite fibrosis, unlike in silicosis.

INTRODUCTION TO EXPERIMENTS

The lesions of human silicosis have been successfully reproduced in experimental animals by the introduction of dust into their tissues (Wainwright and Nichols, 1905; Mavrogordato, 1918; Gardner, 1920, 1929; Gye and Purdy, 1922; Policard 1931; Kettle, 1932-34; Miller and Sayers, 1936; Belt and King, 1945; Ray, King and Harrison, 1951). Since the species of animal used for these purposes are short-lived, the time factor in such experiments must of necessity be curtailed and the reciprocal factor of dosage increased. In spite of these minor adjustments, the lesions produced, though not identical with those in man, are nevertheless very similar in the type of tissue reaction and the cells participating in them.

On the basis of such experiments, Miller and Sayers (1934) classified dusts as pathogenic or otherwise, depending on the lesions they produced. It was found that finely particulate silica, in either colloidal or soluble form, was capable of producing proliferative lesions in animals (Mavrogordato, 1918; Gye and Purdy, 1922; Kettle, 1924; Policard, 1931), while the so-called inert dusts produced very little reaction. The modifying effects of certain dusts on the action of silica has been studied by Kettle (1934), Denny, Robson and Irwin (1937), King, Wright, Ray and Harrison (1950).

According to the solubility theory of silicosis (King, 1947) the silica solubility of a dust as determined with Ringer's solution under standard conditions, bears a definite relation to its fibrogenicity. This has been found to be so in

the majority of cases. In animal experiments, however, it is not possible to estimate how much silica actually goes into solution in the tissues during the production of fibrosis, because the ratio of solid to liquid and the rate of renewal of the liquid are not known. It may be possible to assess the loss in silica - due to solubility - of an injected dust by determining how much dust could be recovered from the lungs and lymph nodes of animals at varying intervals of time after injection. This recovery of injected dust, or its rate of "elimination", is also important from another point of view. It is likely that some dusts may be eliminated more rapidly than others, particularly those which stimulate phagocytosis readily. It is reasonable to assume that those dusts which are phagocytosed and cleared away from the lungs in a short space of time have less chance of dissolving in the tissue fluids and so producing severe lesions, while those which remain longer in the lungs are more likely to do so. A study of the amount of dust left behind in the lungs and lymph nodes at varying intervals of time after the introduction of a known quantity of that dust would, perhaps, provide the answer to this surmise and reveal whether, in actual fact, a correlation exists between the pathogenicity of a dust and its rate of elimination from the lungs. A comparison of the rate of elimination of a known pathogenic dust, such as quartz, and an inert one like corundum, of equal particle size, would be of interest in studying the correlation between pathogenicity and removal of the dust from the lungs. It would also help

to determine the loss in silica, due to solubility, of the quartz dust.

It was once believed that the incidence of pneumoconiosis among coalminers in the South Wales coal-fields differed from that among workers in the coal-fields of Durham, Yorkshire and Cumberland. The coal mined in South Wales is mainly anthracite, which is of high rank (high carbon content and low volatility), whereas the coal in the other areas is chiefly bituminous and of low rank (low carbon content and high volatility). As a result of this alleged difference in the incidence of pneumoconiosis in the two regions, many workers have carried out animal experiments with anthracite and bituminous coal dusts to see whether there was any difference in the pathogenicity of the two. In spite of repeated attempts, both by inhalation and injection methods, the lesions produced by anthracite and bituminous coal dusts in the lungs of experimental animals have been similar as far as histological appearances were concerned (Belt and King, 1945). Although the histological lesions produced by these two types of coal are identical, the possibility exists that there may be some difference between these two dusts, perhaps in the rates at which they are eliminated from the lungs, even while their particle size remains equal. This could be determined quite easily by estimating chemically the amount of the particular dust remaining in the lungs at specific periods after the injection of a known quantity of it. It would also be interesting to

see whether the rate of elimination of a mixture of the two types of coal dust differs in any way from that of the individual dusts, their particle size remaining equal.

The rate of elimination of a dust can be determined by means of other methods besides chemical estimations. A known quantity of dust which has been previously irradiated, and whose radioactivity per unit weight is known, is injected into the lungs of experimental animals and the radioactivity remaining in the animal's thorax measured at regular intervals by making counts with a Geiger counter. The degree of radioactivity may be taken as a measure of the amount of dust in the lungs.

Many mineral dusts have been used in the experimental production of silicosis in animals, both by inhalation and injection. The different forms of free silica - crystalline and amorphous - have been found to give rise to progressive nodular fibrosis (Mavrogordato, 1918; Gye and Purdy, 1922; Gardner, 1938; King, 1947). Gardner injected various forms of pure silica including the two conversion forms of quartz - tridymite and cristoballite - intravenously into rabbits, and found that tridymite was the most pathogenic of them all. King, Mohanty, Harrison and Nagelschmidt (1953a) injected different forms of pure silica - tridymite, cristoballite, quartz and fused silica - of equal particle size and in equal amounts, into the lungs of rats and showed that tridymite was much more toxic than any of the other forms, producing the maximum fibrosis in the minimum time. The reason for the

extreme toxicity of this dust was not clear and they suggested that a further study of several samples of this dust might prove useful in throwing some light on the problem. The study of the reactions produced by varying doses of tridymite dust would reveal whether the dust is as toxic in smaller doses, and give some idea as to its toxicity when compared with quartz dust of the same particle size - 2μ - which size, according to Gardner, is capable of producing classical lung pathology.

The effect of coal on the pulmonary lesions produced by silica has been the subject of much controversy. Some workers believed that coal had an inhibitory action on silica (Oliver 1902; Collis 1915; Haldane, 1923), while others held that coal enhanced the lesions produced by silica (Ray, King and Harrison, 1951). The latter workers found that small amounts of quartz, when mixed with anthracite and bituminous coal dusts, produced more marked lesions in the lungs of experimental animals than the same amount of quartz by itself. Since this is so with mixtures of coal and quartz dusts, it is possible that the same might be true when mixtures of a more toxic dust, such as tridymite, and coal are used. Very small amounts of tridymite, when introduced into the lungs, should normally be removed by the protective mechanisms in operation without the formation of any lesions; but when these same quantities are introduced together with coal dust in larger amounts, the clearing mechanisms of the lung may break down, resulting in the formation of pulmonary lesions.

Silicosis remains a grave occupational problem in spite of the many attempts made to solve it. Much of the experimental work done in this connection has been along the lines of preventing the occurrence of the disease as far as possible. Some workers have advocated the use of an antidotal dust, such as aluminium, which is believed to have an inhibitory effect on the action of silica (Denny, Robson and Irwin, 1937). This view has been open to much criticism, as aluminium dust by itself is reported to have produced pulmonary fibrosis in workmen inhaling it. A different approach to the problem of silicosis has been made by Kennedy, Pare, Pump, Beck, Johnson, Epstein, Venning and Browne (1951), whose work was based on the fact that cortisone and A.C.T.H. have been found to exert a beneficial effect in a number of disorders, including the so-called "mesenchymal diseases" such as rheumatic fever, lupus erythematosus and periarteritis nodosa (Hench, Slocumb, Barnes, Smith, Polley and Kendall, 1949; Howard, 1949). Kennedy and his co-workers treated two cases of beryllium granulomatosis of the lungs with cortisone and A.C.T.H., and reported some degree of improvement in respiratory function and in the X-ray appearances of the lungs. They were, however, unable to obtain the same measure of success in their treatment of a case of pulmonary silicosis, although some improvement in the clinical condition of the patient was evident. In experimental animals, cortisone has been found to retard the migration of dust-laden phagocytes in the reticulo-endothelial system (Spain, Molomut and Haber, 1950), and in

the peritoneum (Policard and Tuchmann-Duplessis, 1951). The effect of cortisone on developing granulation tissue in artificially-produced wounds in experimental animals has been widely studied. As a result of these studies, cortisone has been found to inhibit the development of granulation tissue in rabbits (Ragan, Howes , Plotz, Meyer and Blunt, 1949) and mice (Spain, Molomut and Haber, 1950), and bring about a quantitative reduction of it in rats, while it had no significant effect on granulation tissue in guinea-pigs (Bangham, 1951). On the basis of these findings, Harrison, King, Dale and Sichel (1952) studied the effect of cortisone on developing silicotic fibrosis in the lungs of rats, and found that the main effect was on the migration of dust-laden phagocytes which remained loosely scattered throughout the lungs and within alveoli. This interference with the accumulation of dust cells was found to retard the development of silicotic nodules. Magarey and Gough (1952 (a)), demonstrated that cortisone inhibited the fibrosis produced by quartz when injected into the peritoneal cavity in mice, and depressed it in rats,and that it had no effect on established silicotic fibrosis in the rabbit's peritoneum (Magarey and Gough,1952(b)).

The natural sequel to these discoveries would be the investigation of the effect of cortisone on established silicotic fibrosis in experimental animals, although it seems unlikely that mature fibrous tissue could be acted on by cortisone.

The rôle of infection in pneumoconiosis is of great importance as the majority of silicotic patients are known to die of infection, particularly tuberculosis. A high incidence of pulmonary tuberculosis in different trades involving the inhalation of siliceous dusts has long been recognised. Haldane (1914) maintained that "the disease is the same the world over and the end comes through tuberculosis." Gough (1947a) stated that the infective type of pneumoconiosis was of frequent occurrence among coal-miners, the infection most commonly associated being tuberculosis.

The relation between tuberculosis and pneumoconiosis is not very clearly understood; that one influences the pathological lesions produced by the other is definite, but how they do so is less well known.

Price (1932) found that silica stimulated the growth of the tubercle bacillus in vitro, while Lanza and Vane (1934), Sweaney (1936), Middleton and Heffernan (1936) and Irvine (1938) found that the morbidity and mortality rates from tuberculosis were higher among workers exposed to silica dust than to other dusts. Experimentally, it has been found that when attenuated or dead tubercle bacilli are introduced into the lungs of animals along with silica, proliferating lesions are produced which do not occur when the organisms alone are used (Kettle, 1924 and 1934; Gardner, 1929; Cummins , 1940). Kettle (1934) proved from his experiments that when kaolin or dead avirulent tubercle bacilli were injected into the lungs of guinea pigs no permanent fibrotic lesions appeared, while the combined action

of dust and dead organisms resulted in a considerable amount of fibrosis. Gardner (1929), Policard (1939) and Senn (1939) showed that latent tubercular foci in animals could be reactivated by the inhalation of silica dust. Much experimental work on the combined action of dust and tubercle bacilli on the lungs of animals needs to be carried out before any definite conclusions can be arrived at regarding the exact rôle infection plays in pneumoconiosis.

From the foregoing facts it is apparent that several aspects in the field of pneumoconiosis research warrant closer investigation. The following experiments were therefore devised, in the hope that they might, in some way, help to clear at least a few of the many problems that face the research worker in this field :-

Section I Study of the rates of elimination of various dusts from the lungs of rats, and the tissue reactions produced by them.

A) (i) Quartz dust
(ii) Aluminium oxide dust

B) (i) Anthracite coal dust
(ii) Bituminous coal dust
(iii) Mixture of anthracite and bituminous coal dust.

C) Radio-active tantalum carbide dust

Section II The action of variable amounts of tridymite on the lungs of rats.

Section III The action of anthracite and bituminous coal dusts mixed with small amounts of tridymite on the lungs of rats.

Section IV The effect of cortisone on established silicotic fibrosis in the lungs of rats.

Section V The influence of coal, mine dust and kaolin on avirulent tuberculous infection produced by dead organisms (B.C.G.) in the lungs of rats and guinea-pigs.

EXPERIMENTAL TECHNIQUE

This chapter deals with the routine procedures that have been employed in the experiments described in this work. They include the preparation of a dust before its injection into experimental animals; the technique of intratracheal injection which has been the method adopted in all the experiments embodied in this thesis; histological and micro-incineration techniques. A brief description of the method of assessing the lung pathology and the grading of fibrosis from histological preparations is also included.

A dust, prior to its use in experiments, should be particle-sized. Most workers are agreed that the average particle size of minerals found in the lungs of pneumoconiotic patients is about 1.2μ in diameter (Greenhow, 1865; McCrae, 1913; Watkins-Pitchford and Moir, 1916; Cooke, 1933). Particles $>$ 12μ are not pathogenic with the exception of asbestos fibres. Gardner and Cummings (1933) showed that quartz particles $<$ 3μ are more fibrogenic than larger ones. According to Tebbons and Drinker (1945), the potency of a silicosis-producing dust increases inversely with the particle size. Silica particles below 4μ induce fibrosis easily. Dust samples used for experimental purposes should therefore preferably have a size distribution below 5μ.

The silica solubility of a dust is also of importance with regard to the pathogenicity of a dust, and all dusts used for experimental purposes should have their

solubilities determined. This is done by taking a known quantity (200 mg.) of dust in a definite amount (10 ml.) of buffered Ringer's solution of pH 7.2, and keeping the mixture agitated in a slow shaker for a week. The amount of silica in the filtrate is determined by the colorimetric method. From these figures the amount of silica dissolved in 100 ml. of buffer solution is calculated, and this is known as the silica solubility of the particular dust.

The methods available for the study of the reactions produced by various dusts in the tissues of experimental animals are many. Kettle (1932) injected suspensions of dust into the subcutaneous tissues of animals to study the reactions produced. He maintained that this was a quicker method of obtaining information regarding the pathogenicity of a dust. Stuber (1934) injected suspensions of dust into the main lymphatic in the popliteal space of animals to study the reactions in the neighbouring lymph glands. Miller and Sayers (1936) produced lesions in the anterior abdominal wall of mice by injecting dust suspensions intra-peritoneally. Intravenous injection of dusts has been used in the study of the reactions in the liver and spleen by Giese (1934), Siegmund (1935), Koppenhofer (1936), Harrison, Wright and King (1950).

The effect of dust on the pleural tissues was studied by McCord et al (1936) by the injection of dust suspensions into the pleural cavity. Gardner et al (1935) used the inhalation method for the introduction of dust into the lungs of animals. Animals were placed in specially constructed dusting chambers and exposed to the required

concentration of dust for several hours daily over a period of months. Kettle and Hilton (1932) injected dust suspensions in normal saline into the lungs by the intratracheal route, while Naesland (1938-39) used the insufflation method.

In all the experiments recorded herein, Kettle and Hilton's (1932) technique has been employed, with slight modifications by Belt and King (1945) and by King, Mohanty, Harrison and Nagelschmidt (1953(b)). This method has much to commend it, as in addition to being one of the most effective, it has the advantage of producing lesions in the same organ as in the naturally occurring disease (Hadfield and Garrod, 1948).

The dust for injection after previous determinations of particle size and silica solubility, was weighed into a screw-capped bottle, the amoun weighed being in excess of the actual requirements for the animals to be injected. The suspension was made up in sterile physiological saline, so that the dose of dust to be injected into each animal was contained in 1 ml. in the case of rats, and 1.5 ml. in the case of guinea-pigs. The suspension was well-shaken in a mechanical shaker for about half and hour and then autoclaved for 20 minutes at a pressure of 15 lb/sq.inch. At this stage, a small amount of rat or guinea-pig serum, as the case might be, was added to the suspension in those instances where the dust particles did not appear to suspend well, e.g. coal. The sterilized suspension was kept shaking till it was drawn into the syringe for injection.

The experimental animals (rats and guinea-pigs) were

lightly anaesthetized with ether and placed in the supine position on an operating board, to which they were strapped. The tongue was retracted by means of a small tongue clip, and a medium-sized aural speculum, which was fitted to an auroscope, was introduced into the mouth as far down as the oropharynx. The throat was swabbed clear of mucus and the vocal cords touched with a 2% solution of anethane to produce temporary local anaesthesia. A blunt needle (4" x 14 gauge for rats, and 6" x 16 gauge for guinea-pigs), was passed between the cords, taking care to keep the tip of the needle in contact with the ventral wall of the trachea, so that the vibrations produced by the needle gliding over the tracheal rings could be felt. When the needle was felt hitting the carina, it was withdrawn about 2 mm. The sterile dust suspension for use in that particular experiment was then injected. This suspension, which was kept agitated in a mechanical shaker prior to its injection, was withdrawn through the rubber washer of the container into a 2 ml. syringe fitted with a short sharp needle ($1\frac{1}{2}$" x 20 gauge). The actual amount of suspension injected varied with the species of animals used, but on an average was made up to contain the required concentration of dust in 1 ml. of the suspending medium in the case of rats and 1.5 ml. in guinea-pigs. In order to ensure that the correct amount of suspension was injected into the lungs, an excess of 0.25 ml. was drawn into the syringe, since this was found to be the volume which generally remained behind in the needle and syringe after the injection was made. A small

amount of air - about 0.5 ml. - was also drawn into the syringe to facilitate shaking of the suspension in, and its ejection from, the syringe. The loaded syringe was detached from its needle, attached to the needle in the trachea and the suspension injected quickly and forcibly to obtain good dispersal of the dust in the lungs. The jet of suspension hitting the carina was expected to enter both lungs in approximately equal amounts. The needle was withdrawn quickly, soon after the injection, when a period of momentary apnoea was noticed in the animal. This was followed by a few deep inspirations and a gradual return to the normal respiratory rhythm. No regurgitation of the injected suspension was seen, and there were no immediate post-operative deaths among the animals.

Histo-pathological Technique

Routine post-mortems were carried out on all the animals which were killed at regular intervals and on those which were found dead. In the case of those animals which were killed, the fixative, 10% formol saline (about 10 ml. in rats and 15 ml. in guinea-pigs), was injected intratracheally after expressing air out of the lungs, before the thoracic cavity was opened. The trachea was exposed in the neck, the fixative injected, and the trachea ligatured and divided and the lungs dissected out together with the mediastinal structures. The injection of the fixative before removing the lungs from the thorax prevented them from becoming over-distended and the alveoli from rupturing.

In those animals which were found dead, the fixative was injected after removal of the lungs from the thoracic cavity, since satisfactory replacement could not be obtained otherwise, and in order to study the naked-eye appearances of the lungs and pleura *in situ*.

After preliminary fixation, blocks were selected through the long axes of both lungs at the level of the hilum, to include the hilar lymph nodes and to take maximum representative areas of the lung for histology. Fixation was completed in fresh fixative, the blocks dehydrated in alcohol, embedded in paraffin wax and sectioned at 5μ. Five or six serial sections were mounted from each block. The first was stained by Gordon and Sweet's (1936) silver impregnation method, and the second with haematoxylin and eosin. In some cases, Van Gieson's stain was used and in relevant experiments Ziehl-Neelsen's carbol-fuchsin and phenol auramine (fluorescent microscopy) were used to demonstrate acid-fast organisms.

Micro-incineration was carried out on most sections to reveal the presence and distribution of mineral matter in the lesions. Slides were incinerated at 500°C. for 1 hr., allowed to cool and then washed for half an hour in concentrated hydrochloric acid, rinsed in distilled water and dry-mounted by ringing round the cover-slip with paraffin wax.

Assessment of Lung Pathology

In assessing the degree of fibrosis brought about by various fibrogenic agents, five stages of the progression of the fibrotic process have been recognized histologically

(Belt and King, 1945). All histological preparations were carefully examined and the pulmonary fibrosis seen in the most advanced lesions in each case was graded according to Belt and King, whose classification is as follows :-

Grade 1 fibrosis: With ordinary nuclear stains the lesions appear cellular, while on reticulin staining a loose network of reticulin is seen. No collagen is demonstrable at this stage (Fig.1).

Grade 2 fibrosis: The lesions are cellular with the reticulin fibres becoming thicker and arranged in a compact manner. There may or may not be some collagen (Fig. 2).

Grade 3 fibrosis: The lesions are slightly cellular and composed mainly of collagen fibres (Figs.3 & 4).

Grade 4 fibrosis: The lesions are wholly acellular and fully collagenous (Figs. 5 & 6).

Grade 5 fibrosis: The lesions are acellular, collagenous and confluent (Fig. 7).

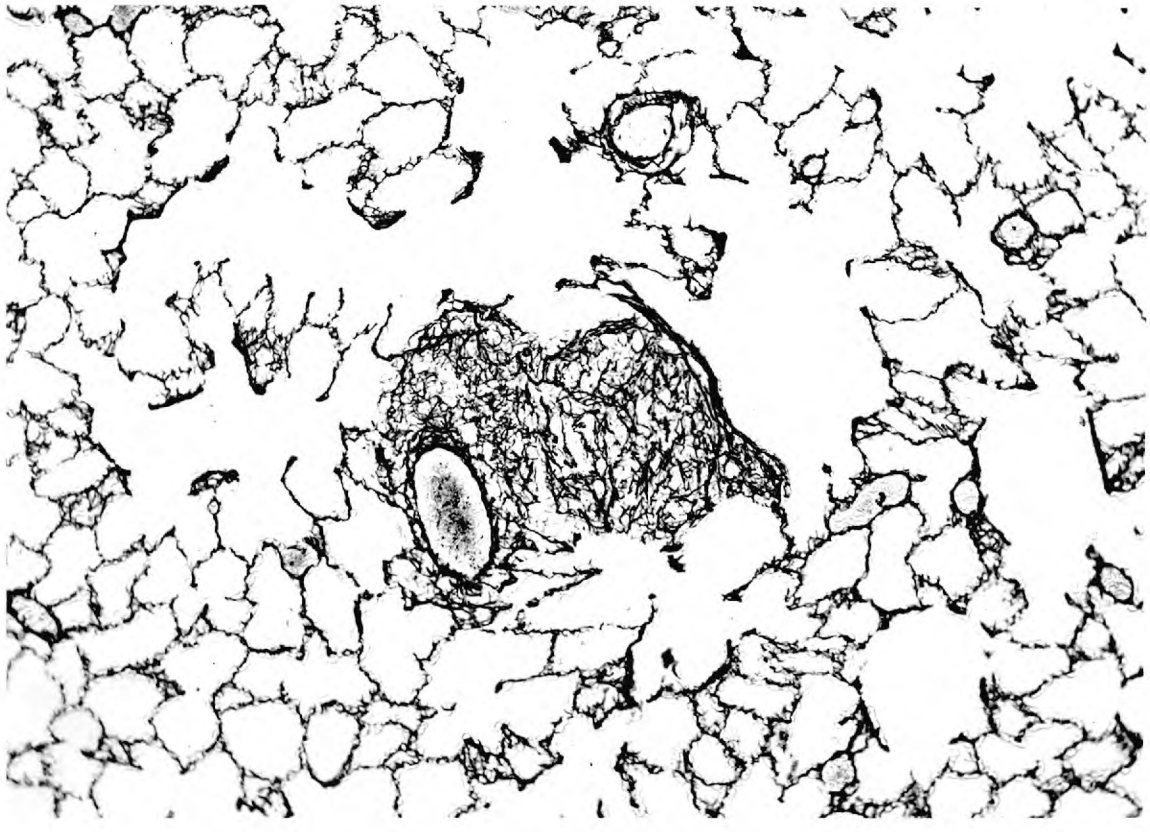


Fig. 1. Rat lung (50 mg. of quartz intratracheally), showing a nodular lesion of grade 1 fibrosis. Fine reticulin fibres are seen arranged in the form of a loose network. Silver impregnation. (X 130).

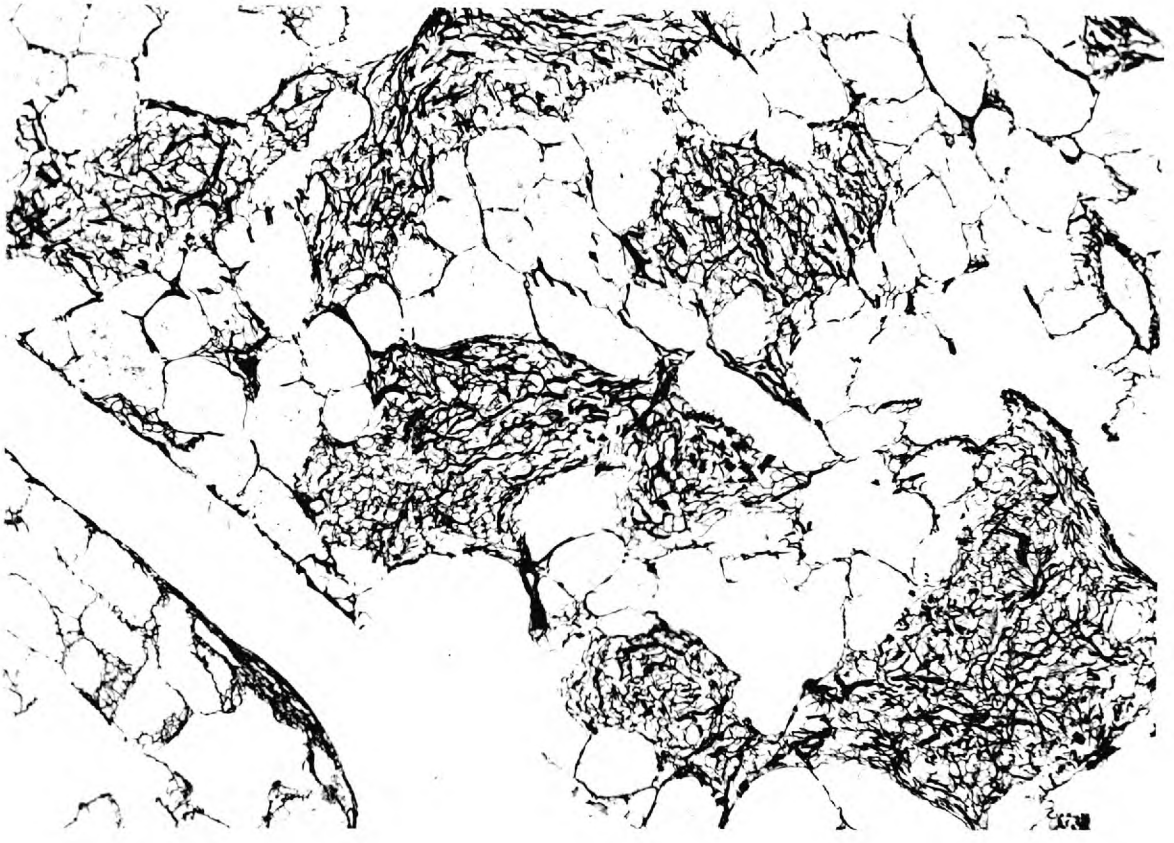


Fig. 2. Rat lung (50 mg. of quartz intratracheally), showing nodular lesions of grade 2 fibrosis. Reticulin fibres are arranged in a compact manner. Silver impregnation. (X.130).

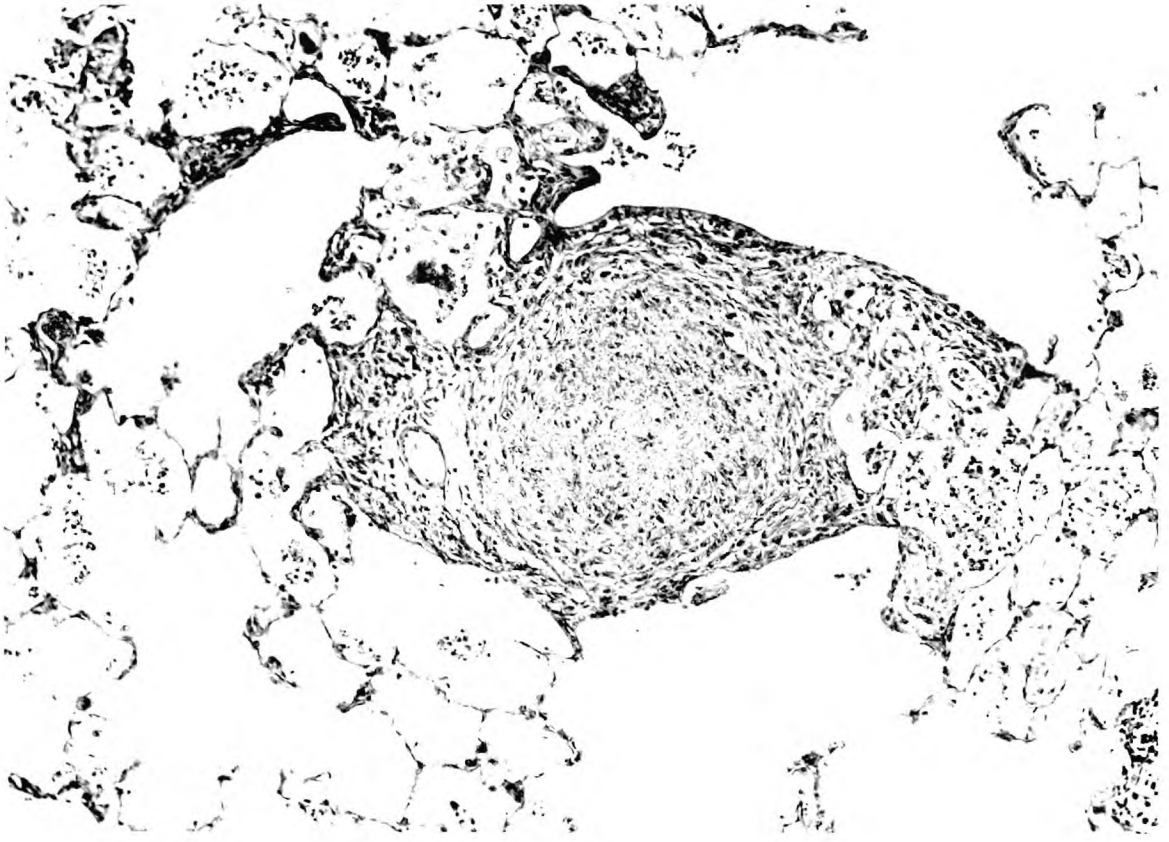


Fig. 3. Rat lung (50 mg. of quartz intratracheally) with a lesion of grade 3 fibrosis. Nuclear staining shows some cellularity. Haematoxylin and eosin. (X.130.).

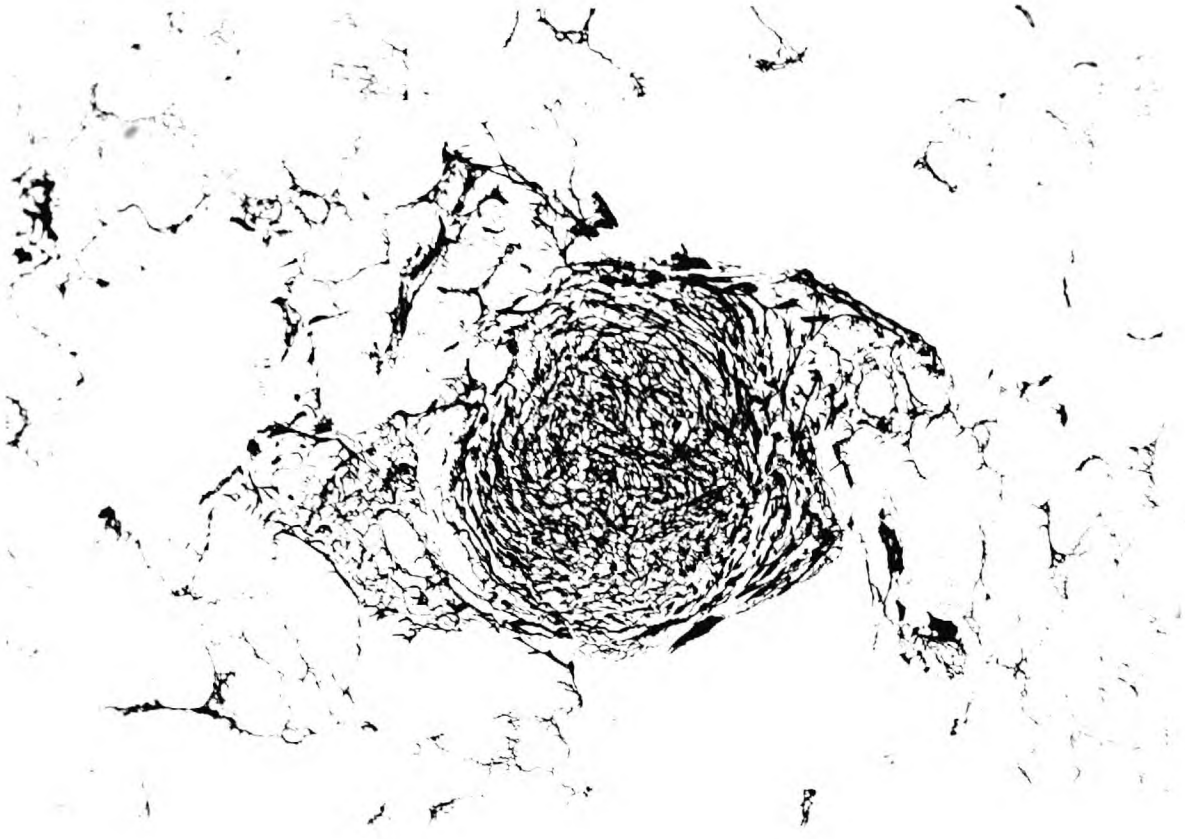


Fig. 4. Grade 3 lesion shown in Fig. 3 stained for reticulin. Thick fibres, mainly collagen, are arranged in a compact manner within the lesion (grade 3 fibrosis). Silver impregnation. (X.130).

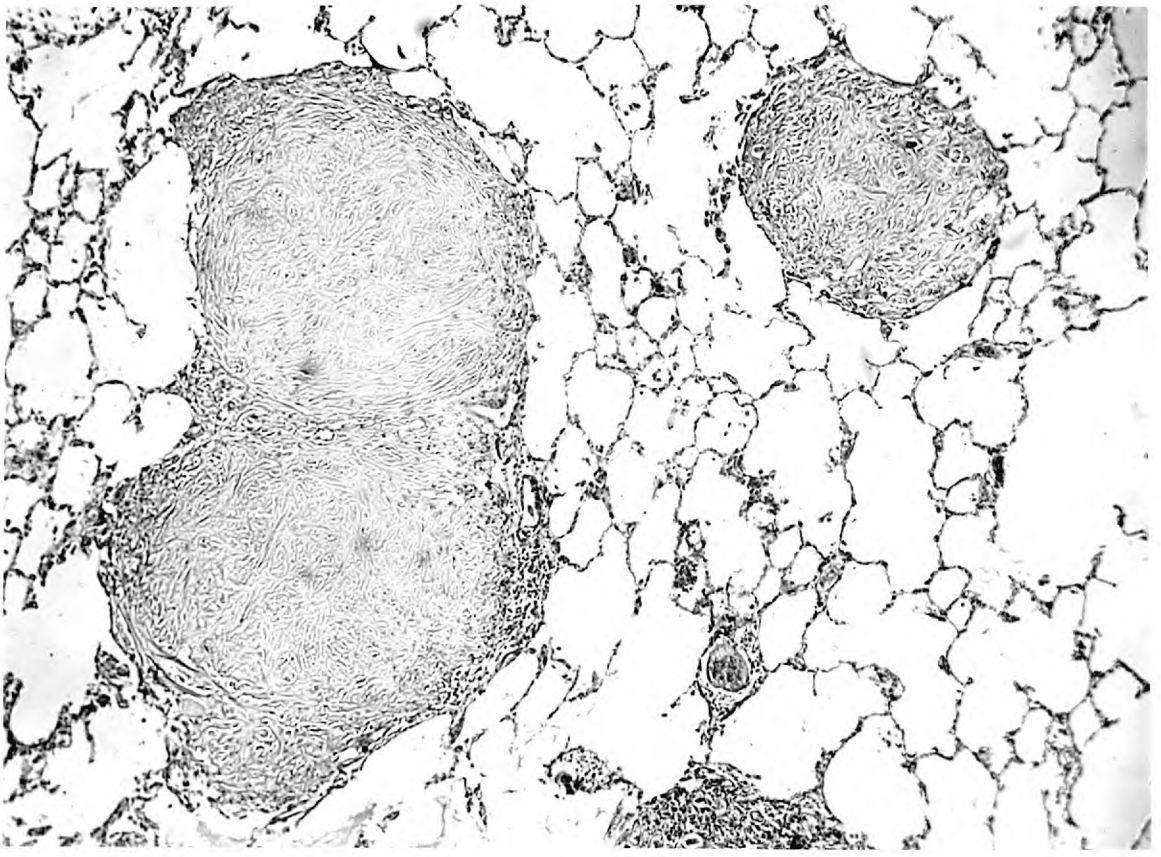


Fig. 5. Rat lung (50 mg. of quartz intratracheally), with lesions showing grade 4 fibrosis. Lesions are mainly acellular. Haematoxylin and eosin. (X.75.)

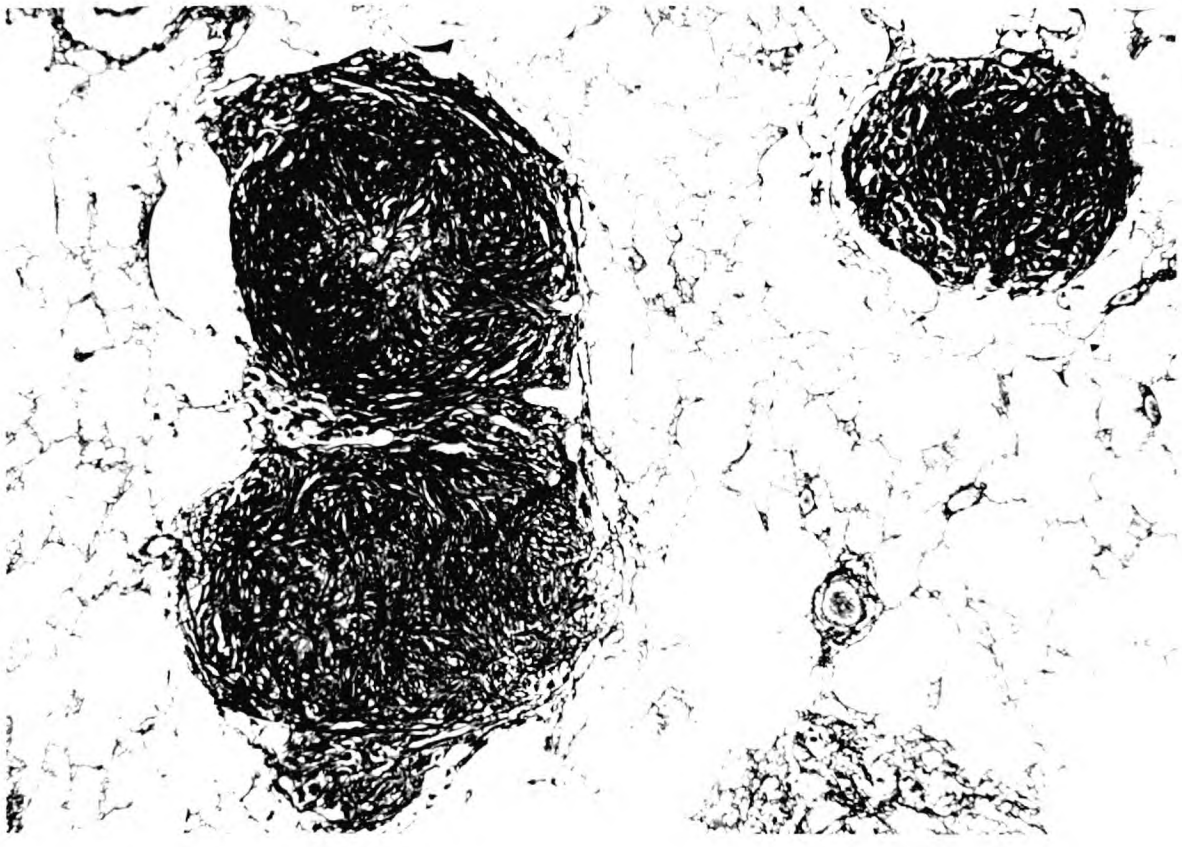


Fig. 6. Grade 4 lesions shown in Fig. 5 stained for reticulin. Nodules are composed of compact collagen (grade 4 fibrosis). Silver impregnation. (X.75).

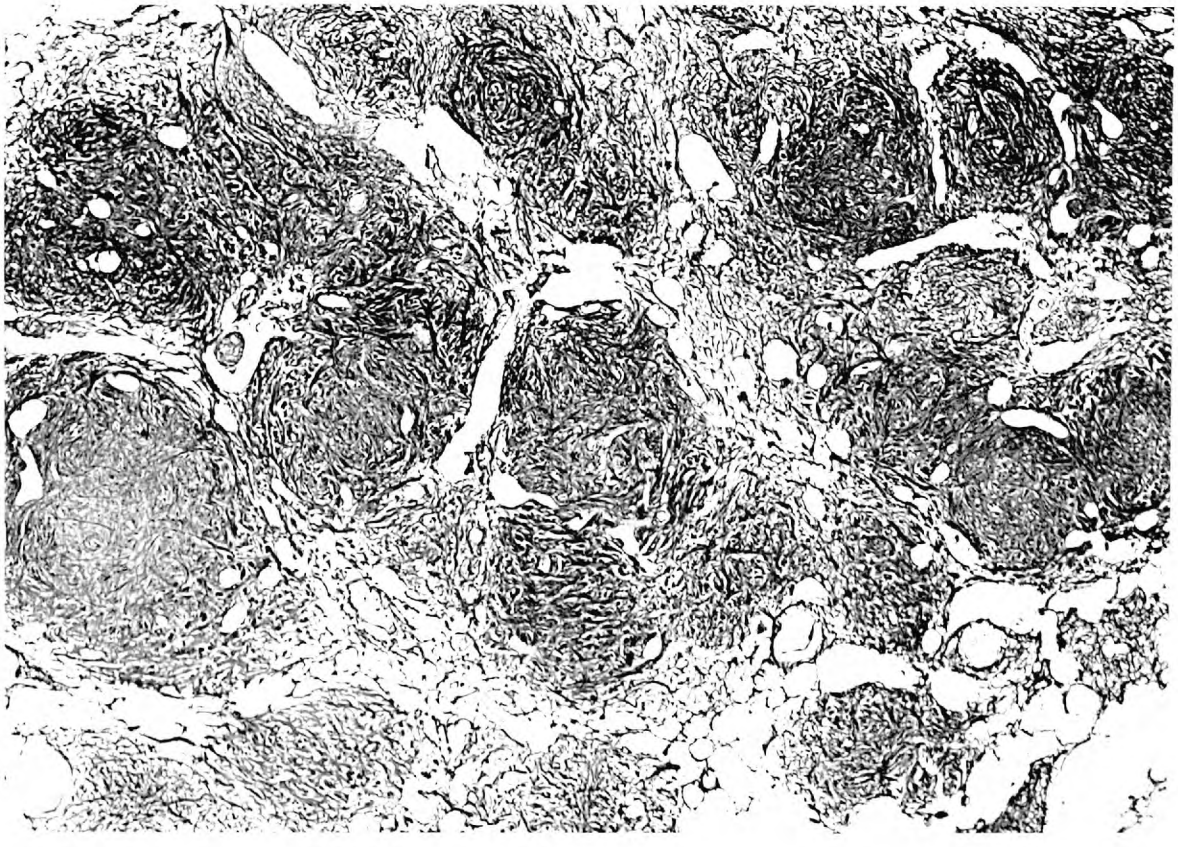


Fig. 7. Rat lung (75 mg. of quartz intratracheally), with lesions showing grade 5 fibrosis. Lesions are confluent and fully collagenous. Silver impregnation. (X.54.).

SECTION I

SECTION I

STUDY OF THE RATE OF ELIMINATION OF VARIOUS DUSTS FROM THE LUNGS OF RATS AND THE TISSUE REACTIONS PRODUCED BY THEM

A. (i) QUARTZ DUST

(ii) CORUNDUM (Al_2O_3)

B. (i) ANTHRACITE COAL

(ii) BITUMINOUS COAL

(iii) MIXTURE OF ANTHRACITE AND BITUMINOUS COAL DUSTS

C. RADIO-ACTIVE TANTALUM CARBIDE

The production of silicotic fibrosis in the lungs of rats by the intratracheal injection of fibrogenic dusts has been widely studied along quantitative lines. According to the solubility theory of silicosis, in the majority of cases a definite relation is known to exist between the fibrogenic properties of a dust and its silica solubility, as determined with Ringer's solution under standard conditions (King, 1947). Although the determination of the silica solubility of a dust *in vitro* by this method presents no difficulties, its determination in animal tissues during the production of fibrosis is extremely difficult. In actual fact, it is not possible to estimate in animal experiments just how much silica is lost from a fibrogenic dust, such as quartz, by solubility during the production of fibrosis, because the ratio of solid to liquid and the rate of renewal

of the liquid are unknown. However, an attempt may be made at assessing the loss in silica, due to solubility, of a dust injected into experimental animals by determining the amount of dust which can be recovered from their lungs and hilar lymph glands after varying intervals of time. One of the main difficulties in estimating this loss in intratracheally injected dusts is that, besides loss in silica due to solubility, there may be a certain amount lost from mechanical causes, such as the removal of the dust from the lungs via the bronchi by coughing, or by way of the stomach as a result of swallowing dust regurgitated from the bronchi. This difficulty may be overcome by using as a control a dust which is known to be insoluble, such as aluminium oxide. Samples of these two dusts of similar particle size distribution, when injected intratracheally, would probably have similar losses as far as mechanical causes are concerned, but the aluminium oxide would not show any loss due to solubility, while the quartz would.

The amount of dust recovered from the lungs of animals at varying intervals of time after its injection might be important from another point of view. The formation of fibrous lesions in silicosis is a chronic process usually extending over a long period, and therefore it is reasonable to assume that dusts which produce such lesions are likely to remain in the tissues longer than those which do not. Dust particles which are cleared away from the lungs rapidly have less chances of going into solution in the tissues and setting

up fibrous reactions than those which remain there longer. It is not known whether this is actually so, but the determination of the rate of elimination of a fibrogenic and an inert dust would give the answer to it.

The occurrence of pneumoconiosis among coalworkers has been recognized for some time past, and it was once believed that the incidence of the disease among workers in anthracite coal mines in the South Wales coalfields was higher than that in the bituminous mines. Investigations carried out by the Medical Research Council on the incidence of pneumoconiosis in South Wales have shown that this is not so (Hart and Aslett, 1942). Nevertheless, it is found that workers in the South Wales mines, where the coal mined is mainly anthracite and of high rank (high carbon content and low volatility), were more liable to contract pneumoconiosis than those in the coal mines of Durham, Yorkshire and Cumberland, where the coal is bituminous and of low rank (low carbon content and high volatility). This higher incidence of pneumoconiosis in South Wales has not been explained, but may be due partly to the fact that the mines there have been dustier than those in the other areas (Fletcher, 1951). Chemical analyses have revealed that there was much more dust in the lungs of miners from South Wales than in those from Cumberland. The high incidence of disease among miners has led many workers to study the reactions produced by anthracite and bituminous coal in the lungs of experimental animals. Belt and King (1945) studied the effects of pure coal dust (dust containing 90% or

more of anthracite, bituminous or steam coal) on the lungs of rats and found that both anthracite and bituminous coal produced identical lesions, which were minimal and consisted of the simplest type of foreign-body reaction, even up to 500 days. Histologically, no differences have been found in the lesions produced by these two types of coal, whether used alone or mixed with other dusts such as quartz (Ray, King and Harrison, 1951). Similar results have been obtained by others and on the face of it there does not seem to be any difference between anthracite and bituminous coal. This is true as far as the lesions produced by these dusts in the lungs are concerned, but there is no evidence as to whether they differ in any other respect, such as the rate of elimination from the lungs. It seemed worthwhile studying the rate at which anthracite and bituminous coal dust are removed from the lungs of experimental animals when introduced in equal amounts of similar particle size and ash content. The behaviour of a mixture of equal amounts of the two substances, with regard to the rate of elimination, could also be studied together with the tissue reactions produced by all these substances.

Chemical estimations of the amount of injected dust recoverable from the lungs of animals can be carried out only after death. *In vivo* determinations may be made by injecting a radio-active dust, whose degree of activity per unit weight is known, and following it up by measuring with the Geiger counter the degree of activity present in the animals at different periods. The degree of activity would give an

indication of the amount of dust left in the lungs, while total body measurements indicate how long activity lasts. Histological studies of these lungs, compared with those injected with inactive dust, would reveal any differences, apart from radiation-induced changes.

Plan of Experiments

Several experiments were set up using various dusts and intended mainly for analysis and recovery of dust, with only occasional studies of the lung histology.

(A) Quartz and Corundum: The first series was intended for the study of the loss of dust, both pathogenic and inert, from the lungs and lymph nodes at varying periods of time. It was also hoped to determine if there was any loss in silica, due to solubility, of quartz dust. Quartz dust and synthetic corundum (Al_2O_3) were used, the two samples being of comparable size. The corundum was to serve as a control, as it was expected to have similar losses as the quartz in so far as mechanical causes were concerned, but not show any loss due to solubility. The injected dust was recovered from the lungs of animals at different periods up to 365 days, and the histology studied occasionally.

(B) Anthracite and Bituminous Coal: Anthracite and bituminous coal of equal particle size and similar ash content were injected into the lungs of animals. A third group of animals was injected with a mixture of equal amounts of anthracite and bituminous coal. Animals from all the groups were killed at regular intervals up to 400

days and recoveries of dust made on the lungs and hilar lymph nodes. Histological studies were carried out on the lungs.

(C) Tantalum Carbide: Radio-active tantalum carbide was injected into the lungs of rats so that each received 25-50 microcuries of the isotope. The animals were housed in metabolism cages and their excretions assayed for activity. In vivo measurements of activity were made on the animals at intervals and after 250 days the surviving ones killed and the lung activity measured. Histological examinations of the lungs were also made.

During the course of measurements it was found that the activity in the animals was too high to enable accurate counts to be taken, and a second group of animals was injected with 5-10 microcuries of the isotope each. Measurements were carried out as before and the animals killed and the lung histology studied at 240 days.

To serve as a control, a few animals were injected with inactive tantalum carbide and the tissue reactions produced were studied histologically at 250 and 300 days.

DESCRIPTION OF SAMPLES USED FOR INJECTION

(A) Quartz: This was a Belgian glass sand of high purity. A fraction of nominal size, 0.8 - 1μ, was prepared by repeated centrifuging in water. The actual size distribution, determined with the electron microscope, is shown in Table 1, together with the data obtained by chemical analysis. Figure 1A shows the electron micrograph of the dust.

TABLE 1

SIZE DISTRIBUTION AND ANALYSIS OF QUARTZ AND ALUMINA DUSTS

("nominal" size 0.8-1μ)

Particle Size μ	Snowit - Quartz Per Cent		Alumina Per Cent	
	By No.	By Mass	By No.	By Mass
< 0.11	9.5			
0.11 - 0.16	11.9		1.6	
0.16 - 0.23	7.5		7.0	
0.23 - 0.32	4.2		5.9	
0.32 - 0.45	2.5	0.1	9.2	0.2
0.45 - 0.64	3.3	0.5	9.1	0.7
0.64 - 0.9	18.5	7.1	11.6	2.4
0.9 - 1.3	27.8	29.5	26.4	14.9
1.3 - 1.8	11.6	35.2	16.5	26.7
1.8 - 2.6	3.2	27.6	8.9	40.1
2.6 - 3.6			0.6	7.8
3.6 - 5.1			0.2	7.2

Particles Counted	805	497
Estimated surface Area (m^2/g)	2.16	1.12
SiO_2 (%)	98.9	-
Ignition Loss (%)	0.6	1.39
Silica Solubility (mg/100 ml)	5.85	-

Synthetic Corundum: A sample of similar size distribution to the quartz was prepared from synthetic corundum, made by heating pure aluminium hydroxide for several hours at 1500°C. The size distribution of the dust and chemical data are given in Table 1, and the electron micrograph is shown in Figure 1B.

(B) Anthracite Coal: A low ash (3.14%) fraction of anthracite coal from Pentramawr Colliery, South Wales, was turbine-ground and a fraction below 10μ separated by air elutriation (Roller apparatus). Starting with this material, a fraction below 2μ was prepared by centrifuging in water after the addition of sufficient teepol to make the coal wettable. The material below 2μ equivalent diameter was spun down by prolonged centrifuging, boiled with water to remove as much of the teepol as possible and finally dried.

The particle size distribution and other data are given in Table 2.

Bituminous Coal: A low ash fraction (3.65%) of coal from Haig Pit, Cumberland, was treated in a similar manner to the anthracite coal, and a fraction under 2μ obtained. Table 2 shows the particle size distribution.

(C) Tantalum Carbide: A sample of tantalum carbide was obtained from a commercial source and a fraction below 2μ equivalent diameter prepared by sedimentation in water.

Table 3 shows the size distribution of the sample.

Radio-active Tantalum Carbide: A sample of the above was irradiated in the aluminium pile at Harwell to give

TABLE 2

SIZE DISTRIBUTION AND ANALYSIS OF ANTHRACITE AND BITUMINOUS COAL DUSTS

("nominal" size, < 2 μ)

Particle Size μ	Anthracite Coal Per Cent		Bituminous Coal Per Cent	
	By No.	By Mass	By No.	By Mass
0.11 - 0.16			7.84	0.02
0.16 - 0.23			9.3	0.07
0.23 - 0.32			11.03	0.19
0.32 - 0.45			17.63	0.8
0.45 - 0.64			15.2	2.07
0.64 - 0.9	1.4	0.1	17.97	6.73
0.9 - 1.3	3.5	0.9	9.99	10.38
1.3 - 1.8	73.0	51.2	5.97	17.78
1.8 - 2.6	20.9	41.1	2.98	24.89
2.6 - 3.6	1.2	6.7	0.83	19.84
3.6 - 5.1			0.003	17.24
Estimated Surface Area (m^2/gm)	2.6		3.2	
Ash (%)	3.14		3.65	

TABLE 3

SIZE DISTRIBUTION AND ANALYSIS OF TANTALUM CARBIDE

Particle Size μ	Per Cent By No.	Per Cent By Mass
0.16 - 0.23	13.7	0.5
0.23 - 0.32	22.9	1.8
0.32 - 0.45	24.2	4.9
0.45 - 0.64	21.6	13.9
0.64 - 0.9	11.1	19.6
0.9 - 1.3	4.6	22.6
1.3 - 1.8	1.5	21.6
1.8 - 2.6	0.4	15.1
Specific Surface (m^2/gm)	8.5	

adequate γ ray activity for *in vivo* measurements. The half-life of the isotope was 120 days. The activity in 50 mg. of dust was 25-50 microcuries for the first series of animals. This sample was mixed with inactive tantalum carbide in such proportions as to contain 5-10 microcuries of the isotope in 50 mg. dust injected into the animals in the second series.

Preparation of Dust Suspensions for Animal Injection

(A) Quartz and Corundum: 2g. of quartz dust and 2.8g. of corundum were weighed into screw-capped bottles and 40 ml. sterile physiological saline added to each bottle, so that the concentrations were 50 mg. quartz dust per ml. of suspension in one case and 70 mg. corundum per ml. of suspension in the other. The suspensions were thoroughly shaken for about half an hour in a microid flask-shaker, autocalved for 20 minutes (15 lbs. pressure) and then re-shaken till the time of injection. Each animal received 1 ml. suspension containing 50 mg. quartz or 70 mg. corundum. The difference in dosage was to allow for the difference in densities of the two materials, and to obtain approximately equal numbers of particles.

(B) Anthracite and Bituminous Coal: Amounts of 3g. of anthracite and 3g. bituminous coal were weighed into screw-capped bottles and 28.5 ml. sterile physiological saline added to each. The suspensions were shaken, autoclaved and 1.5 ml. rat serum (aseptically prepared) added to each suspension to help the particles to suspend better. The

final concentration was 100 mg. of anthracite or bituminous coal per ml. of suspension, and was the amount injected into each animal in the respective groups.

Anthracite and Bituminous Mixture: 1.5 g. anthracite and 1.5 g. bituminous coal were weighed, intimately mixed and transferred to a screw-capped bottle. The suspension was made up as for the anthracite and bituminous coal alone, with 28.5 ml. sterile physiological saline and 1.5 ml. rat serum. The concentration of dust was 100 mg. of the mixture (50 mg. anthracite and 50 mg. bituminous) per ml. of suspension, which was the dose each animal received.

(C) Tantalum Carbide: Amounts of 0.8 g. of the radio-active sample of tantalum carbide containing 25-50 microcuries of the isotope per 50 mg. dust, and 0.4 g. of the sample containing 5-10 microcuries per 50 mg. dust, were weighed into bottles and 16 ml. and 8 ml. sterile physiological saline added to the respective bottles. The suspensions were made as before and each of the animals in the two groups received 1 ml. suspension containing 50 mg. dust (containing 25-50 microcuries or 5-10 microcuries of isotope), this being the final concentration of the suspensions.

0.4 g. of the inactive dust was weighed and the suspension prepared in 8 ml. sterile physiological saline to contain 50 mg. dust per ml. Each animal was injected with 1 ml. suspension.

Animals

(A) Quartz: 35 male Albino rats of the Worcester strain

of average weight 350g. were used.

Corundum: 35 male rats were injected. They were black and white hooded rats of the Medical Research Council strain and were of average weight, 225 g.

(B) Anthracite, Bituminous and Mixture of Anthracite and Bituminous Coal: 20 male rats were used in each of these experiments. They were albinos of the Worcester strain, of average weight 350 g.

(C) Radio-active Tantalum Carbide: 10 male, albino rats of the Worcester strain of average weight 300 g. were used for injection of radio-active dust containing 25-50 microcuries of the isotope.

5 male, black and white hooded rats of the Medical Research Council strain of average weight 225 g. were used for injection of the dust containing 5-10 microcuries of the isotope per dose.

Tantalum Carbide: 6 male, black and white hooded rats of the Medical Research Council strain, of average weight 225 g. were used for injections of inactive tantalum carbide.

Experimental Procedure

The animals were lightly anaesthetized with ether and the dust suspensions injected intratracheally via the mouth, following the technique of Kettle and Hilton (1932) modified by King et al (1953 (b)), a detailed description of which has been given earlier.

Duration of Experiments

(A) The experiments in which quartz and corundum were injected were extended to 365 days. Two animals from each group were killed immediately after injection, and 24 hours, 48 hours, 7 days and 15 days after; thereafter, one animal from each group was killed at monthly intervals, except when the histology was studied when two animals were killed. Killings were carefully matched in both groups. Chemical analyses were carried out on the lungs and lymph nodes at all stages. Histology was studied at 150 days in the case of quartz, and at 150, 240, 330 and 365 days in the case of corundum.

(B) In the series of experiments set up with anthracite, bituminous and a mixture of the two coal dusts, one animal from each group was killed at monthly intervals for 180 days, then at 60 day intervals till 300 days and finally at monthly intervals till 400 days. Histological examination of the lungs and chemical analyses on the lungs and lymph nodes were carried out regularly.

(C) In vivo measurements of activity were carried out on the animals injected with radio-active tantalum carbide (25-50 microcuries) at 30 days and 60 days after injection. All surviving animals were killed at 250 days and the lungs, hilar nodes and carcass measured for activity.

In the case of animals injected with the smaller dose of the isotope, in vivo measurements were made at 17, 28, 56 and 100 days after injection, and the animals killed at 240 days.

All animals in these groups were housed in metabolism cages to enable the excreta to be assayed for activity.

In the control group, the animals injected with inactive dust were killed at 250 and 300 days. The lung histology was studied in all three groups.

Procedure Adopted for in vivo Measurements on Animals Injected with Radio-active Dust

In the group of animals injected with radio-active tantalum carbide containing 25-50 microcuries of the isotope per 50 mg. dust, in vivo measurements were made on the head and neck, thorax and abdomen separately. The animals were anaesthetized with intraperitoneal injections of a solution of nembutal and placed in a specially constructed apparatus consisting of two metal (lead) cylinders. By adjusting these cylinders over the animal's body, it was possible to shield off most of it and leave exposed only the particular part on which measurements were being made. This ensured that only the radiations emanating from the exposed part of the body were being recorded by the Geiger counter. Counts were made per half-minute.

Two sets of in vivo measurements were made, after which the animals had grown to be too large to fit the apparatus. The last measurement was made after killing the animals, the lung count, lymph node count and the carcass count being recorded separately, per minute.

In the second group of animals injected with 5-10 microcuries of the isotope, measurements were made on the whole animal - total body count - as it was apparent that in the earlier procedure, where counts were made on the head and neck, thorax and abdomen separately, the readings were not very accurate, owing to the difficulty in obtaining precise demarcation between these regions externally, which resulted in a considerable overlap of values. Apart from this, the measurements were carried out in the same way.

PATHOLOGICAL TECHNIQUE

Routine post-mortem examinations were carried out on all animals which were killed and on any found dead.

In the early stages (till 30 days) of the experiments with quartz and corundum, one pair of lungs from each group was injected with 10% formol-saline and fixed before chemical estimations of dust were carried out on them. The other pair of lungs was used unfixed. The tracheo-bronchial lymph nodes were dissected out and analysed separately after 30 days, when they were found to be enlarged. Blocks were selected along the long axes of the lungs at the level of the hilum - as for histological examination - and analysed separately from 30-65 days. All lungs for chemical analyses from 90 days onwards were injected with air before their removal from the thoracic cavity and then immersed in fixative till the time of analysis.

The lungs of animals used for histological study were treated in the same way in all groups - quartz and

corundum, coal, tantalum carbide. After preliminary fixation of the lungs by the injection of 10% formol saline before their removal from the thoracic cavity, they were left in fixative for some time, and blocks were selected through the long axes of the lungs at the level of the hilum. These blocks were dehydrated, embedded in paraffin wax and serial sections cut at 5μ thickness. These were stained with haematoxylin and eosin and by Gordon and Sweets' (1936) silver impregnation method for reticulin. In addition, with quartz and corundum, sections were micro-incinerated, acid-washed, and the distribution of dust in the lesions studied. Details of these procedures have been given in an earlier section of this thesis.

Chemical Methods

The lungs and lymph nodes (tracheo-bronchial) were analysed for the recovery of injected dust in the case of quartz, corundum and coal.

(A) Quartz and Corundum - Preparation of tissues: The trachea was cut off at the bifurcation and the lungs cut into pieces of convenient size. When analyses were carried out on tissues which had been blocked for histological study, the blocks were de-waxed before use and combined with the remainder of the formalin-fixed lung from which the blocks were taken.

Method - Analyses were carried out in conical, glass-stoppered centrifuge tubes of approximately 10ml. capacity. The pieces of lung were oven-dried and ashed in

the centrifuge tube in an electric muffle at 470°C. (The ashing was started from cold and it took several hours for the oven to reach the maximum temperature). It was necessary to heat for 24 hours to complete the ashing. The ash was then heated with 2N HCl in a water bath for about 1 minute. After cooling, the acid-insoluble residue was centrifuged, dried and repeatedly shaken with distilled water till it was free from chloride, and dried to constant weight at 105°C. The weight of residue was the amount of dust recovered from the lungs.

The tracheo-bronchial lymph nodes were treated in exactly the same way, but their wet weights were recorded before placing them in the centrifuge tubes.

A number of test analyses of the material which had been used for injections, either alone or together with fresh animal lung, yielded recoveries of the order of 96-98% by this method, the amount of sample varying from 10-100 mg. These tests showed that the technique of recovery was good.

(B) Anthracite, Bituminous and Anthracite and Bituminous Coal Mixture - Preparation of tissues:

The paraffin blocks used for the preparation of histological sections were de-waxed with hot benzene, and this tissue combined with the rest of the lung tissue cut up into small pieces.

Method - The material from each pair of lungs was placed in a centrifuge tube of approximately 50 ml. capacity, about 15 ml. concentrated HCl added to it and left in an

oven at 58°C for 24 hours. This mixture was centrifuged for 1 hour and the supernatant fluid pipetted off by suction. 15 ml. of 25% NaOH were then added and the mixture heated on a water bath at 80°C for 1 hour. After the addition of a few drops of absolute alcohol to this to dissolve any fat present, it was centrifuged for 1 hour and the supernatant fluid removed by suction. About 15 ml. of distilled water were then added to the residue, mixed, centrifuged for ½ hour, and filtered through asbestos wool in a Gooch crucible which had been previously prepared and weighed. The residue was finally washed with a mixture of alcohol and ether and dried overnight at 105°C. The crucible was cooled and weighed. The weight of residue represented the amount of dust recovered.

The tracheo-bronchial lymph nodes were treated in the same way after bulking the glands from several animals. This was necessary in order to get enough dust to weigh, since each gland or set of glands contained only a very little. They were bulked as follows :- glands from animals killed in the period 30-120 days; 150-250 days; 300 days; 330 days; 365-400 days.

Test analyses were carried out on samples of anthracite and bituminous coal with and without normal lung tissue, and recoveries of the order of 98-99% were obtained in all cases, showing that the method was satisfactory.

RESULTS

Several animals from the various groups were lost as a result of cannibalism and their lungs were not available for chemical analyses or histological examination.

(A) Quartz - Apart from the animals killed at the regular intervals mentioned above, spontaneous deaths occurred at 19, 24, 31, 53, 57, 65, 88, 158 and 303 days. The lungs of some of these animals were not available for chemical analyses owing to cannibalism. Histological examination of the lungs was made at 150 days.

Corundum - Spontaneous deaths occurred at 6, 9, 23, 32 and 269 days, and most of the lungs in these animals were discarded because of autolysis or infection.

Lung histology was studied at 150, 240, 330 and 365 days. Chemical analyses were carried out on the lungs and lymph nodes of animals killed at regular intervals in the quartz and corundum groups, and the results are given in Table 4.

(B) Anthracite Coal Dust - Animals were killed at regular intervals till 400 days and their lungs used for chemical and histological studies. Some animals were lost by cannibalism at 7, 11, 21, 25, 28 and 335 days.

Bituminous Coal - Spontaneous deaths occurred at 12, 24, 43, 330 and 337 days and the lungs of some of these animals were discarded as they were autolysed.

Anthracite and Bituminous Mixture - The lungs of animals which died spontaneously at 6, 11, 12, 19, 49 and

TABLE 4.

RECOVERY OF QUARTZ AND CORUNDUM INJECTED INTO THE LUNGS OF RATS

Days of Survival	QUARTZ (50 mg injected)					CORUNDUM (70 mg injected)				
	SiO_2/lung (mg)	SiO_2/lung (% of injected)	SiO_2/node (mg)	Recovery (total mg)	Recovery total (% of injected)	Al_2O_3/lung (mg)	Al_2O_3/lung (% of injected)	Al_2O_3/node (mg)	Recovery (total mg)	Recovery Total (% of injected)
0	x 47.5	95		47.5	95	x 50.2	72		50.2	72
1	x 43.3	86.6		43.3	86.6	x 47.3	67.6		47.3	67.6
2	x 40.1	80.2		40.1	80.2	x 46.8	67		46.8	67
7	x 39.6	79.2		39.6	79.2	x 42.5	61		42.5	61
14	x 36.6	73.2		36.6	73.2	x 63.1	90.1	S 1.4	64.5	92
30	39.9	79.8		39.9	79.8	44.3	68.3		44.3	68.3
49	34.4	68.8	1.7	36.1	72	-	-		-	-
60	x 20.6	41.2	x 1.0	21.6	43.2	38	54.3	S 1.4	39.4	56.3
90	18.7	37.4		18.7	37.4	50.8	72.5		50.8	72.5
120	24.7	49.4	4.73	29.5	59	37.2	53.1		40	57
150	x 23.1	46.2		27.9	56	42.2	60.3	2.8	45	64.5
150	H 16.3	32.6		16.3	32.6	H 30.8	44		33.6	48
180	17.2	34.4		21.9	44	32.4	46.3		37.5	53.5
210	15.1	30.2	4.7	19.8	40	32.5	46.3	5.1	37.6	54
240	25.7	51.4		30.4	61	37.0	52.8		42.1	60
240	-	-		-	-	H 39.5	56.4		45.6	65
270	17.0	34.0		25.1	50	24.5	35		24.5	35
300	20.0	40.0	8.1	28.1	56	41.1	58.7		45.3	65
330						30.6	43.7	4.2	34.81	50
330						H 28.8	41.1		33.0	47
365	21.7	43.4	8.1	29.8	60	ǂ 35.2	50.3	ǂ 6.4	41.66	60
365						H 30.1	43		30.1	43

x Average value of two separate analyses

ǂ Average value of four separate analyses

S Average of bulked node analyses (bulking of nodes from two rats)

H Analysis of lung and deparaffined histological blocks

52 days were autolysed or infected and were unfit for histological and chemical studies.

Killings in these three groups were matched and continued for 400 days. All lungs and tracheo-bronchial lymph nodes were analysed for recovery of dust. The results are given in Table 5.

Histological examinations were carried out at regular intervals.

(C) Radio-active Tantalum Carbide (25-50 microcuries of isotope). One animal died at 27 days and two at 88 days. The seven remaining animals were killed at 250 days. Table 6 shows the counts obtained when *in vivo* measurements of activity were made at 30 and 60 days, and those on the lungs and tracheo-bronchial lymph nodes at post-mortem.

Radio-active Tantalum Carbide (5-10 microcuries of isotope). One animal died on the day of injection and another died two days later. The results of *in vivo* measurements made on the three surviving animals are given in Table 7, together with the measurements of their excreta for activity. Both the body activity and the activity of the excreta are expressed as percentages of the total doses deposited in the animals on the day of injection, which count is taken as 100%.

Non-active Tantalum Carbide - Four animals in this group were killed at 250 days and two at 300 days, and their lung histology studied.

TABLE 5

RECOVERY OF ANTHRACITE, BITUMINOUS AND ANTHRACITE PLUS BITUMINOUS COAL DUSTS

(All figures calculated for one rat)

Days of Survival	ANTHRACITE (100mg)		BITUMINOUS (100mg)		ANTHRACITE PLUS BITUMINOUS (100mg)	
	Amount in lungs mg. or %	Amount in nodes mg. or %	Amount in lungs mg. or %	Amount in nodes mg. or %	Amount in lungs mg. or %	Amount in nodes mg. or %
30	68.8		62.9		56.0	
60	69.0		63.9		55.8	
90	67.2	2.3	46.1	1.3	52.8	1.1
120	68.5		45.1		59.0	
150	62.0		42.9		58.0	
180	65.8	1.7	42.4	1.2	59.4	1.8
250	62.0		48.2		59.3	
300	53.0	1.6	40.4	2.8	55.0	6.9
330	50.0	3.5	39.4	5.2	56.0	1.7
365	53.8	4.6	49.6	5.1	51.8	1.0
400	56.4		40.2		53.4	

TABLE 6

MEASUREMENTS ON RATS INJECTED WITH RADIO-ACTIVE TANTALUM CARBIDE (25-50 MICROCURIES OF ISOTOPE)

Period of time (days)....	30 (in vivo)	60 (in vivo)	250 (post-mortem)		
Rat No.	Thorax (Count/min)	Thorax (Count/min)	Lung (Count/min)	Lymph nodes (hilar) (Count/min)	(%)[x]
1	16,800	14,000	9,171	231	2.5
2	27,400	16,600	3,395	127	3.8
3	42,400	41,200	22,823	751	3.3
4	21,000	17,200	5,248	Nil	-
5	51,600	48,800	-	-	-
6	37,800	35,600	-	-	-
7	25,400	17,600	3,814	169	4.4
8	34,600	29,000	13,411	912	6.7
9	56,200	61,000	31,651	1,976	6.2

[x] Expressed as % total count (lung + lymph nodes)

TABLE 7

IN VIVO MEASUREMENTS ON RATS INJECTED WITH RADIO-ACTIVE TANTALUM CARBIDE (5-10 micro-curies) AND ASSAY OF THEIR EXCRETIONS

EXCRETIONS (URINE & FAECES) % OF DAY 1						BODY CONTENT % OF DAY 1					
Days	Rat 1	Rat 2	Rat 3	Average	Average Cum.	Days	Rat 1	Rat 2	Rat 3	Average	Body Content & Av. Cum Excretion
0-2	12.4	11.8	6.9	10.3	10.3	1	100	100	100	100	-
2-4	4.0	4.0	6.0	4.7	15						
4-6	4.9	0.2	3.7	2.9	17.9						
6-8	14.3	1.9	4.6	6.9	24.8						
9-10	1.8	3.2	nil	1.7	26.5						
10-13	15.0	5.8	3.3	8.0	34.5						
13-15	1.8	3.6	4.0	3.1	37.6						
15-17	3.3	7.4	9.1	6.6	44.2	17	x 41.0	48.0	66.0	52.0	96.2
17-24	0.9	9.4	5.1	5.1	49.3						
24-28	6.0	6.0	6.4	6.1	55.4	28	x 42.8	35.6	53.0	43.8	99.2
28-35	3.4	3.8	10.7	6.0	61.4						
35-42	7.5	11.4	13.4	10.8	72.2						
42-49	6.3	4.4	0.4	3.7	75.9						
49-56	1.5	7.5	8.2	5.7	81.6	56	27.5	28.2	33.6	29.8	111.4
56-63	3.3	4.4	10.0	5.9	87.5						
63-70	12.4	6.1	6.3	8.3	95.8						
70-77	1.6	3.3	5.4	3.4	99.2						
77-85	1.3	5.1	4.2	3.5	102.7						
85-100	2.9	1.5	1.2	1.9	104.6	100	10.1	11.5	12.3	11.3	115.9
Total	104.6	100.8	108.9	104.6	104.6						

Body contents and excretions are expressed as percentages of the 'total' doses deposited in the animals, i.e. they are expressed in terms of the total body measurements obtained on day 1.

x Probably error

Cum = Cumulative

HISTO-PATHOLOGICAL FINDINGS

Sections from the lungs of animals in all the groups were examined histologically and the pulmonary fibrosis present in the most advanced lesions in each case graded according to Belt and King (1945), into one of five grades.

(A) QUARTZ AND CORUNDUM

Macroscopic Appearances

Quartz: The lungs of animals killed up to 30 days showed subpleural collections of dust mainly on the dorsal aspect. By 120 days there were discrete areas of fibrosis which became confluent at 150 days and involved large portions of the lungs (Fig. 3A). There were pleural adhesions to the chest wall. The fibrosis increased in amount from this time onwards and at 365 days there was very little normal-looking lung tissue left (Fig,3B).

The tracheo-bronchial lymph nodes showed slight enlargement at 30 days and increased in size and were firm thereafter.

Corundum: The lungs showed collections of dust most marked dorsally, at 30 days. By 150 days a few scattered, whitish areas, which were probably fibrous tissue, were seen (Fig. 4A). There was very little change macroscopically in the lungs from this period till 365 days, when the areas of fibrosis were still small and discrete (Fig.4B).

The tracheo-bronchial lymph nodes showed a mild

degree of enlargement at 30 days, which increased steadily during the course of the experiment.

Microscopic Appearances

Histological examinations of the lesions were made at 150 days in the case of the animals injected with quartz, and at 150, 240, 330 and 365 days in those injected with corundum.

Quartz: At 150 days both lungs showed large, confluent, acellular lesions with a few patent alveoli and some blood vessels lying within them. On reticulin staining they were seen to be composed entirely of thick collagen bundles (grade 5 fibrosis, Fig.5), and micro-incineration revealed much dust within the lesions (Fig.6).

Corundum: The histological appearances were very unlike those seen in the lungs of the animals in the previous group. At 150 days there were a few dust cells lying free within the lung alveoli, while most of them were collected together in the form of nodules which were small and discrete. On reticulin staining these lesions were made up of thick reticulin fibres, arranged loosely for the most part, although they were becoming compact in a few lesions (grade 1 fibrosis, maximal. Fig.7). Micro-incineration revealed a fair amount of dust in the lesions (Fig. 8). By 240 days the lesions were no different to those seen at 150 days. No further changes were observed either at 330 days or 365 days, when the lesions were still discrete and small, with a few reticulin fibres within them, and remained within the maximal

limits of grade 1 fibrosis.

(B) ANTHRACITE, BITUMINOUS AND ANTHRACITE AND BITUMINOUS MIXTURE

There was no difference macroscopically between the lungs of the animals injected with anthracite, bituminous or the anthracite and bituminous coal dust mixture. Histologically, too, the lesions were identical.

Macroscopic Appearances

At 30 days the lungs of the animals in all three groups showed dense collections of coal dust over the dorsal aspect mainly, with a few pleural adhesions between the pleura and the chest wall. The gross appearances of the lungs showed no change from this time till the end of the experiment at 400 days.

The tracheo-bronchial lymph nodes showed slight enlargement at 30 days; the degree of enlargement became more marked in the later stages and the glands were black in colour.

Microscopic Appearances

Anthracite Coal 100 mg. At 30 days both lungs showed dust diffusely distributed; some of it was lying free in the alveoli, while some were within dust cells. There was no evidence of fibrosis (Fig. 9). At 60 days all the dust was within mononuclear cells which were still diffusely distributed. By 90 days there were a few subpleural collections of dust cells and the rest of the dust cells were aggregated to form focal nodular lesions, which were perivascularly

situated for the most part. There was some dust in the peribronchial lymphoid tissue. Reticulin staining did not reveal any fibrosis. By 180 days all the dust cells were aggregated to form nodules which were discrete and irregular in shape. A few scanty reticulin fibres were seen in the nodules, but no definite fibrosis (Fig. 10). Around the nodules there was a mild degree of focal emphysema. From this time till the termination of the experiment at 400 days, the lesions did not progress, there being the same discrete, cellular nodules with the dust tightly packed together, but no fibrosis.

Bituminous Coal 100 mg. At 30 days all the dust was diffusely distributed throughout the lungs and was lying free within the alveoli (Fig. 11). By 90 days there were subpleural collections of dust and commencing nodule formation in the lung fields. The dust cells were collected into localised nodules without any fibrosis. At 150 days the nodules were cellular, discrete and perivascularly situated. Reticulin staining showed a few reticulin fibres surrounding these nodules (Fig. 12). There was some focal emphysema. During the later stages of the experiment the dust in the nodules was more closely collected together, but no fibrosis was evident, even at 400 days.

Anthracite and Bituminous Coal Mixture: Diffuse distribution of dust throughout both lung fields was seen at 30 days. Most of the dust was free in the alveoli, while some were within dust cells (Fig. 13). At 60 days a few subpleural

dust collections were visible. Most of the dust at this stage was within mononuclear cells and showed a tendency to collect together. By 150 days definite nodules were seen with an irregular shape; they were discrete, cellular and perivascular. At 180 days reticulin staining of these lesions did not show anything more than a few scanty reticulin fibrils amid the cells forming the nodules, (Fig.14). There was some focal emphysema in the neighbourhood of the lesions. The lesions remained discrete and cellular with no fibrosis till 400 days, when the experiment was concluded.

(C) RADIO-ACTIVE AND NON-ACTIVE TANTALUM CARBIDE

There were a few differences between the lungs of animals injected with radio-active tantalum carbide and those injected with the non-active dust.

Macroscopic Appearances

Radio-active Tantalum Carbide (25-50 microcuries). The lungs of an animal which died at 27 days were haemorrhagic, and showed deposits of dust on both lung surfaces. Two animals which died at 88 days showed similar changes in the lungs. By 250 days, when all surviving animals were killed, only occasional deposits of dust were seen on the lung surfaces. Four of the seven animals had multiple abscesses and areas of necrosis in their lungs. Bacteriological examination and culture of the contents of these abscesses proved that they were sterile.

The tracheo-bronchial lymph nodes were enlarged and black.

Radio-active Tantalum Carbide (5-10 microcuries). The lungs of the animal which died on the second day after injection were congested and there was some dust on the surface. There were no apparent dust collections on the lung surfaces of the animals killed at 240 days.

The tracheo-bronchial lymph nodes were slightly enlarged and black.

Non-active Tantalum Carbide: A few dust collections were seen on the lungs of the animals killed at 240 days and 300 days. The tracheo-bronchial lymph nodes were enlarged and black.

Microscopic Appearances

Radio-active Tantalum Carbide (25-50 microcuries). At 27 days there were dust cells lying free within the alveoli, while a few were aggregated. Some dust particles were free. There was marked hyperplasia of the peribronchial lymphoid tissue. By 88 days the dust cells were collected together in nodules which did not show any fibrosis. At 250 days the lungs showed very occasional nodular lesions without any fibrosis (Fig. 15). Emphysematous areas were seen in some cases, the spaces dilated to very large proportions. Parts of the lung were necrotic. Apart from this there was no evidence of the usual radiation changes occurring in the lungs.

Radio-active Tantalum Carbide (5-10 microcuries). In the lungs examined after two days the dust was seen lying free within the alveoli. At 240 days a few nodular collections of dust cells were seen, in which no reticulin fibres were

demonstrable by silver impregnation (Fig. 16). There was very little dust in the lungs at this stage. The peribronchial lymphoid tissues showed hyperplasia. A mild degree of emphysema was present.

Tantalum Carbide (Non-active). At 250 days a few nodular collections of dust cells were seen situated perivascularly. No fibrosis was seen on reticulin staining (Fig. 17). There was some hyperplasia of peribronchial lymphoid tissue. At 300 days the lesions were similar to those at 240 days, and showed very little dust in the lungs.

DISCUSSION

The degree and amount of fibrosis produced in the lungs by quartz and corundum of comparable size and amount were very different. Whereas quartz produced the maximum fibrosis with fully collagenous confluent lesions (grade 5) at 150 days, corundum merely produced lesions which remained within the maximum limits of grade 1 fibrosis, even at 365 days. In spite of this difference in the tissue reactions produced by these two dusts, their recoveries from the lungs at varying intervals were, on the whole, very similar, as seen from Table 4. There was a rapid loss of about 40% by weight of the dusts during the first 60 days, and after this the total recovery was fairly constant. It is possible that the early loss of material in the case of the corundum was due to the dust being removed via the bronchi and then being swallowed with mucus, as no loss due to solubility during the production of tissue lesions is likely to occur

with this dust. It is reasonable to assume from the available data that the same is probably true for the quartz. It would seem, therefore, that loss of material due to solubility during the production of lesions in the tissues is too slight to be detected by the technique employed in these experiments. There does not seem to be any relation, either, between the fibrogenicity of a dust and the rate at which it is eliminated from the lungs. About 30 days after the injection of dust, the tracheo-bronchial lymph nodes were large enough to be dissected out and analysed. There was more dust in the lymph nodes of animals injected with quartz than in those of animals injected with corundum. This difference was more marked in the later stages at 365 days. Contrary to expectations, it seems as if fibrogenic dust is more rapidly transported from the lungs than an inert dust. Moreover, this transport of dust appears to take place after fibrosis is established, when the dust would be more likely to be firmly lodged in the tissues.

The results obtained in the case of the different types of coal were quite different. Histologically, the lesions produced in the lungs by anthracite and bituminous coal of comparable particle size and ash content, were identical - discrete, cellular nodules surrounding which were a few reticulin fibrils, with no evidence of fibrosis even at 400 days. The lesions produced by a mixture of anthracite and bituminous coal were no different to those produced by either alone. Belt and King (1945) obtained similar results in their studies on the action of pure

anthracite and bituminous coal on the lungs of rats. The lesions in both cases were minimal and consisted of the simplest type of foreign-body reaction even at 500 days. Wright (1951) found, similarly, that rats failed to develop fibrosis in their lungs after exposure to an atmosphere of coal dust for long periods of time, even though he was able to demonstrate the presence of large amounts of dust in them. Badham, in Australia, exposed rabbits to coal dust inhalation for periods up to seven years, but no fibrosis was evident in their lungs (Fletcher, 1951). Although anthracite and bituminous coal behave in an identical manner with regard to the lesions they produce in the lungs of experimental animals, yet they differ in the rates at which they are eliminated from the lungs (Table 5 and graph). In the early stages of the experiment - during the first 60 days - the differences between the amounts of dust recovered were slight, there being more anthracite coal in the lungs than bituminous coal. By 90 days, however, about 33% of the injected dust was lost in the case of anthracite, while the bituminous coal showed a loss of nearly 54%. From this period onwards, there was a definite difference in the amounts recovered from the lungs, there being more anthracite coal remaining in the lungs than bituminous coal at all stages. The recoveries from the lungs of animals injected with the mixture of anthracite and bituminous coal gave intermediate values, being between those for anthracite and bituminous alone from 90 days onwards. Making allowances for unavoidable experimental errors, the

recovery values from the lungs of these three substances are, on the whole, significant. Recoveries on the bulked tracheo-bronchial lymph nodes showed no significant differences between the three groups till 250 days, after which there was more dust in the nodes of animals injected with bituminous coal than in the other two, which was what was to be expected. It is not possible to draw any definite conclusions from the lymph node recoveries, although when studied in conjunction with the recovery from the lungs it would appear from the available data that bituminous coal dust is eliminated more rapidly from the lungs than anthracite coal.

The results of <u>in vivo</u> measurements made on rats injected with radio-active tantalum carbide containing 25-50 microcuries were disappointing and difficult to interpret. The values were not very accurate as the counts made were much too high for accurate assessment, too much radio-active tantalum carbide having been used. Nevertheless, there was a diminution in the activity measurements up to 60 days (Table 6), and it is likely that this persisted, since despite inconsistencies in the counts made after post-mortem removal of the organs at 250 days, the lung activity had, in most cases, fallen to between one half and one-eighth of what it was at 30 days. In fact, in some cases it had fallen even below this. The tracheo-bronchial lymph nodes had 2 to 7% of the total activity, which compares fairly well with the percentages of coal found in the lymph nodes (Table 5) and of corundum (Table 4), but is less than those

for quartz. These inert, nearly non-pathogenic, dusts appear to be moved less rapidly than the pathogenic quartz.

In the group of animals injected with the smaller dose of isotope (5-10 microcuries), in vivo measurements were made on the animal as a whole and the total body activity, as well as the activity of the excreta, expressed as percentages of the total doses deposited in the animals as represented by the measurements obtained soon after injection (Table 7). Nearly 48% of the total activity deposited in the animals was lost by 17 days, 65% by 28 days and 70% by 56 days, while only 12% of the activity remained at 100 days. Assay of the excreta for activity showed a corresponding increase with decrease of body activity. These results suggest that most of the radio-active dust was eliminated fairly rapidly from the lungs, as the decrease in activity cannot be accounted for in any other way, since the isotope has a half-life of 120 days and radio-active decay would not be an important factor in the diminution of activity.

Neither the radio-active dust nor the non-active tantalum carbide produced pulmonary fibrosis in rats. In the animals injected with radio-active dust containing the larger dose of the isotope the lungs showed large areas of necrosis and sterile abscesses, but apart from this tendency there were no radiation-induced changes similar to those known to occur in human beings.

SUMMARY

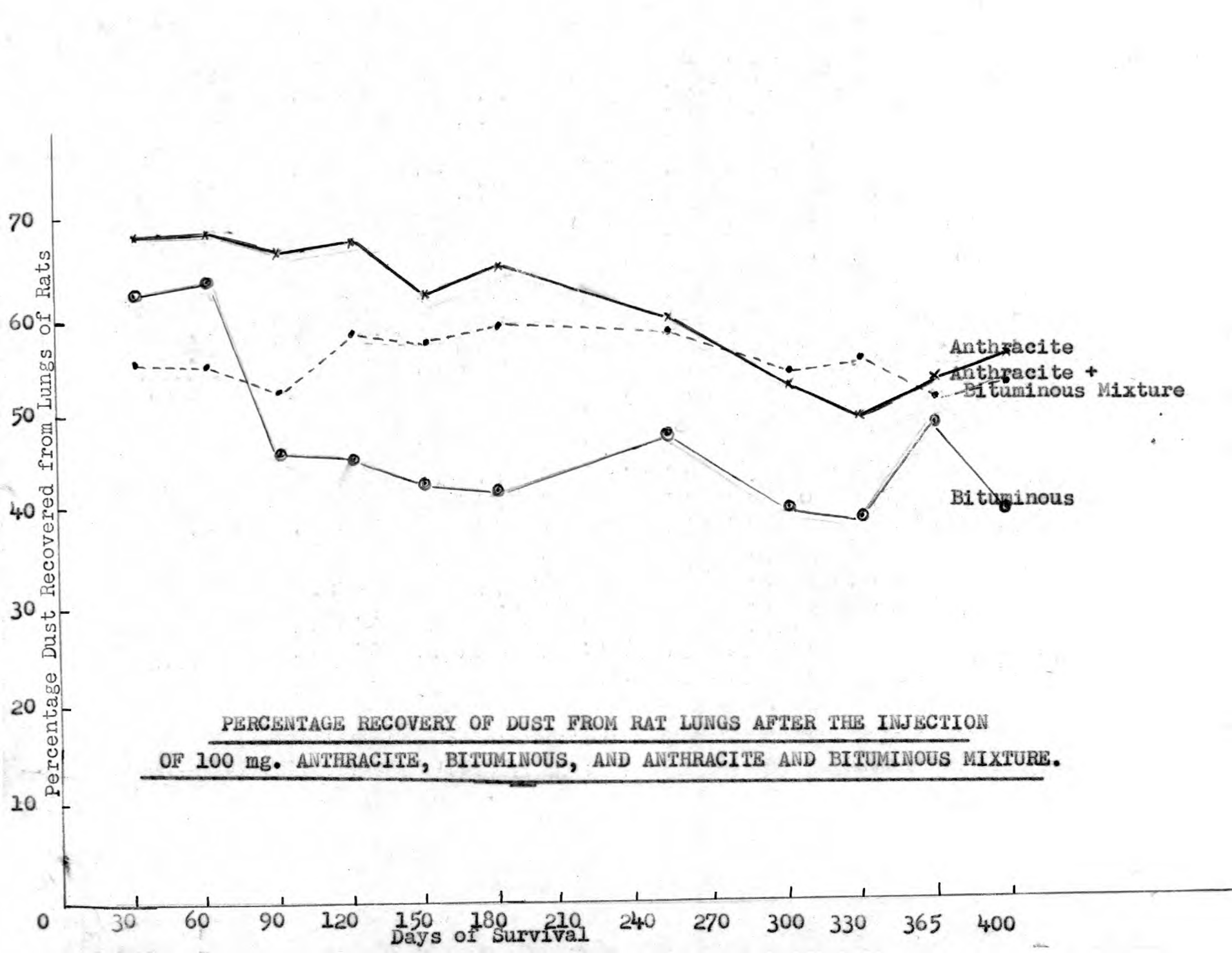
The recovery of quartz and corundum injected into

the lungs of rats was studied at varying periods after injection. The main loss occurred during the first 100 days, when 40% of the material injected was lost. The total recoveries for quartz which produced grade 5 fibrosis in 150 days and for corundum, which produced no more than grade 1 fibrosis (maximal) at 365 days, were so similar that loss of silica due to solubility of the dust in animal tissues is likely to be very small. Fibrogenic material (quartz) moved to the lymph nodes in greater amounts than the nearly inert substance corundum.

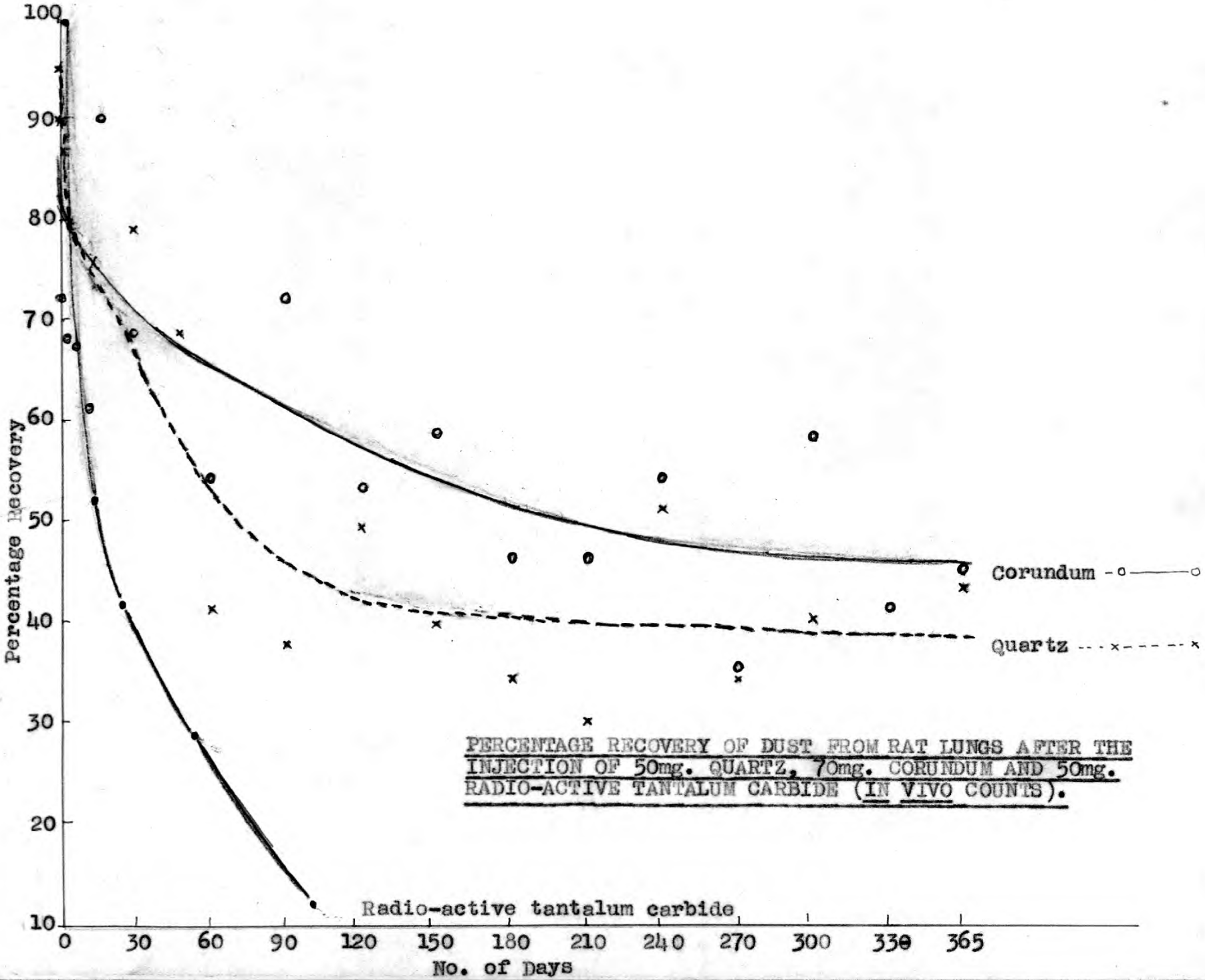
Anthracite and bituminous coal dust of similar particle size, and a mixture of the two, produced identical lesions in the lungs of rats - minimal reaction without any fibrosis, even at 400 days. Recoveries of these two dusts from the lungs yielded very different results, bituminous coal being found in smaller amounts than anthracite. By 120 days only about 30% of the anthracite was eliminated from the lungs, while nearly 55% of the bituminous was removed during the same period. Recoveries for the mixture of the two coals gave intermediate values. There seemed to be more dust in the lymph nodes of animals injected with bituminous coal than in the other two groups.

Rats injected with radio-active tantalum carbide containing 25-50 microcuries or 5-10 microcuries of isotope, exhibited a diminution in activity as seen by the results of <u>in vivo</u> measurements during the course of 250 days. In those animals injected with the larger dose, the lung activity fell

to less than $\frac{1}{8}$ the original, while in the animals injected with the smaller dose only 12% of the total dose deposited in the animals remained at the end of 100 days. Neither the radio-active nor the non-active dust produced pulmonary fibrosis in rats.



PERCENTAGE RECOVERY OF DUST FROM RAT LUNGS AFTER THE INJECTION OF 100 mg. ANTHRACITE, BITUMINOUS, AND ANTHRACITE AND BITUMINOUS MIXTURE.



PERCENTAGE RECOVERY OF DUST FROM RAT LUNGS AFTER THE INJECTION OF 50mg. QUARTZ, 70mg. CORUNDUM AND 50mg. RADIO-ACTIVE TANTALUM CARBIDE (IN VIVO COUNTS).



Fig. I A. Electron micrograph of quartz dust (Snowit) nominal size, 0.8-1 μ. (1 μ = 6mm.) (X 6000).



Fig. I B. Electron micrograph of synthetic corundum (Alumina), nominal size 0.8 - 1 μ (1 μ = 6 mm.) (X. 6,000).



Fig. 2. Radiograph of normal rat lung, air-inflated, for comparison with dust-injected lungs. Shows normal lung markings with alveoli and vessels. (X 1.5)



Fig. 3 A. Radiograph of rat lung, 150 days after injection of 50 mg. quartz, showing replacement of normal lung tissue by fibrous areas; very little normal tissue seen in the peripheral parts of the right lung only. Air inflated. (X. 1.5).



Fig. 3 B. Rat lung, 365 days after injection of 50 mg. quartz. Fibrosis seen in the greater part of the lungs. Air-inflated. (1.5).



Fig. 4 A. Radiograph of air-inflated rat lung, 150 days after injection of 70 mg. synthetic corundum. Shows lung markings in most areas with very little fibrosis. (X. 1.5).

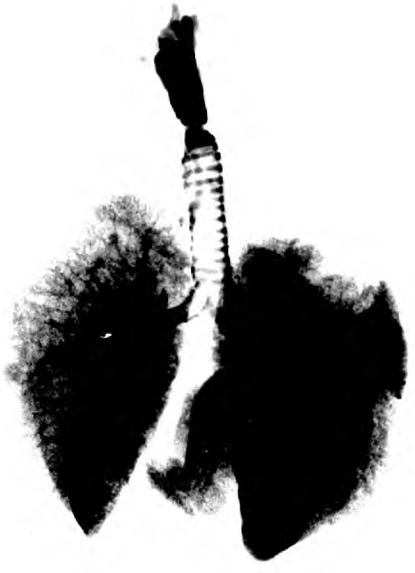


Fig. 4 B. Radiograph of rat lung, 365 days after injection of 70 mg. synthetic corundum. There is some fibrosis, but most of the lung fields appear clear. Air-inflated (X.1.5).

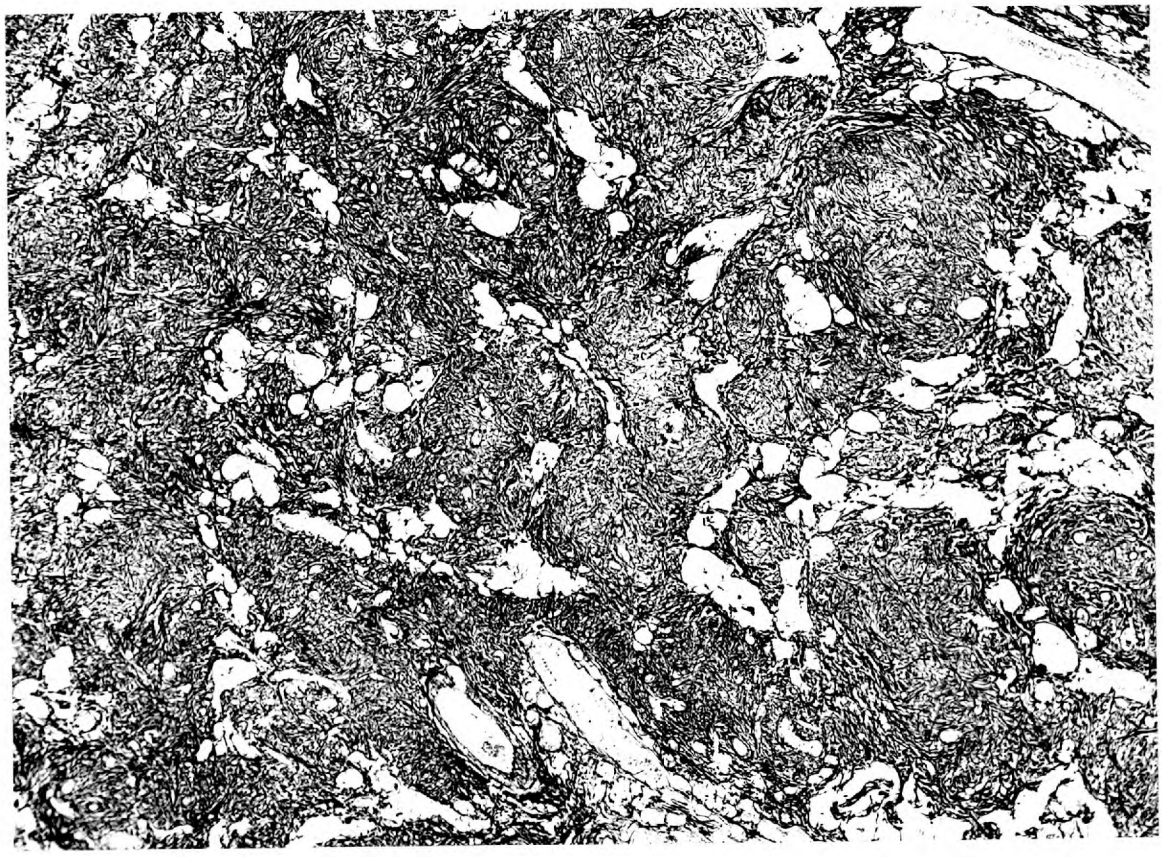


Fig. 5. Rat lung, 150 days after injection of 50 mg. quartz. Shows confluent lesions which are totally collagenous (grade 5 fibrosis). Silver impregnation. (X. 39).

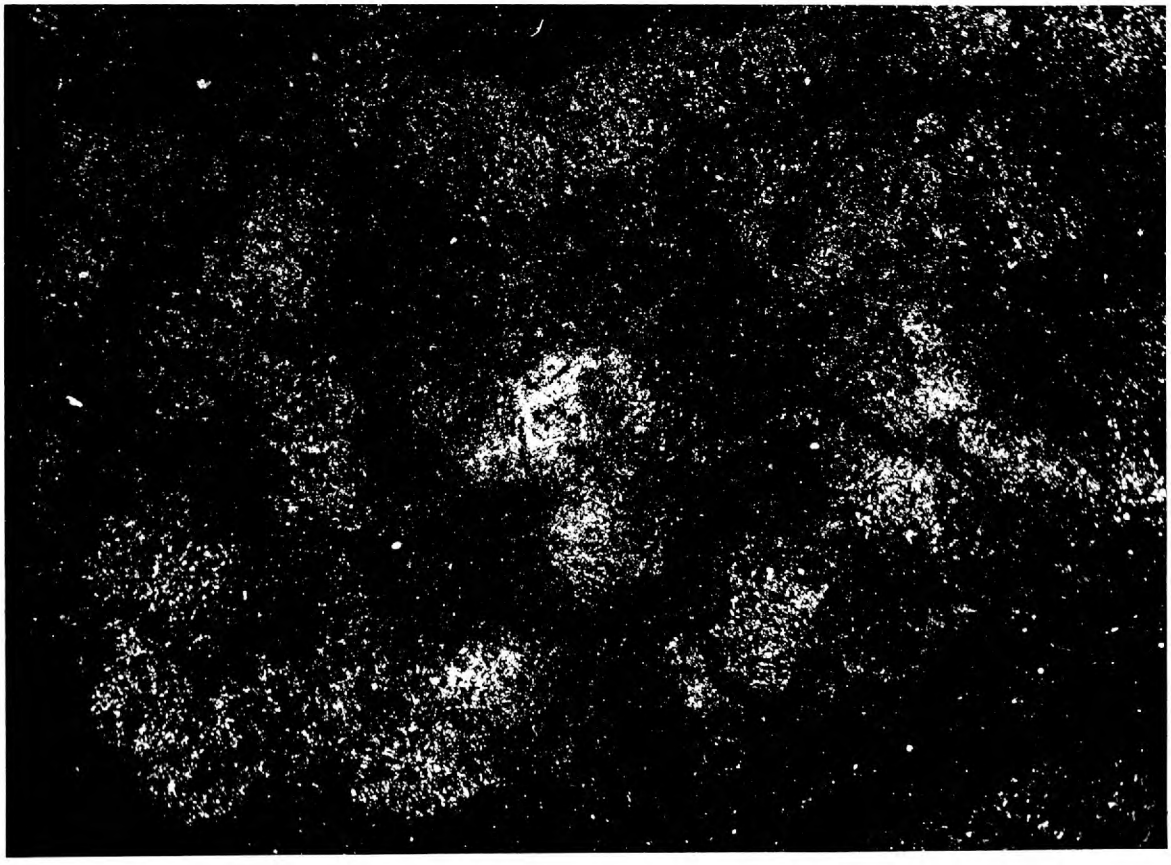


Fig. 6. Serial section to Fig. 5 photographed under dark-ground illumination to show the distribution of dust in the lesions. Micro-incinerated preparation. (X. 39).

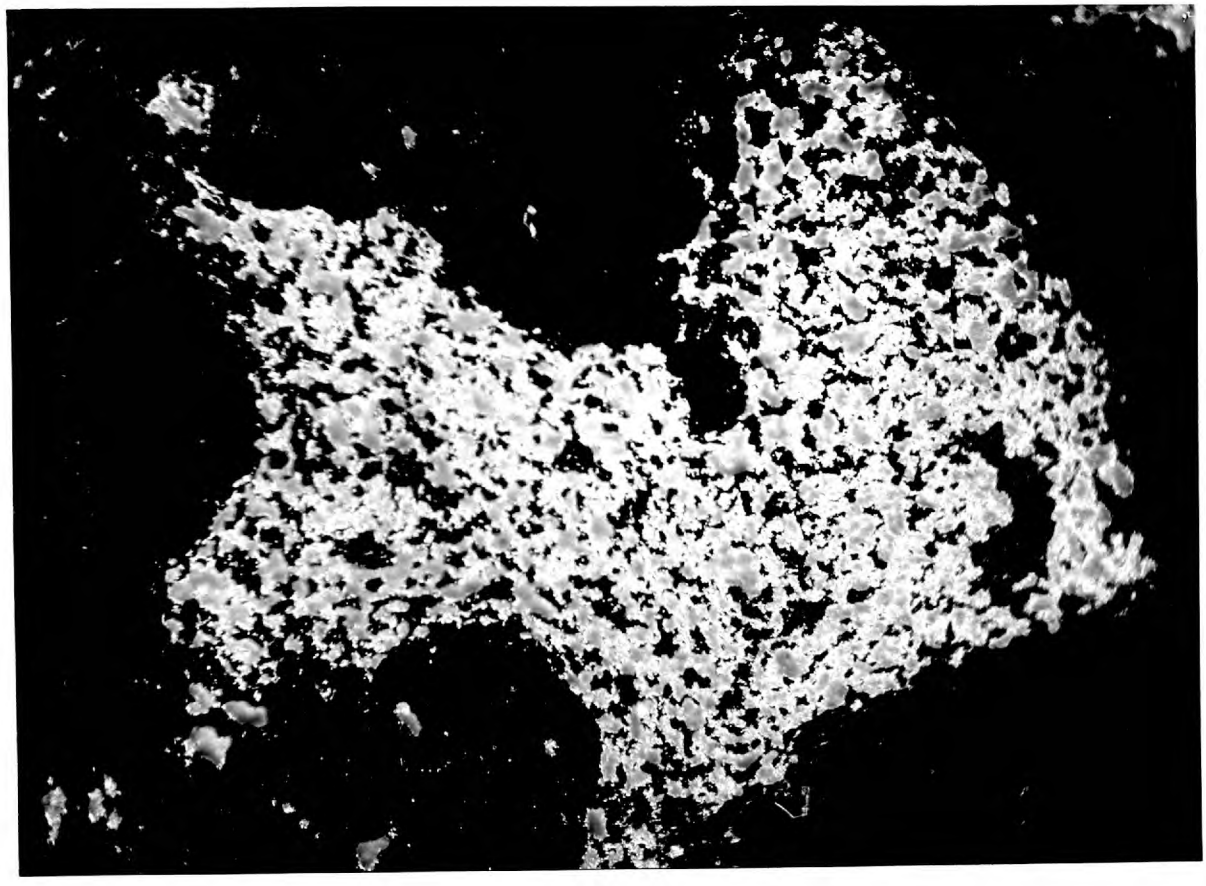


Fig. 8. Same nodule as in Fig. 7. Micro-incinerated preparation showing a large amount of dust in the lesion. (X.175).

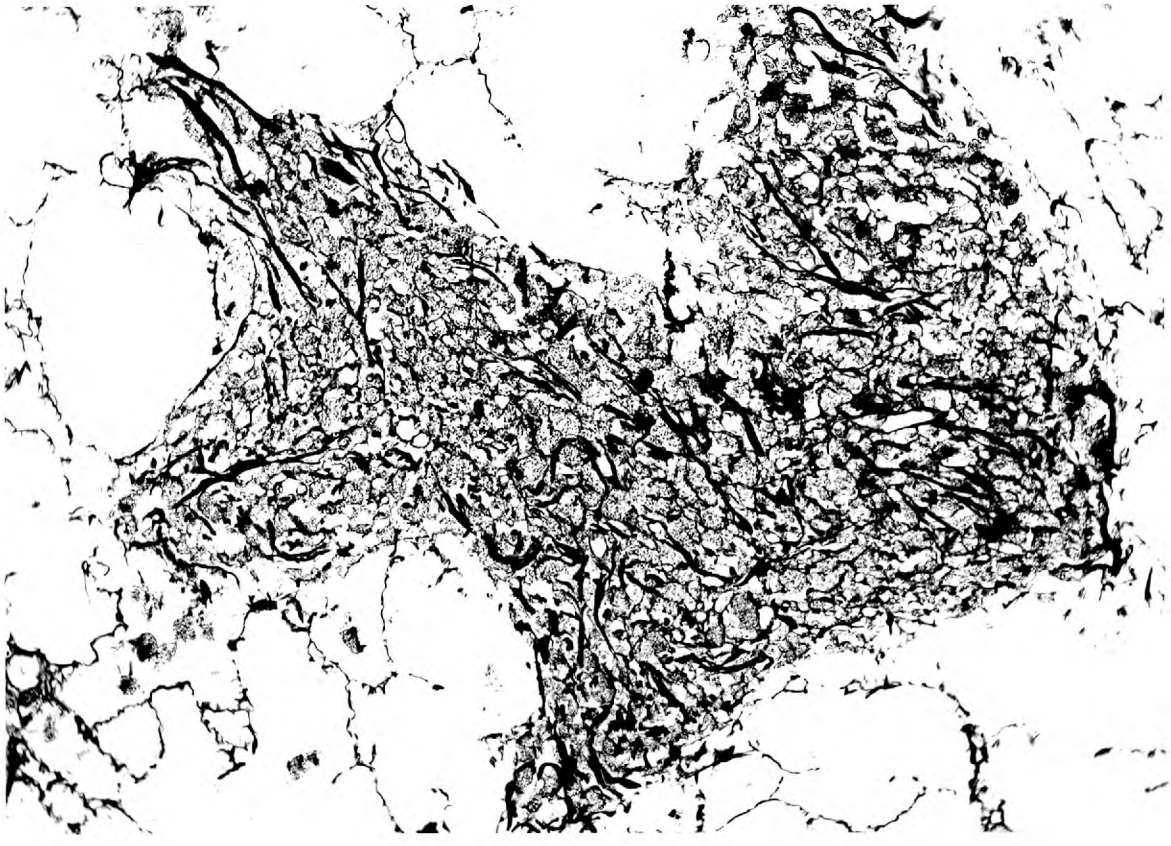


Fig. 7. Rat lung, 150 days after injection of 70 mg. corundum. (Al_2O_3). Nodular lesions with thick reticulin fibres loosely arranged (grade I fibrosis, maximal). Silver impregnation. (X. 175).

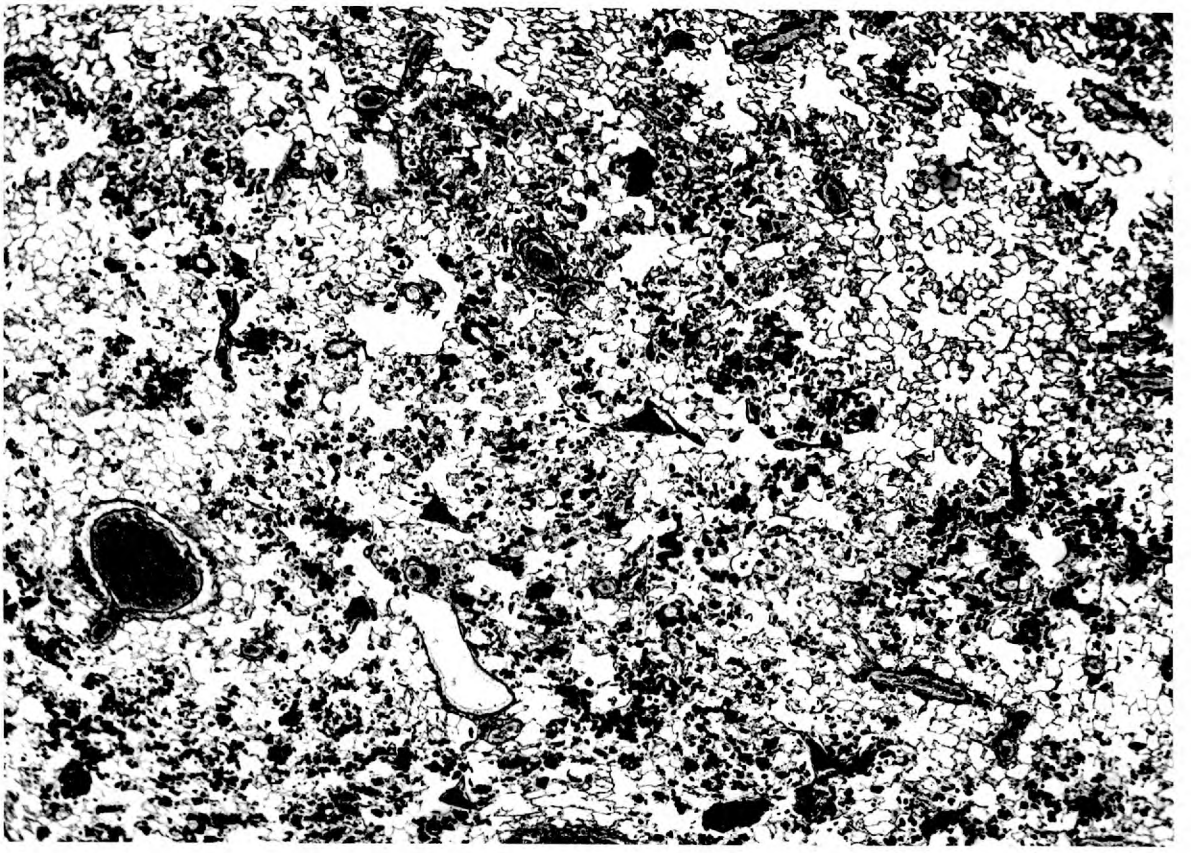


Fig. 9. Rat lung, 30 days after injection of 100 mg. anthracite coal. Dust distributed diffusely throughout the lung within alveoli. Silver impregnation. (X. 42.)

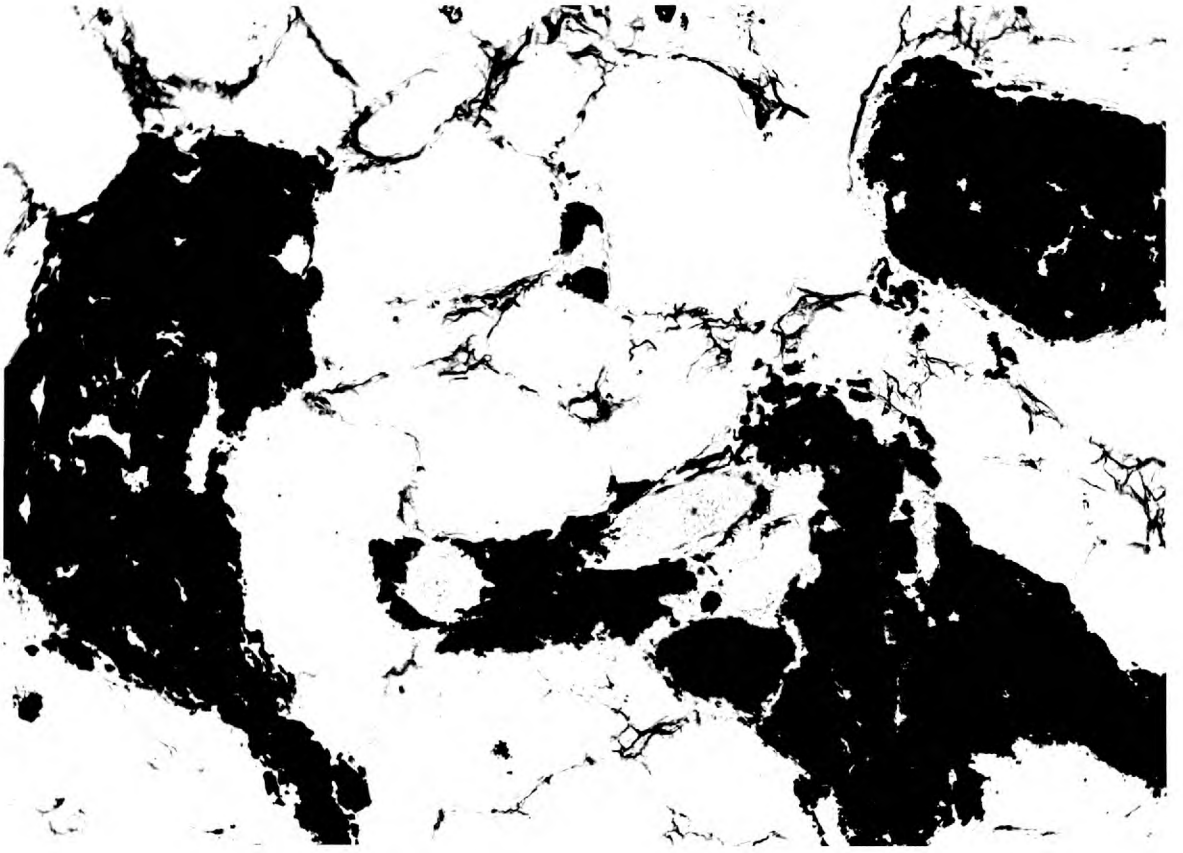


Fig. 10. Rat lung, 180 days after injection of 100 mg. anthracite coal. Dust collected into focal nodules which are compact. No evidence of fibrosis. Silver impregnation. (X. 220).

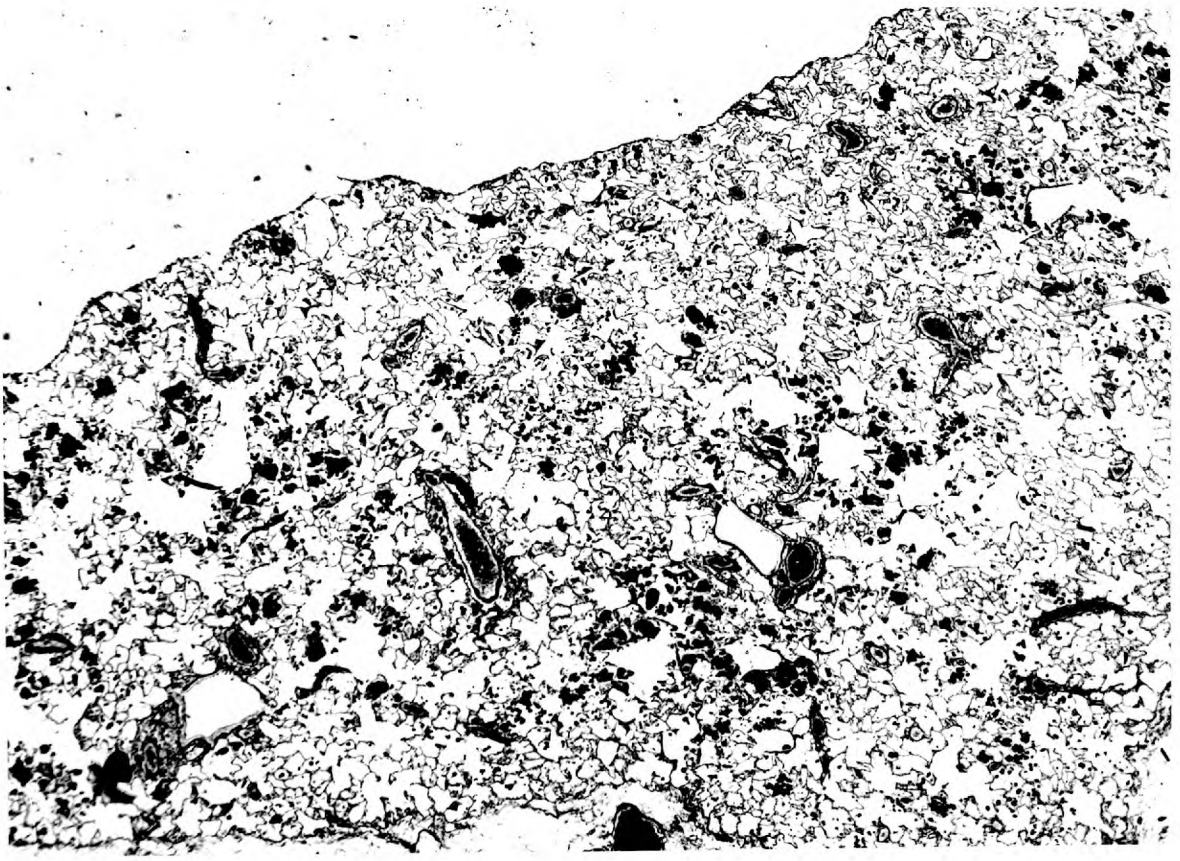


Fig. 11. Rat lung, 30 days after injection of 100 mg. bituminous coal. Dust diffusely distributed throughout lung alveoli. Silver impregnation. (X. 42.)

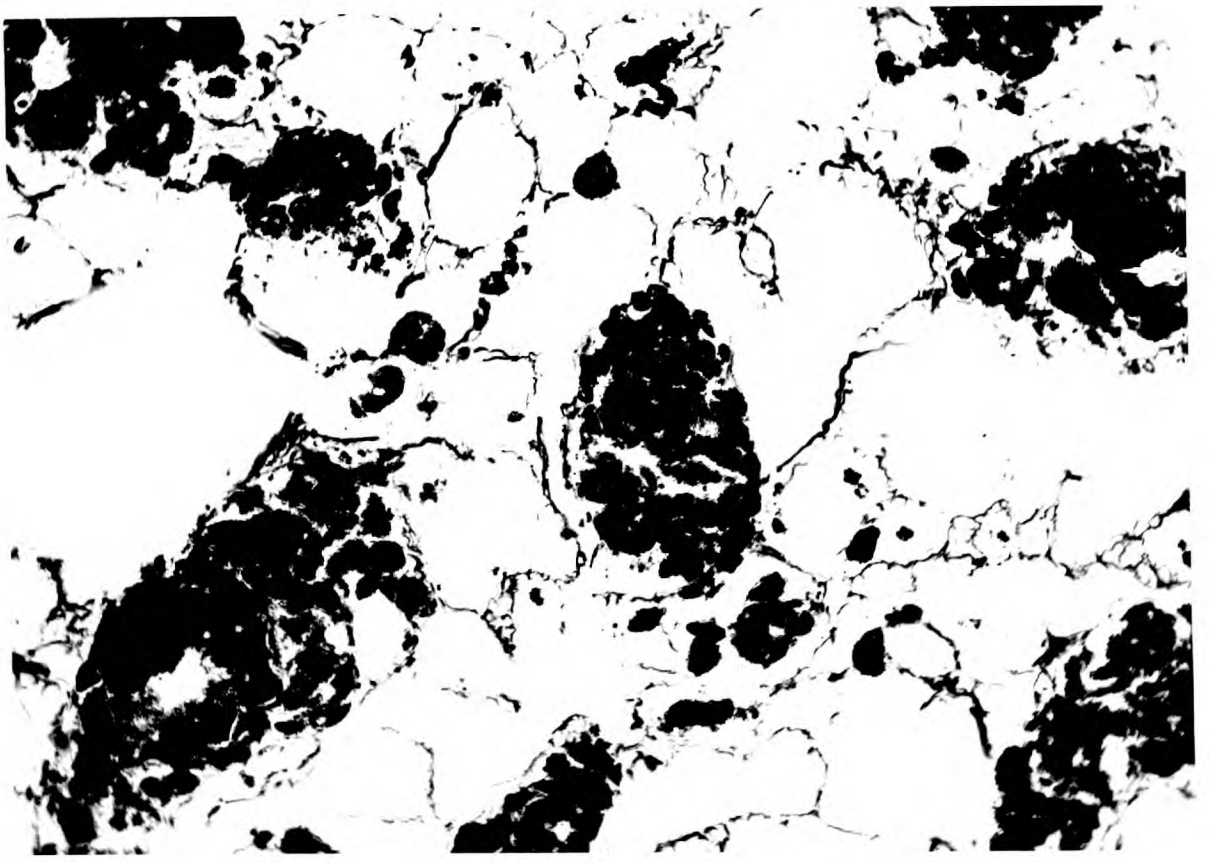


Fig. 12. Rat lung, 150 days after injection of 100 mg. bituminous coal. Focal aggregations of dust with no reticulin fibrosis. Silver impregnation. (X. 220).

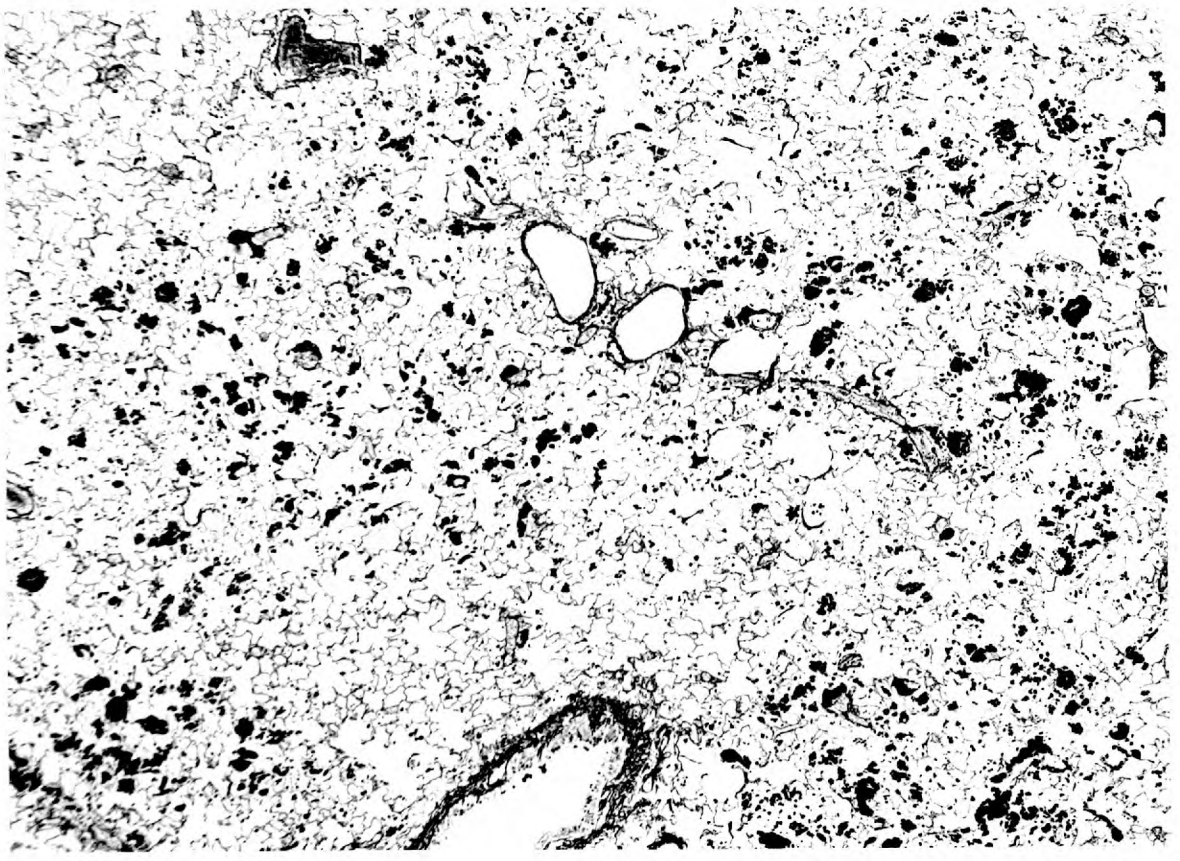


Fig. 13. Rat lung, 30 days after injection of 100 mg. anthracite plus bituminous mixture. Dust distributed diffusely throughout the lung. Silver impregnation. (X. 42.)

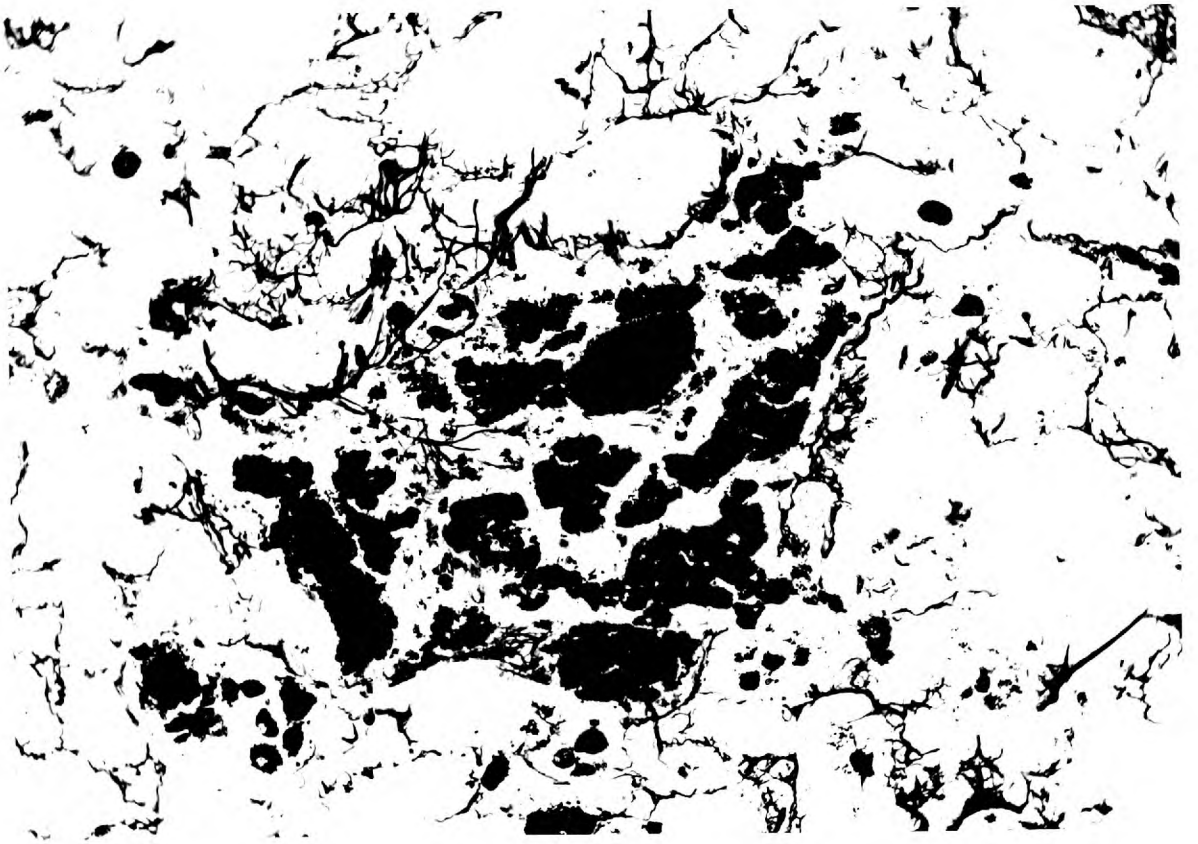


Fig. 14. Rat lung, 180 days after injection of 100 mg. anthracite and bituminous mixture. Dust collected into nodules with scanty reticulin fibres surrounding them, but no definite fibrosis. Silver impregnation. (X.220).

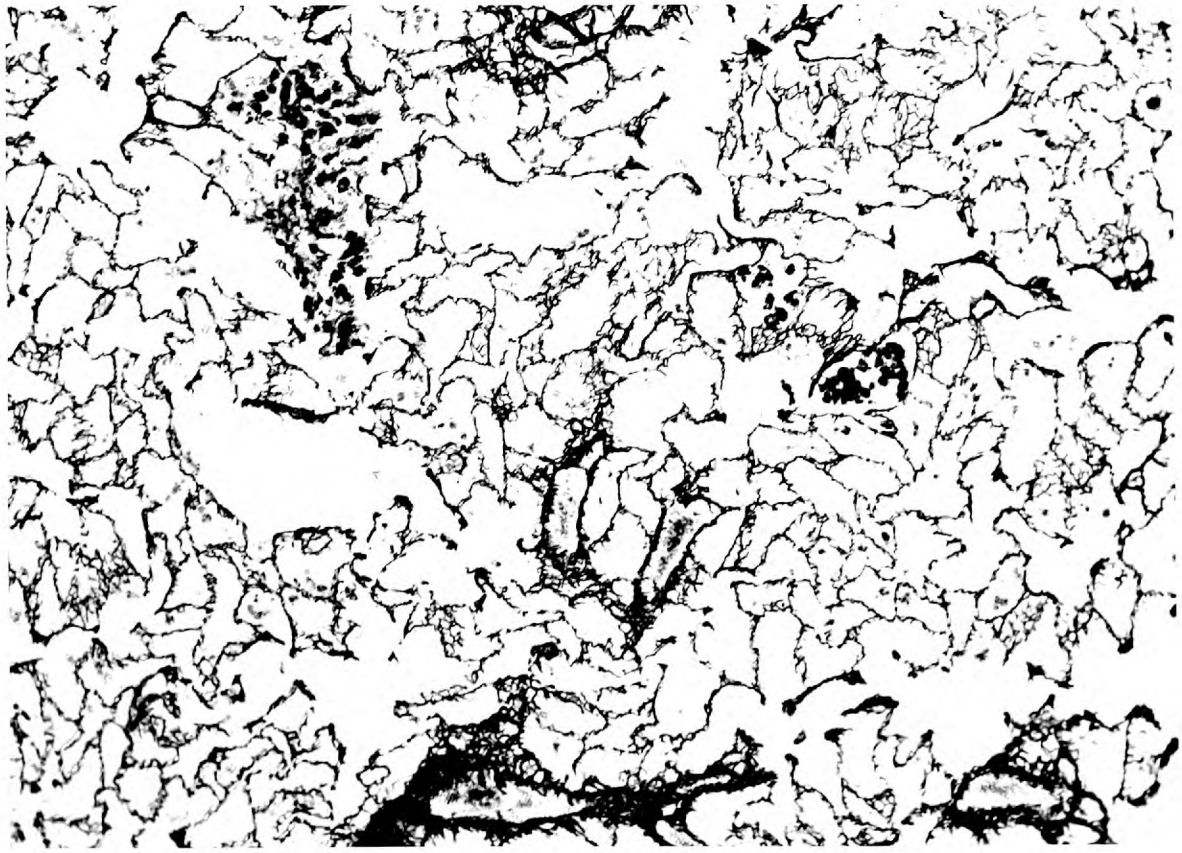


Fig. 15. Rat lung, 250 days after injection of 50 mg. radio-active tantalum carbide containing 25-50micro-curies of isotope. Small collections of dust, without any fibrosis. Silver impregnation. (X. 130).

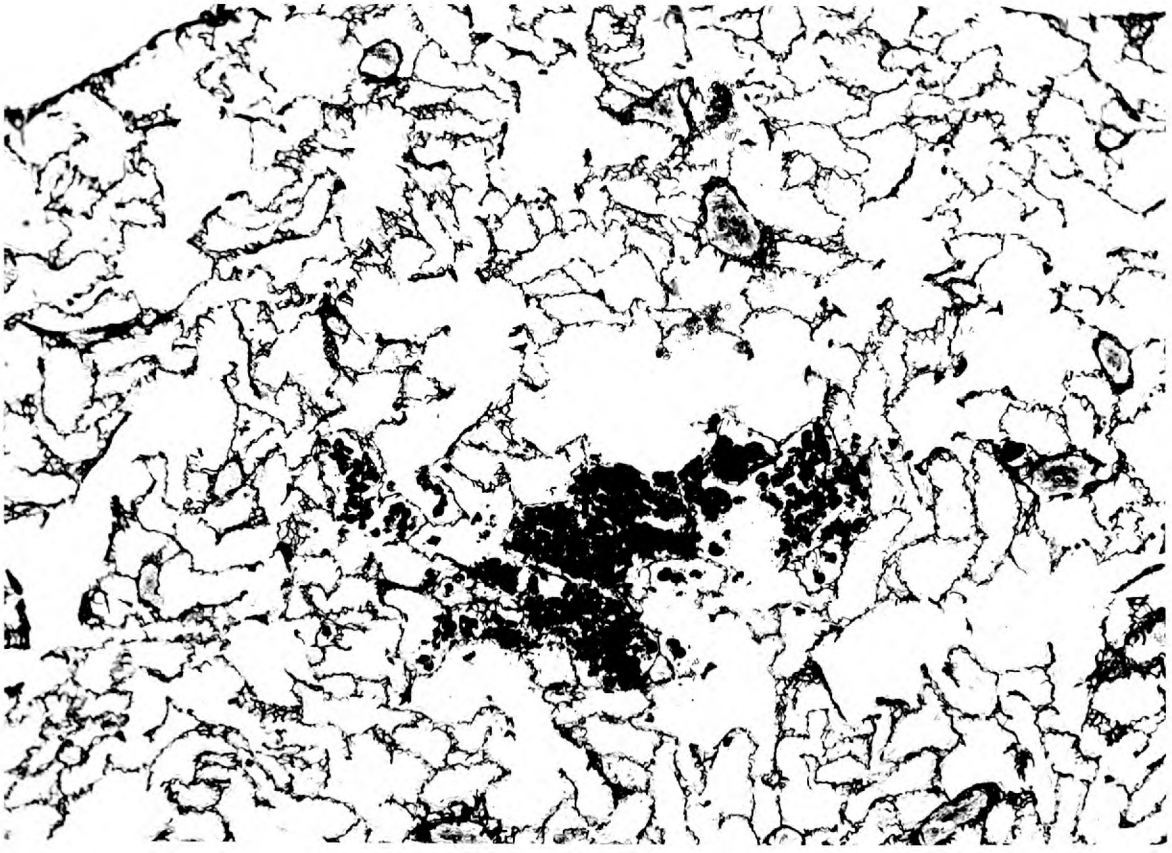


Fig. 16. Rat lung, 240 days after injection of 50 mg. radio-active tantalum carbide containing 5-10 microcuries of isotope. Small, irregular dust lesions with no fibrosis. Silver impregnation. (X. 130).

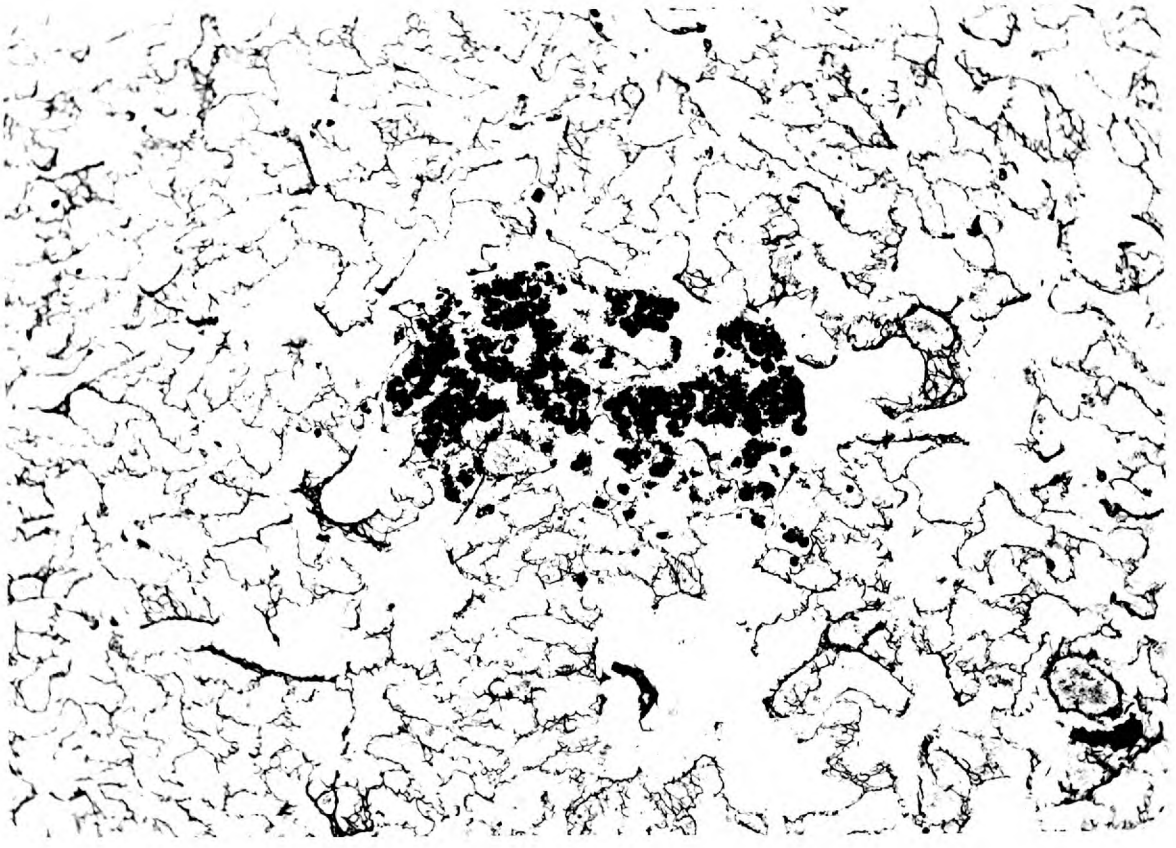


Fig. 17. Rat lung, 250 days after injection of 50 mg. tantalum carbide (non-active). Focal collections of dust without any increased reticulin. Silver impregnation. (X. 130.).

SECTION II

SECTION II

THE ACTION OF VARIABLE AMOUNTS OF TRIDYMITE ON THE LUNGS OF RATS

It is an accepted fact that pure silica can produce tissue reactions in experimental animals similar to those found in human silicosis. Various forms of pure silica are known to exist in nature and are inhaled by workers employed in industries making use of these dusts. Several modifications of pure silica exist at room temperature and of these, quartz, cristobállite and tridymite are crystalline forms, while fused silica is a solid.

Although many forms of silica have been investigated in a general way, very little was known as to the difference in pathogenicity between the various forms of pure silica till recently. Investigations on the rate and severity with which the different forms of silica act were made by Gardner and Cummings (1933) and later by Gardner (1938). They studied the reactions produced by the different forms of pure silica when introduced into rabbits by the intravenous route. Quartz produced nodular fibrosis of the liver on intravenous injection, while the most severe and rapid lesions were obtained with tridymite. From the results of these experiments, Gardner arranged the different forms of silica according to the intensity of the reactions produced by them. He found that tridymite was most pathogenic, cristoballite came next and the others followed with chalcedony and tripoli, quartz, and vitreous silica in that order.

King, Mohanty, Harrison and Nagelschmidt (1953(a)) injected pure forms of tridymite, cristoballite, quartz and fused silica of similar silica solubility and uniform particle size - $< 2\mu$ - in equal amounts into the lungs of rats, and studied the reactions produced by them. They found that tridymite produced the most fibrosis in the shortest period of time, cristoballite produced more fibrosis than quartz in the same period, while fused silica produced less. The reaction produced in the tissues by tridymite was dramatic; the maximum degree of fibrosis was reached in 60 days, while cristoballite took 240 days to produce the same degree of fibrosis and quartz 270 days; and fused silica failed to produce lesions of the same severity even up to 450 days. The reason as to why the tridymite was so much more fibrogenic than the other forms of pure silica was not clear. It may be mentioned, however, that in the course of preparation, the tridymite had been in contact with hydrofluoric acid at one stage. This raised the question as to whether the extreme pathogenicity of the tridymite could, in any way, have been due to the hydrofluoric acid, as King, Mohanty, Harrison and Nagelschmidt (1953(c)) found that treatment of a sample of quartz with hydrofluoric acid enhanced its pathogenicity. It was therefore decided to use a sample of tridymite similar to the previous one in every way except in that it had not been in contact with hydrofluoric acid, and to study the reactions produced in the lungs of experimental animals by variable amounts of this dust. A sample of the dust was also used after electro-dialysis to

ensure its further purification by the removal of any adsorbed electrolytes.

Experiments were set up using tridymite in doses of 12.5 mg., 25 mg., 50 mg. and 25 mg. of the electro-dialysed dust per rat, injected intra-tracheally. Animals were killed at regular intervals and the lung pathology studied.

Group I - 12.5 mg. tridymite/rat

Group II - 25 mg. tridymite/rat

Group III - 25 mg. electro-dialysed tridymite/rat

Group IV - 50 mg. tridymite/rat

Description of Samples

Synthetic tridymite was prepared from Lochaline sand with a flux. The flux was dissolved after heating by prolonged boiling with hydrochloric acid. The resulting tridymite was very pure and a fraction below 2 microns was prepared at the Safety in Mines Research Establishment, Sheffield, by sedimentation in water after grinding the material in an agate mortar.

The above sample was electro-dialysed for twenty hours between platinum electrodes to remove any adsorbed electrolyte.

The tridymite was kindly supplied by Mr. Badger of the North Thames Gas Board Laboratory.

The chemical analyses, solubilities and particle size distribution of the two samples are given in Table 1, and the electron micrographs in Fig. 1.

Preparation of Dust Suspensions for Injection

Amounts of 0.1875 g., 0.375 g. and 0.75 g. of the tridymite sample and 0.375 g. of the electro-dialysed sample

TABLE 1

PARTICLE SIZE DISTRIBUTION AND ANALYSIS OF DIFFERENT SAMPLES OF TRIDYMITE USED IN ANIMAL EXPERIMENTS

Sample	Tridymite		Tridymite (Electro-dialysed)	
Size in u	Per Cent By No.	By Mass	Per Cent By No.	By Mass
0.23 - 0.32			22.4	0.3
0.32 - 0.45	12.5	0.7	22.1	0.9
0.45 - 0.64	22.4	3.4	18.4	2.1
0.64 - 0.9	29.4	12.3	14.1	4.4
0.9 - 1.3	27.3	31.8	9.4	8.3
1.3 - 1.8	6.2	20.7	7.8	19.6
1.8 - 2.6	1.6	15.0	4.0	28.2
2.6 - 3.6	0.6	16.1	1.8	36.2
Specific Surface $m^2/gm.$	2.3		1.7	
SiO_2 (%)	99.3		99.2	
Ignition loss (%)	0.10		0.57	
Silica Solubility (SiO_2 mg/100 ml.)	10.7		9.05	

were weighed into 25 ml. screw-capped bottles and 15 ml. of sterile physiological saline added to each, so that the final concentrations of the suspensions were 12.5 mg., 25 mg., 50 mg. tridymite and 25 mg. electro-dialysed tridymite per ml. of suspension. The mixtures were thoroughly shaken for about half-an-hour in a microid flask-shaker, autoclaved for 20 minutes at 15 lb. pressure and then re-shaken till the time of the injection. Each animal received 12.5 mg., 25 mg., 50 mg. and 25 mg. of the respective dust suspensions.

Animals

Ten male rats were used in each of the four experiments. They were the black and white hooded variety of the Medical Research Council strain and had an average weight of 200g.

The animals were lightly anaesthetized with ether and the dust suspensions injected intratracheally following the technique of Kettle and Hilton (1932), modified by Belt and King (1945) and by King, Mohanty, Harrison and Nagelschmidt (1953(b)), a detailed description of which has been given earlier.

There was no regurgitation of the dust and no immediate post-operative deaths.

Duration of Experiments

One rat from the group of animals injected with 12.5 mg. of dust, two from the group injected with 25 mg., two from the group with 25 mg. electro-dialysed dust, and five from the group which had 50 mg. were lost by death or cannibalism. There was a large number of deaths in the last group (50 mg.) during the first week of the experiment, due mainly to infection. It was, therefore, not possible to obtain equal days of survival in all

cases. However, the killing of animals in the various groups was so adjusted as to enable the lung pathology to be studied till the maximum degree of fibrosis was produced by the different amounts of dust injected. The first experiment (12.5 mg. dust) was extended to 300 days, and the second and third experiments (25 mg. of dust, and 25 mg. electro-dialysed dust) to 100 days. In the case of the last group, where the animals were injected with 50 mg. of dust, the experiment ran for 75 days only as none of the animals survived beyond that time.

Pathological Technique

One animal from each group was killed at monthly intervals as far as was possible. In the group which received the electro-dialysed dust this was done at fortnightly intervals.

Routine post-mortem examinations were carried out both on the animals which were killed, as well as on those found dead. The lungs were fixed in 10% formol-saline and blocks selected along their long axes at the level of the hilum, for histological study. The blocks were dehydrated, cleared, impregnated with paraffin wax, embedded, and serial sections made at 5 μ. These sections were stained with haematoxylin and eosin and Gordon and Sweets' silver impregnation; another was micro-incinerated and acid-washed and used to study the distribution of dust. The details of these procedures have been recorded earlier.

PATHOLOGICAL FINDINGS

The lungs of some animals were not available for histological examination owing to cannibalism. The lungs of

most of the animals which were found dead showed areas of bronchopneumonic consolidation with a certain amount of necrosis. These lungs were not included among those used to assess the degree of fibrosis due to dust.

Sections from the lungs were examined and the pulmonary fibrosis seen in the most advanced lesions in each case was graded according to Belt and King (1945), into one of five grades as previously described.

The pathological gradings so obtained, together with the number of days of survival of the animals and the mode of their death, have been summarized in Table 2.

Gross Appearances of the Lungs

The gross appearances of the lungs of the animals killed periodically from the four groups showed some variations.

12.5 mg. Tridymite. The group which received the smallest dose of dust (12.5 mg.) showed in the early stages some scattered subpleural collections of dust, greyish-white in colour and most marked over the dorsal aspects of the lungs. At 90 days, small, discrete areas of fibrosis were seen over both lungs and these appeared larger by 150 days. There was hardly any change from this time till 300 days, when one lung showed confluent areas of fibrosis and the other patchy fibrosis.

25 mg. Tridymite. Two animals from this group died during the first fortnight of the experiment. Their lungs were haemorrhagic and the dust lesions less prominent than in those examined later. At 30 days there were subpleural dust collections with well-marked fibrotic areas dorsally, which became confluent by 60 days.

TABLE 2

SUMMARY OF THE PATHOLOGICAL CHANGES IN THE LUNGS OF RATS BROUGHT ABOUT BY VARIABLE AMOUNTS OF TRIDYMITE

Days of Survival	12.5 mg.		25 mg.		25 mg. (Electro-dialysed)		50 mg.	
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
1 - 9			D(1)	-	K(1)	2 †	D(5)	-
10 - 15			D(1)	-	K(1)	2M	K(1)	2 †
16 - 30	K(1)	1 †	K(1)	2 †	K(1)	3M †	K(1)	3M
31 - 45			D(1)	-	K(1)	5 †		
46 - 60	K(1)	3 †	K(1)	5 †	K(1)	5	D(1)	5 †
					Eaten (2)			
61 - 75					K(1)	5	D(2)	5
76 - 90	K(1)	3	K(1)	5	K(1)	5		
91 - 100			D(3)	5				
101 - 120	K(1)	3	D(1)	5	K(1)	5		
121 - 180	K(2)	4						
181 - 299	K(2)	4						
	Eaten (1)	-						
300	K(1)	5 †						

† Sections from the lungs of these animals are reproduced in the figures.

K = Killed; D = Died; the numbers ( ) indicate the number of deaths or killings during that period.

M = Maximum within the indicated grade of fibrosis.

25 mg. Electro-dialysed tridymite. The first animal was killed at 9 days. The lungs showed the usual subpleural dust collections and, in addition, there were definite areas of fibrosis distributed throughout both lungs. At 15 days the fibrotic areas were beginning to become confluent, and by 45 days these confluent areas were larger. Part of the lung tissue was replaced by confluent fibrosis at 75 days and these areas increased in amount till the end of the experiment (100 days).

50 mg. Tridymite. Within the first week five animals in this group died. Their lungs were haemorrhagic and parts were firm due to consolidation. The animal killed at 15 days had subpleural dust collections dorsally, with discrete areas of fibrosis. At 30 days these areas were more marked, while both lungs of an animal dying at 50 days showed confluent fibrosis. The last two animals died at 72 days and their lungs showed massive confluent fibrosis with very little normal-looking areas left behind.

The tracheo-bronchial lymph glands in all these groups were enlarged to several times the normal size and were firm.

Microscopic Appearances of the Lungs

The lesions produced in the lungs by the various amounts of tridymite injected were similar in the type of reaction and differed merely in their severity. The smallest amount (12.5 mg.) produced a fair quantity of fibrosis at 30 days, progressing to grade 3 at 60 days, grade 4 at 150 days and the maximum fibrosis (grade 5) at 300 days. 25 mg.

tridymite produced fibrosis rapidly, reaching the maximum at 60 days. There was hardly any difference between the reactions produced by 25 mg. of tridymite and the same quantity of electro-dialysed dust. The most marked lesions were in the lungs of the animals which received 50 mg. of the dust, being grade 3 at 30 days and grade 5 at 52 days, involving nearly all the lung tissue.

In all groups, microscopically there was subpleural drift of dust and the pleura overlying the lesions showed thickening. Dust cells were present in some alveoli, in the interstitial tissues and lymphoid follicles. There was marked hyperplasia of the peribronchial lymphoid tissues.

12.5 mg. Tridymite. This dose produced mild lesions in the lungs in the early stages, but the maximum degree of fibrosis was not reached till 300 days. The lungs of the animal killed at 30 days showed a good dust cell reaction and a marked peri-bronchial lymphoid reaction. In many areas the dust cells were aggregated to form small, discrete, nodular lesions which were irregular in shape and mainly perivascular in distribution. These lesions were composed entirely of cells and on reticulin staining a loose network of fine reticulin fibres was seen within them (grade 1 fibrosis. Fig. 2). An occasional nodule showed a small amount of collagen. At 60 days the nodular lesions were still discr[illegible] and their centres were somewhat acellular. The loose reticulin network was replaced by collagen fibres which were thick and compact and seemed to occupy most of the

nodules (grade 3 fibrosis. Fig. 3). There was no appreciable difference in the histological picture from this time till 150 days, when the lesions were larger, while most of them were almost completely acellular. A few such lesions, however, were slightly cellular at the periphery. The nodules were composed wholly of thick collagen fibres tightly packed together in the form of a whorl (grade 4 fibrosis). From this period till 300 days there was little change in the lesions except for an occasional one which showed signs of commencing confluence. At 300 days the right lung showed many lesions which were acellular, confluent and fully collagenous (grade 5 fibrosis; Figs. 4 and 5), while the left lung presented a similar picture but with few confluent lesions.

25 mg. Tridymite. The animal killed at 30 days showed a good dust cell reaction in the lungs with a well-marked peribronchial lymphoid reaction. There were, in addition, many discrete cellular lesions perivascularly distributed, with thick and compact reticulin fibres. There was some collagen in many of the nodular lesions (grade 2 fibrosis. Fig. 6). By 60 days the lesions had progressed so rapidly that the nodules were mainly acellular, confluent and collagenous (grade 5 fibrosis. Figs. 7 and 8). About half the lung area examined was replaced by these confluent lesions. At 90 days the fibrosis had increased in quantity and the area of lung involved was greater than at 60 days. The two remaining animals died at 97 and 110 days respectively and their lungs presented the same histological appearances as those of the animal killed just before. Pleural thickening was noticed in the sections

examined from the lungs of animals killed in the later stages.

25 mg. Electro-dialysed Tridymite. The first animal killed in this group was at 9 days and the lungs showed a few dust cells lying within the alveoli and hyperplasia of the peri-bronchial lymphoid tissues. There were, also, numerous discrete, nodular lesions composed of aggregations of dust cells and situated perivascularly in the majority of cases. A few nodules showed, on reticulin staining, thick reticulin fibres arranged in a compact manner, while most of the others contained some collagen tightly packed (grade 2 fibrosis. Figs. 9 and 10). At 15 days the nodules had enlarged in size, were acellular for the most part and mainly composed of collagen (grade 3 fibrosis, Fig. 11). The lesions seen at 30 days were similar to the ones seen at 15 days. By 45 days the lesions were greatly advanced and nearly all the nodular areas were confluent, acellular and fully collagenous (grade 5 fibrosis. Figs. 12 and 13). A few nodules were still discrete and slightly cellular at the periphery. From this period onwards till the close of the experiment at 105 days the fibrosis seemed to progress quantitatively, so that there was very little lung tissue which was not involved. The picture was one of massive confluent fibrosis in both lungs. Pleural involvement was also present in the later stages.

50 mg. Tridymite. During the first week five animals died and their lungs showed varying degrees of pneumonic consolidation. One animal's lungs had multiple abscesses. In assessing the pulmonary fibrosis present in the lungs as a result of the injection of dust, the lungs which presented the appearances of infection /or consolidation were not included in the series.

The lungs of the animal killed at 15 days showed a good dust cell reaction and a marked peribronchial lymphoid reaction. There were many nodular lesions scattered throughout both lungs. They were discrete, cellular and had coarse, compact reticulin fibres with a certain amount of collagen (grade 2 fibrosis. Fig. 14). At 30 days the lesions still remained discrete although they were larger and somewhat acellular. On reticulin staining they were found to be composed mainly of compact collagen (grade 3 fibrosis). The next stage at which the lungs were examined was at 52 days, when one of the animals was found dead. The right lung was almost wholly replaced by large, confluent areas of fibrosis which were acellular and collagenous (grade 5 fibrosis. Figs. 15 and 16). Within such areas a few alveoli remained patent, and some new formation of blood vessels could be seen. The left lung presented a similar appearance, except that the amount of lung substance involved in the fibrotic process was much less than in the right one. This was probably due to unequal dispersal of dust in the two lungs at the time of injection. By 72 days the remaining animals in this group were dead. The lesions in their lungs were similar to those seen in the lungs of the animals killed previously (52 days), with the one difference that the extent of lung involvement by the pathological process was much greater, both lungs being almost totally replaced by massive confluent fibrosis, leaving hardly any normal functioning tissue.

DISCUSSION

The different quantities of tridymite used in these experiments produced very rapid fibrosis in the lungs of rats, as compared with other forms of pure silica. The smallest amount injected - 12.5 mg. - produced lesions which, according to the type of grading of fibrosis used (Belt and King, 1945), were of grade 1 severity in 30 days. These lesions progressed to grade 3 fibrosis by 60 days, grade 4 by 150 days and reached the maximum grade of fibrosis (grade 5) at 300 days. When double this amount of dust was used - 25 mg. - the process of fibrosis was hastened, grade 2 being reached at 30 days and grade 5 at 60 days. The same amount of dust injected after electro-dialysis produced fibrosis even more rapidly, grade 2 fibrosis being reached at 9 days, grade 3 in 30 days and grade 5 in 45 days. With 50 mg. of dust the tissue reaction was very pronounced. Grade 2 fibrosis was reached by 15 days, grade 3 by 30 days and grade 5 by 52 days; the actual amount of lung tissue involved by the fibrotic process was very great - almost the entire lung substance examined being replaced by massive areas of fibrosis. This dose appeared to be too toxic and the animals did not tolerate it well, there being no survivors after 72 days.

On comparing the severity of the lesions produced in animal lungs by these two preparations of tridymite at particular periods, it would seem at first that the electro-dialysed sample acted more rapidly in producing fibrosis than the other. A closer investigation, however, shows that this

apparent discrepancy is due to experimental variation. For instance, from the results, 50 mg. of tridymite is found to produce grade 5 fibrosis in 52 days, while 25 mg. of the electro-dialysed sample produced lesions of the same severity in 45 days. But it is possible that the maximum fibrosis in the former group might have been found to be present earlier than 52 days, if tissues had been examined between 30 and 52 days. Probably the maximum grade of fibrosis was produced before the period at which the tissues were examined and the fibrous lesions increased in quantity as was seen by the amount of lung tissue involved as compared with that in the animals receiving 25 mg. of dust. It is to be regretted that equal survival periods could not be obtained with the different groups of animals and that their killings could not be matched better, as this would have thrown more light on the subject. But this was not possible owing to the loss of a number of animals during the early stages of the experiments in some groups.

King, Mohanty, Harrison and Nagelschmidt (1953(a)) found from their experiments that 50 mg. of tridymite produced far more reaction in the lungs of rats than the same amount of the other crystalline forms of pure silica - cristoballite and quartz - of the same particle size and silica solubility. In their studies, 50 mg. of tridymite produced lesions of grade 2 severity in 30 days, grade 3 in 45 days and grade 5 in 60 days; cristoballite produced grade 2 lesions in 30 days, grade 3 in 60 days and grade 5 in 240 days; quartz produced grade 2 lesions in 60 days, grade 3 in 90 days and grade 5 in 270 days. In the

present series of experiments, the action of the tridymite was even more rapid, grade 5 being reached at 52 days. From these results it may be stated that the severity of the reaction produced by tridymite can, in no way, be attributed to contact with hydrofluoric acid, as was thought earlier. The dust used in the present experiments had not been treated with hydrofluoric acid, but the severity of its reactions in the tissues was in no way less than that of the sample so treated. Further purification of a sample by electro-dialysis does not seem to have made any difference either, as the tissue reactions were just as severe.

There does not seem to be any explanation for this severe tissue reaction produced by tridymite. However, a few facts may be mentioned which may account for it, at least partly. Detailed solubility studies were carried out on samples of tridymite, cristoballite and quartz of equal particle size. The solubilities as determined after a week were similar in these samples; but when solubility measurements were made at intervals of 2 hours, 5 hours, 48 hours, 5 days and 7 days on these same samples, the values for tridymite rose more rapidly than the corresponding values for cristoballite and quartz. If the same process occurs in tissues as it does in test-tubes, it is reasonable to assume that the tridymite goes into solution in the tissues earlier than cristoballite and quartz. According to the solubility theory, these facts indicate that lesions would be produced more rapidly by tridymite than by the more slowly dissolving cristoballite and quartz. Moreover, crystal structure may itself affect the biological properties of the

dust in a way not yet understood.

Tridymite appears to be several times as fibrogenic as quartz, which is also a crystalline modification of pure silica. 50 mg. of quartz dust produced the maximum degree of fibrosis (grade 5) in the lungs in 270 days (King et al, 1953(a)) while equal and half the amounts of tridymite of the same particle size did so in about one-fifth that time (52 days), and a quarter the amount (12.5 mg.) produced the same lesions in 300 days, which is a little longer than the time taken by 50 mg. quartz to produce grade 5 fibrosis.

These findings are in agreement with those of Gardner (1938) who concluded from his experiments that tridymite was the most fibrogenic of the various forms of pure silica, and of King, Mohanty, Harrison and Nagelschmidt (1953(a)), whose experiments also proved that tridymite produces more severe tissue reactions than other forms of pure silica.

SUMMARY

Variable amounts of tridymite dust of high purity and equal particle size were injected into the lungs of rats to study the reactions produced by them. The lowest dose injected - 12.5 mg. - produced the maximum degree of fibrosis in less than a year (300 days), while double the dose (25 mg.) did so in two months, and four times the amount in 45 days. The 50 mg. dose replaced most of the lung tissue by fibrous areas.

Electro-dialysis of the dust did not seem to have any effect in reducing its fibrogenicity, as the maximum fibrosis was produced as rapidly with the electro-dialysed sample as with

the ordinary one, when both were injected in equal amounts.

It is assumed from these experiments that tridymite which is a crystalline modification of pure silica is nearly five times as fibrogenic as quartz - another crystalline modification of pure silica - of equal particle size.

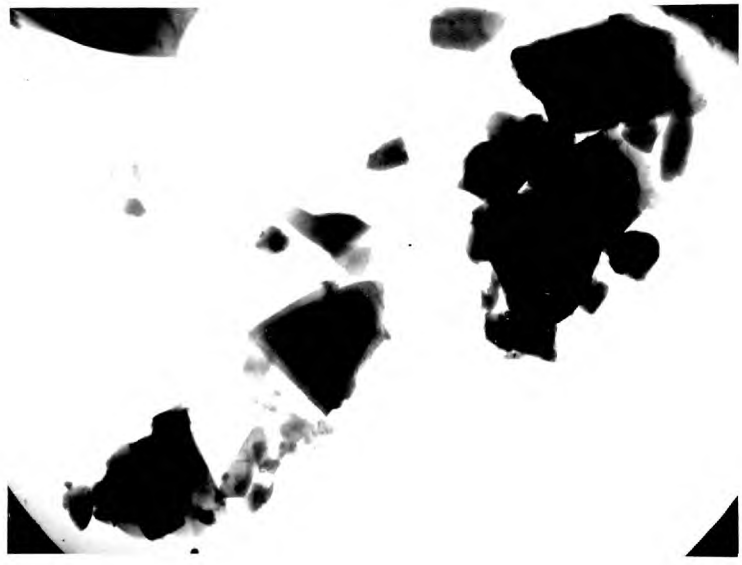


Fig. 1 A. Electron micrograph of tridymite dust $< 2\ \mu$. (1 μ = 6 mm.) (X. 6000).



Fig. I B. Electron micrograph of electro-dialysed tridymite dust. $< 2\ \mu$ (1 μ = 6 mm.) (X. 6000).

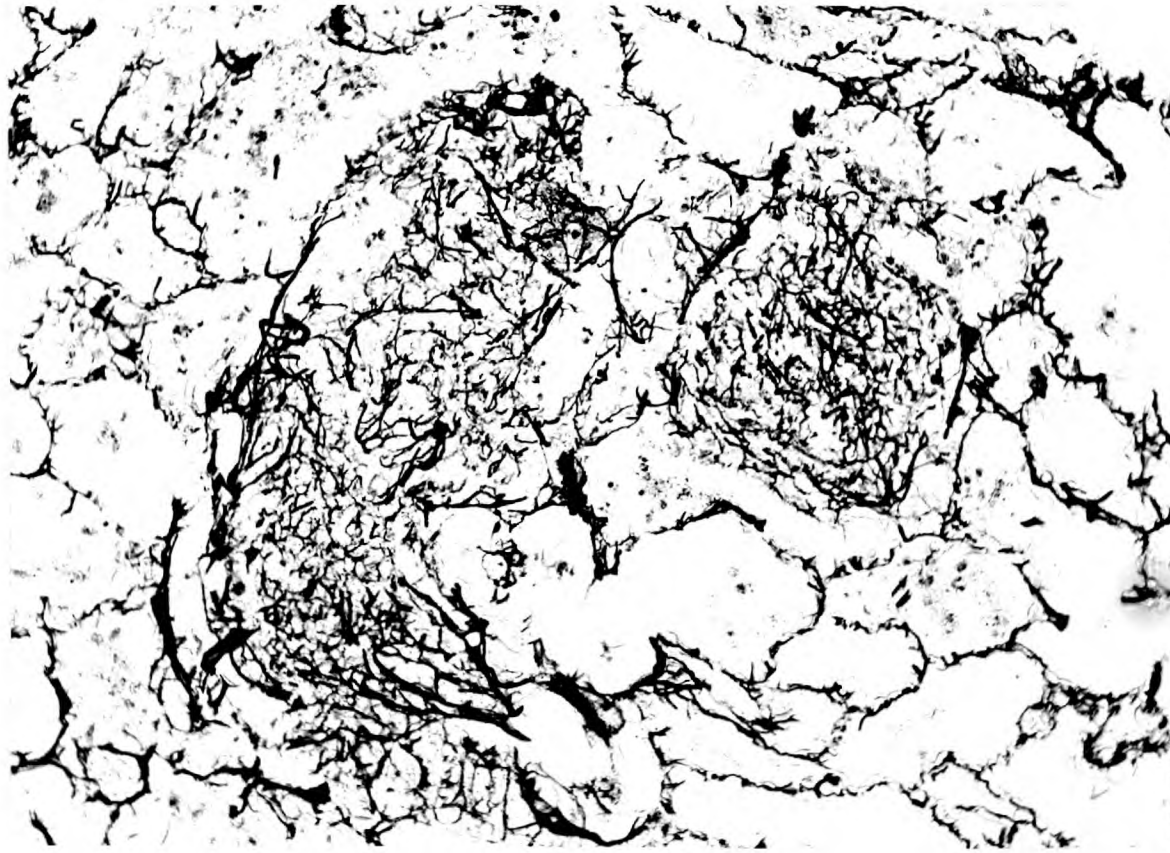


Fig. 2. Rat lung, 30 days after injection of 12.5 mg. tridymite. Nodular lesions showing a loose network of fine reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 210).

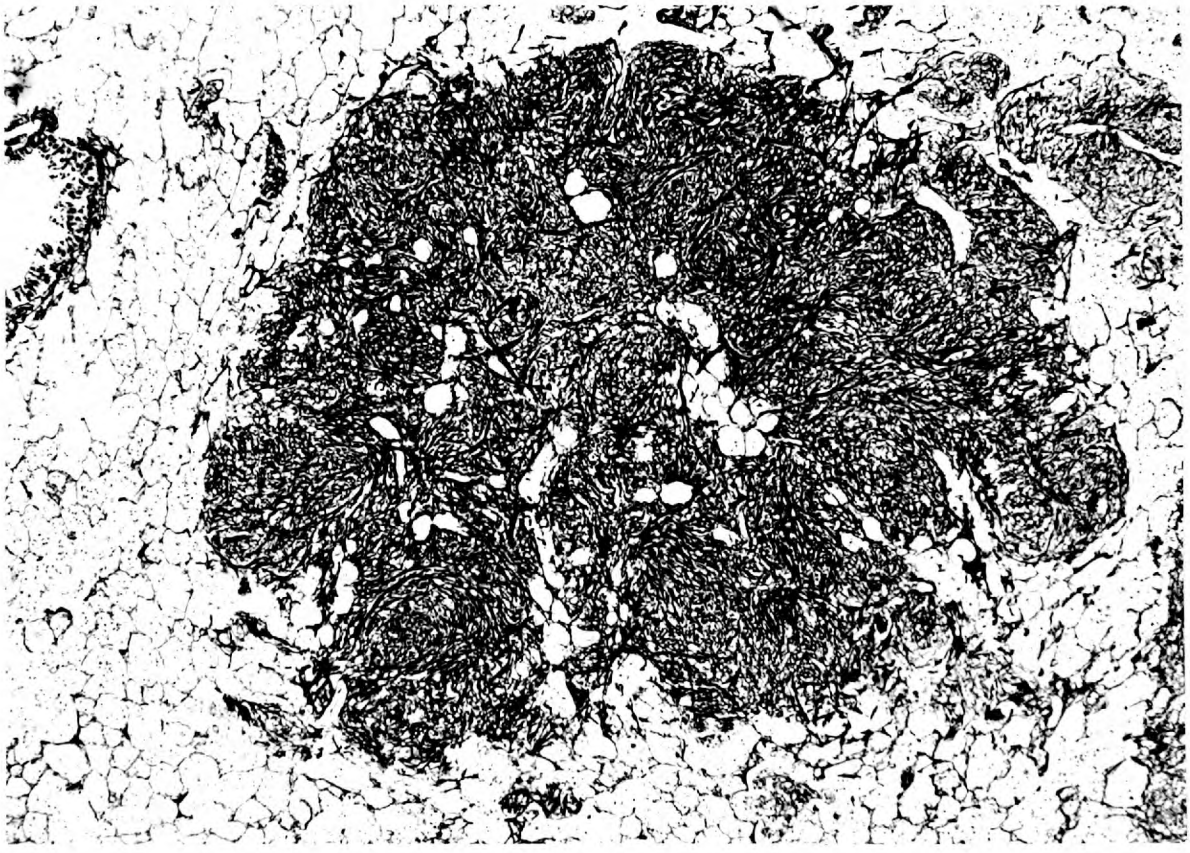


Fig. 3. Rat lung, 60 days after injection of 12.5 mg. tridymite. Shows a silicotic nodule with thick, compact collagen (grade 3 fibrosis). Silver impregnation. (X. 65).



Fig. 4. Rat lung, 300 days after injection of 12.5 mg. tridymite, showing confluent lesions, which are fully collagenous (grade 5 fibrosis). Silver impregnation. (X. 48).

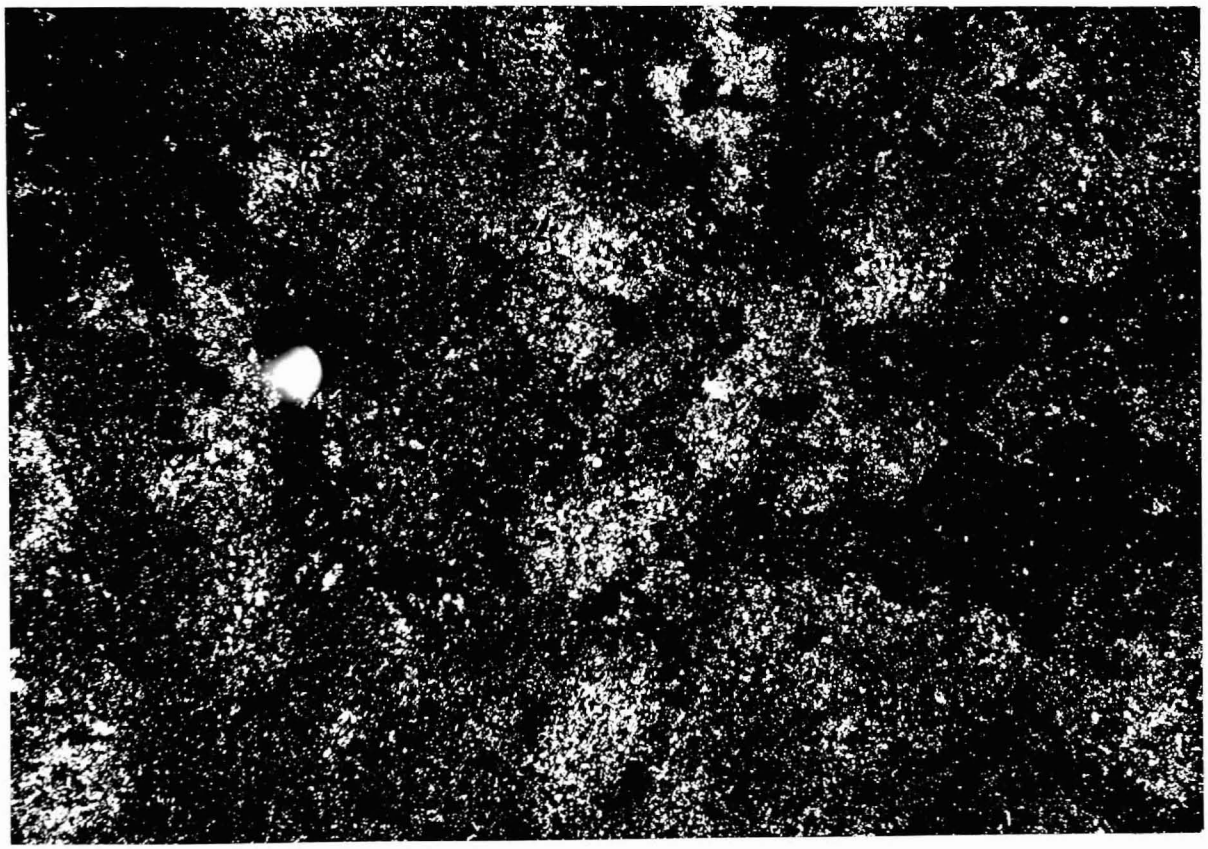


Fig. 5. Serial section to Fig. 4. Micro-incinerated preparation of the same area, showing the distribution of dust in the lesions. (X. 48).

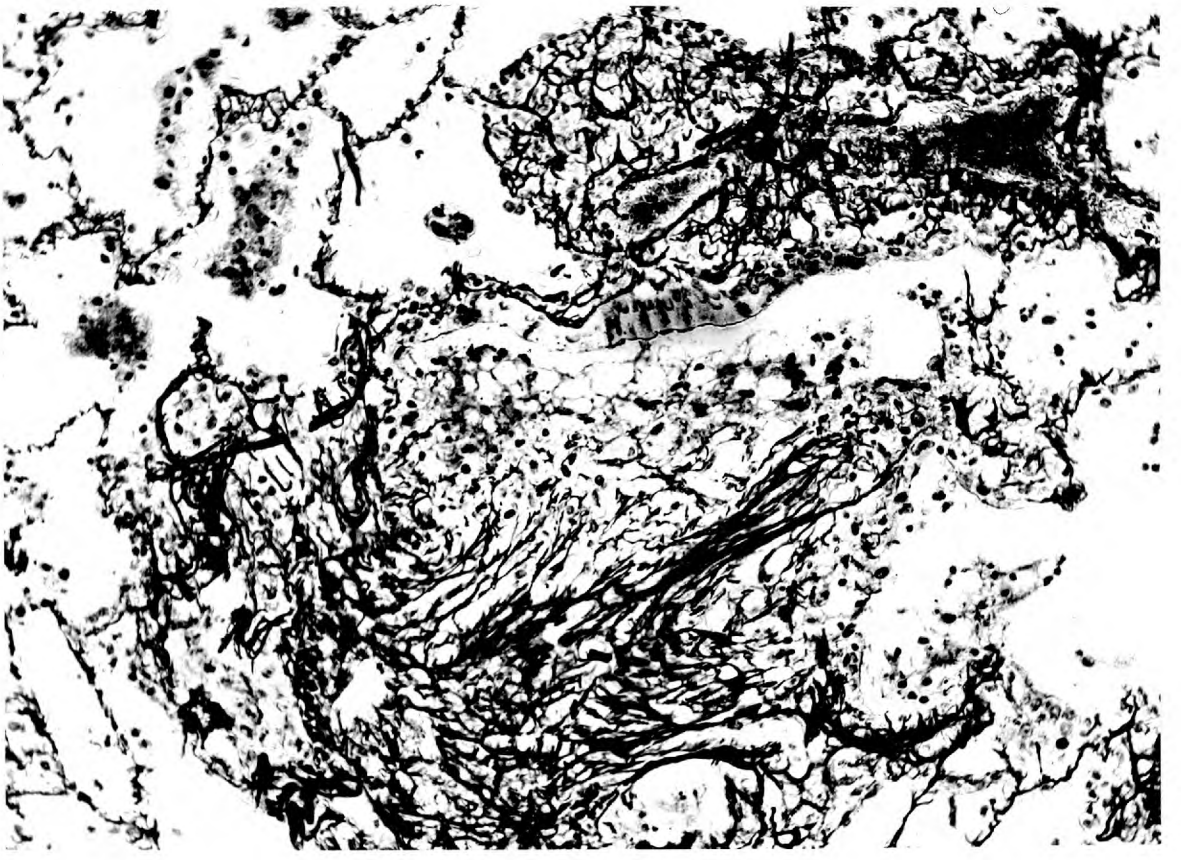


Fig. 6. Rat lung, 30 days after injection of 25 mg. tridymite, showing nodular lesions with thick and compact reticulin and some collagen (grade 2 fibrosis). Silver impregnation. (X. 210).

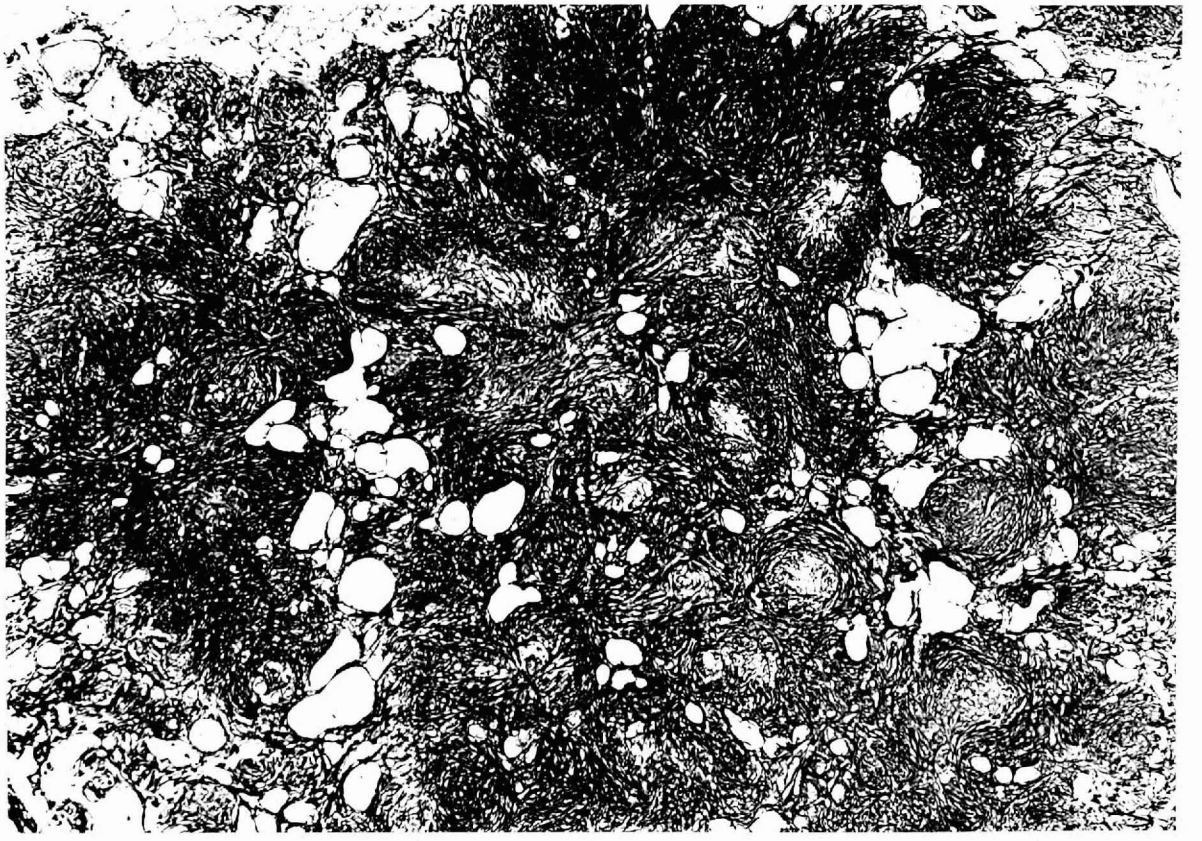


Fig. 7. Rat lung, 60 days after injection of 25 mg. tridymite. Confluent, fully collagenous lesions (grade 5 fibrosis) with a few patent alveoli. Silver impregnation. (X. 48).

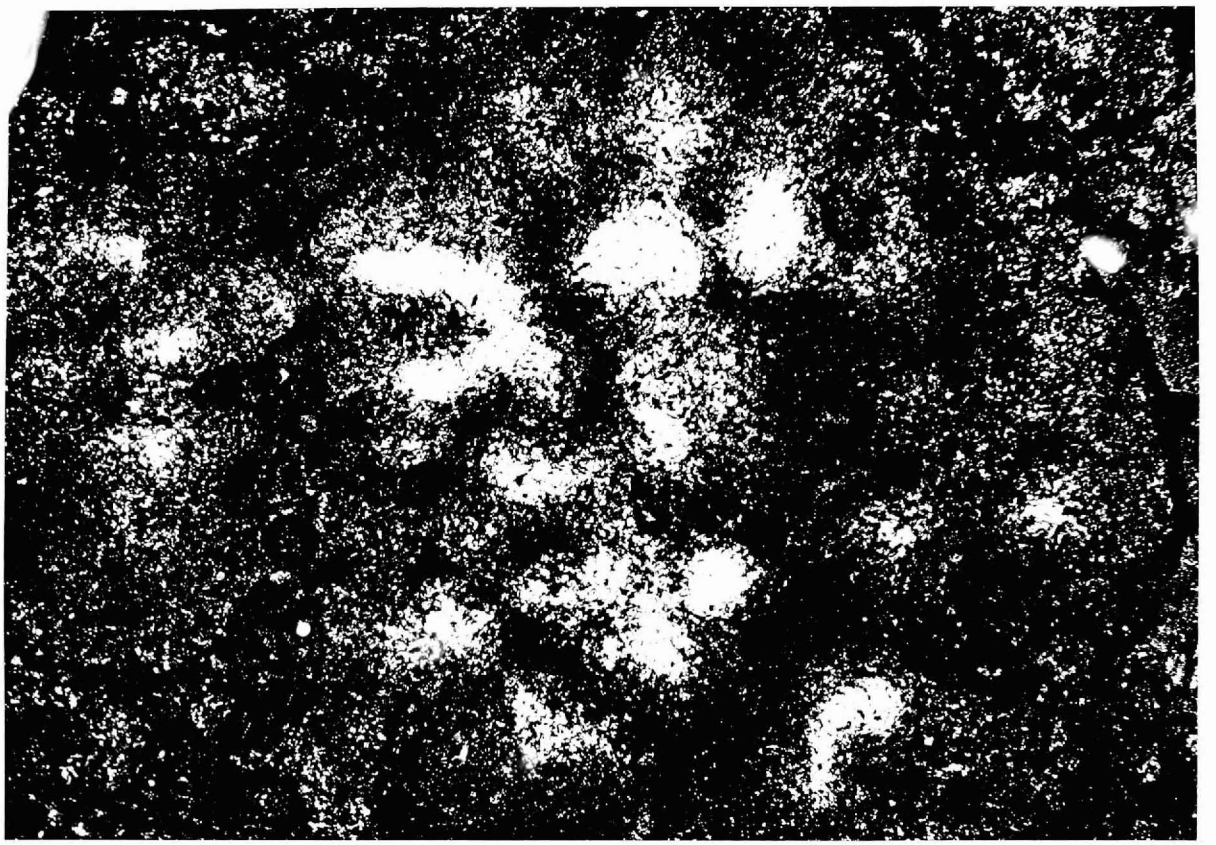


Fig. 8. Serial section to Fig. 7 photographed under dark-ground illumination to show dense collections of dust in the lesions. Micro-incinerated preparation. (X. 48).

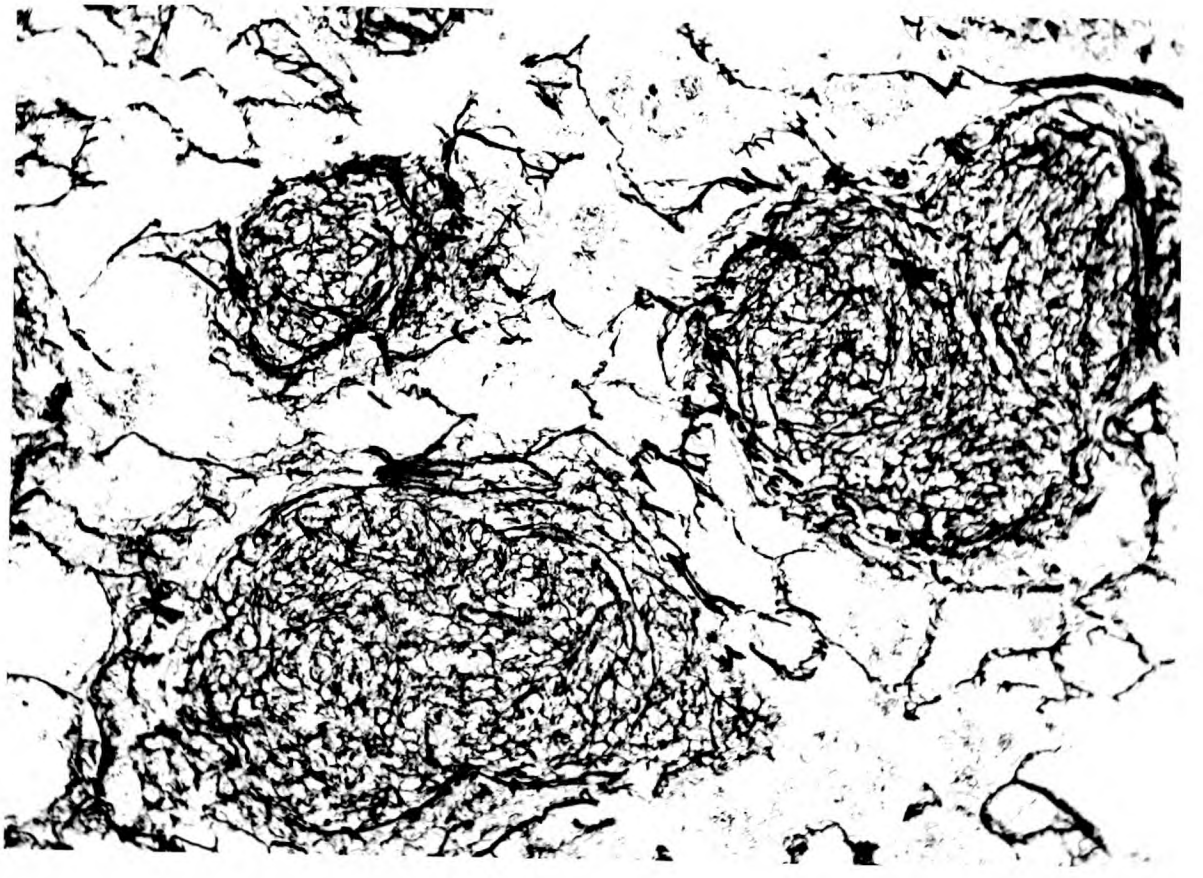


Fig. 9. Rat lung, 9 days after injection of 25 mg. electro-dialysed tridymite. Nodular lesions with compact reticulin (grade 2 fibrosis). Silver impregnation. (×. 200).

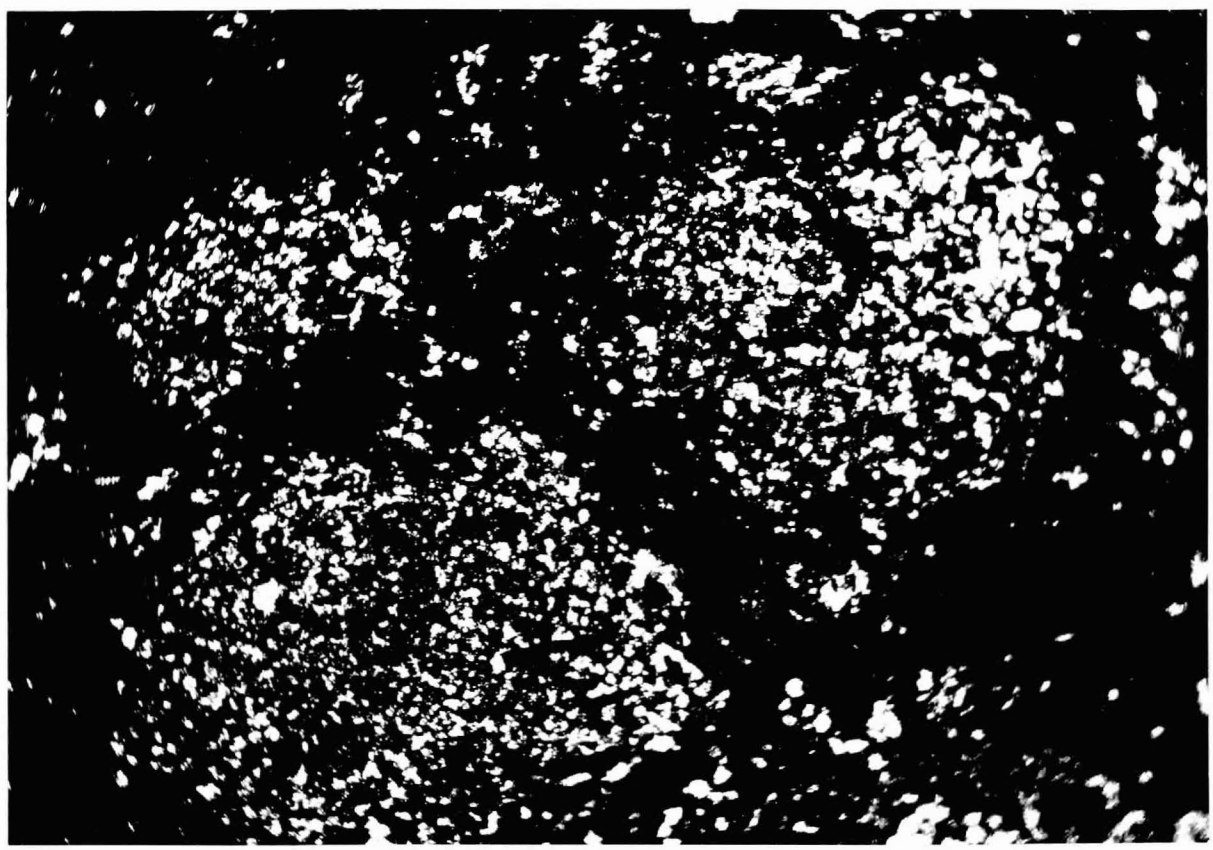


Fig. 10. Serial section to Fig. 9, micro-incinerated to show the distribution of dust in the lesions. (X.200).



Fig. 11. Rat lung, 15 days after injection of 25 mg. electro-dialysed tridymite. Lesions contain compact collagen (grade 3 fibrosis). Silver impregnation. (X. 130).

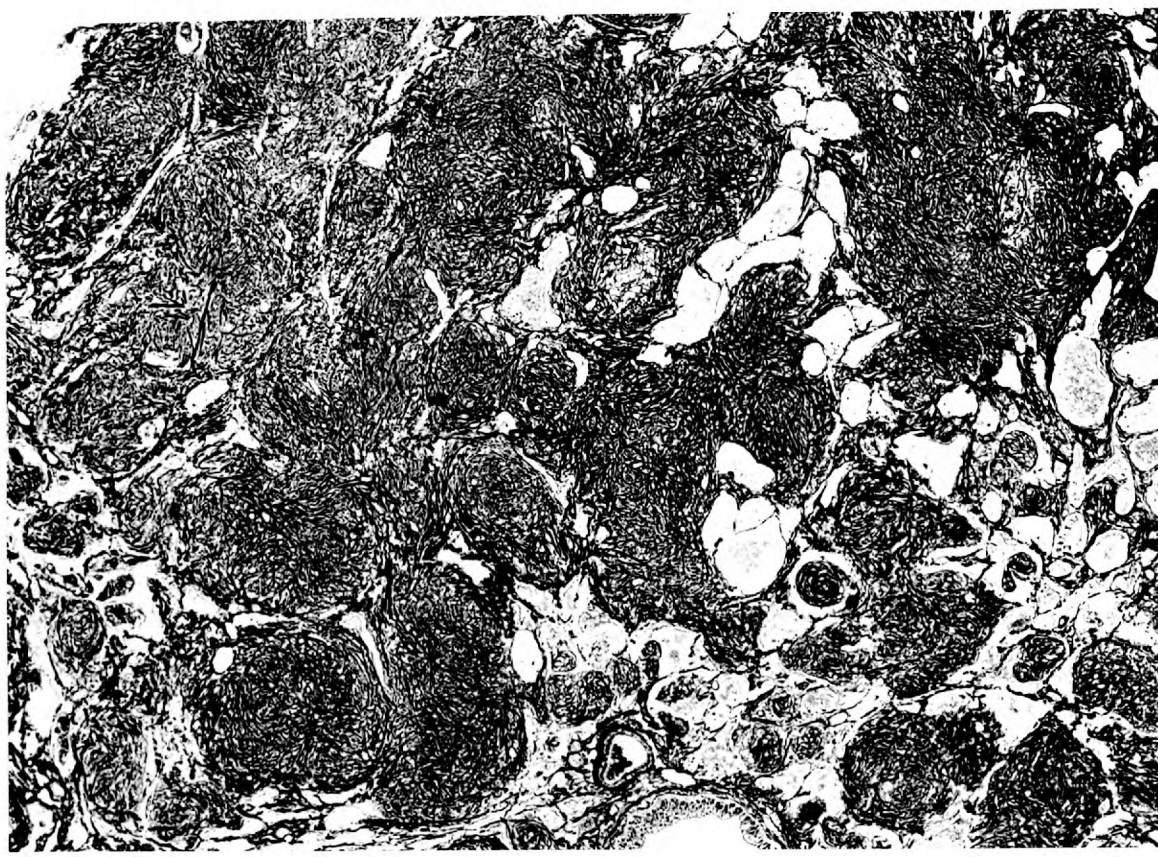


Fig. 12. Rat lung, 45 days after injection of 25 mg. electro-dialysed Aust. Confluent lesions composed entirely of dense collagen (Grade 5 fibrosis). Silver impregnation. (X. 48).

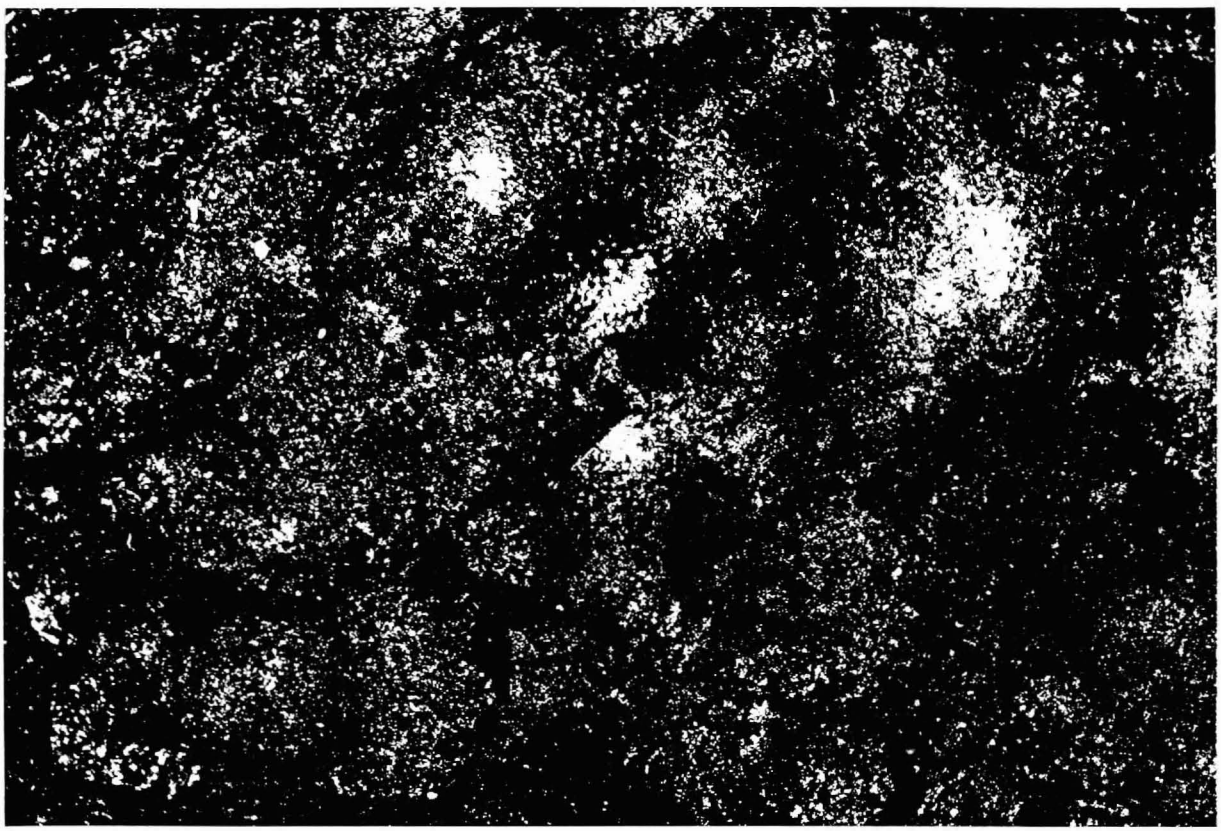


Fig. 13. Serial section to Fig. 12, showing the same area after micro-incineration. Lesions contain large quantities of dust. (X. 48).

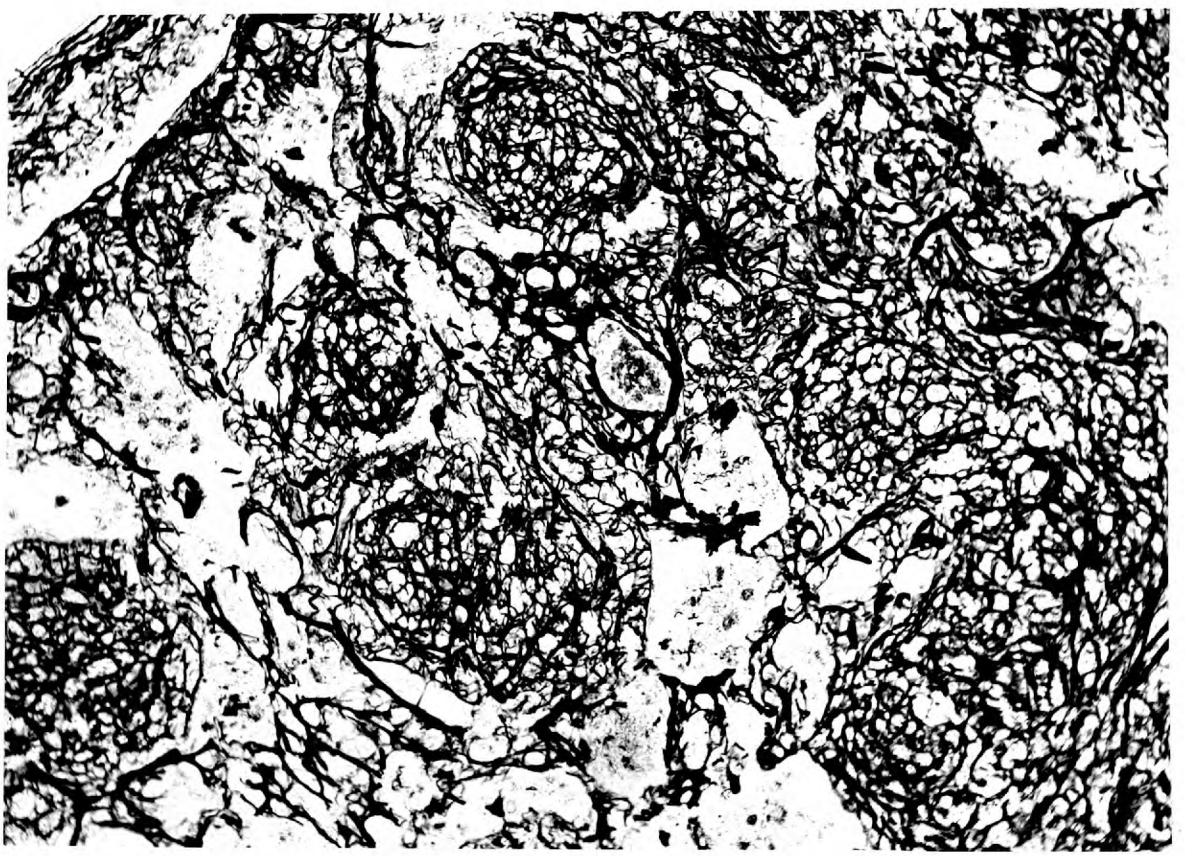


Fig. 14. Rat lung, 15 days after injection of 50 mg. of tridymite. Lesions show thick, compact reticulin and some collagen (grade 2 fibrosis). Silver impregnation. (X. 210).

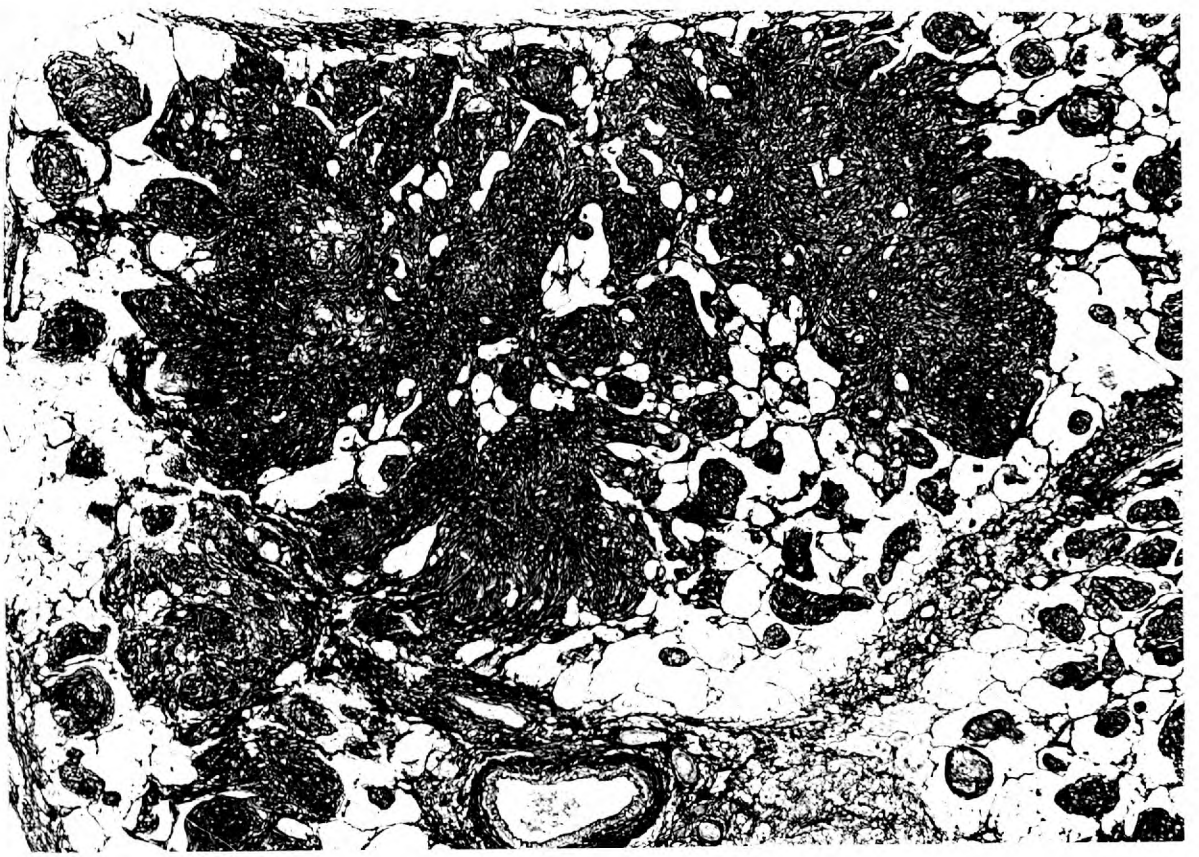


Fig. 15. Rat lung, 52 days after injection of 50 mg. tridymite. Confluent lesions with dense collagen (grade 5 fibrosis). A few patent alveoli and blood vessels seen within the lesions. Silver impregnation. (X. 48).

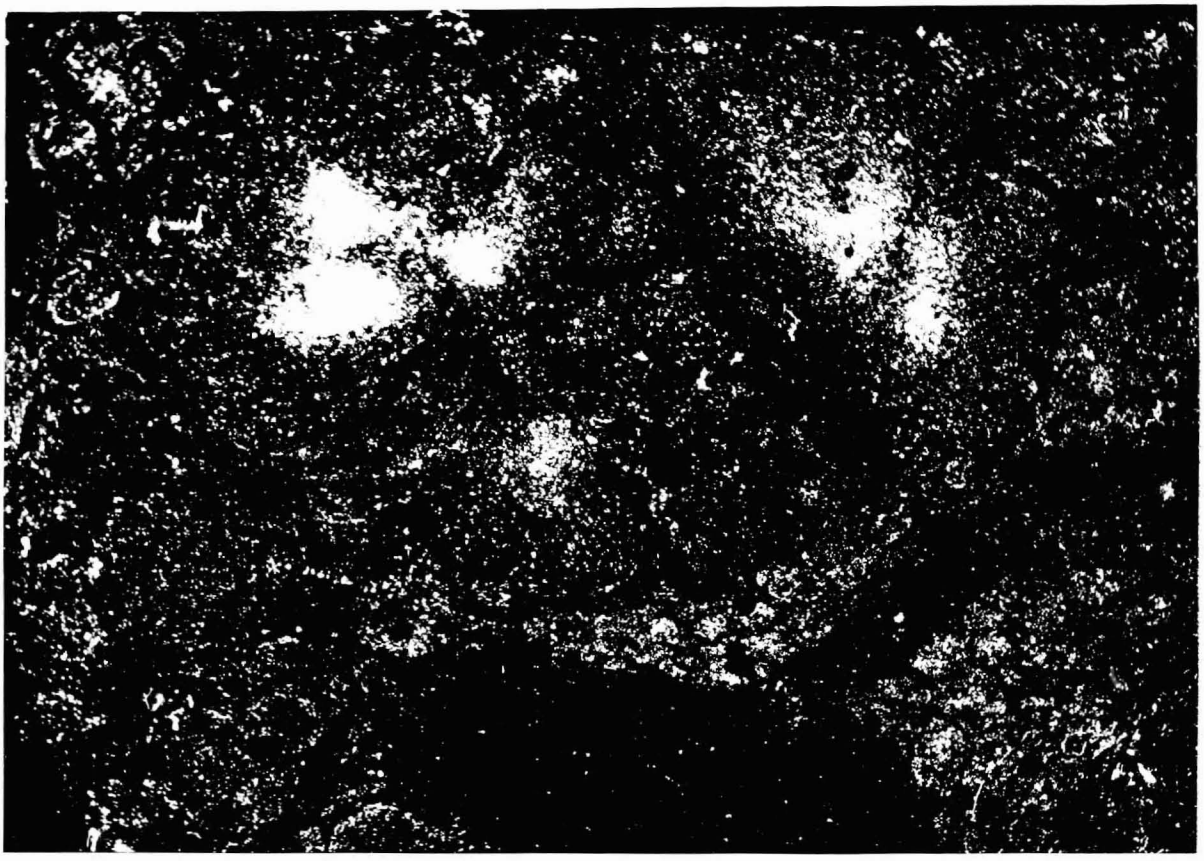


Fig. 16. Micro-incinerated preparation of the serial section to Fig. 15, showing the distribution of dust in the lesions. (X. 48).

SECTION III

SECTION III

THE ACTION OF ANTHRACITE AND BITUMINOUS COAL DUSTS MIXED WITH A SMALL AMOUNT OF TRIDYMITE ON THE LUNGS OF RATS

Experimental studies on animals have shown that pure coal does not produce any permanent lesions in the lungs even after long periods of time (Belt and King, 1945; Wright, 1951; Fletcher, 1951; Section I of this thesis). Belt and King (1945) studied the reactions produced in the lungs of rats by various dusts from the South Wales mines, and found that dusts consisting of 90% or more coal, whether anthracite, bituminous or steam coal, only produced minimal lesions of the simplest type of foreign-body reaction in 250-500 days. Coal dusts containing 1-3% quartz produced mild reticulinosis which was more marked than in the lesions produced by pure coal alone. When, however, the sample of coal used contained more than 12.5% siliceous material, more advanced pulmonary lesions appeared, the fibrosis reaching grade 2 and 3 (compact, collagenous lesions). Artificial mixtures of airborne mine dust from anthracite and bituminous mines, containing pure quartz in amounts varying from 19-30% produced grade 4 fibrosis in 365 days in rats, there being no difference in the lesions produced by the anthracite-quartz and bituminous quartz mixtures.

Ray, King and Harrison (1951 (a) and (b)) showed that a small amount of quartz (2 mg.) when mixed with a large amount of clean coal (98%) produced more severe pulmonary

lesions than the same amount of quartz by itself. The large amount of inert coal dust acted to prevent the loss of quartz through the lymphatics to the lymph nodes, thus causing retention of the quartz dust in the lung, where it exerted its toxic effect with consequent formation of fibrous lesions. The same workers later studied the action of airborne mine dusts (anthracite and bituminous) on varying amounts of quartz, by injecting into rats mixtures of mine dust with 10% and 30% commercial quartz (1951 (c)). They were unable to observe any differences between the lesions produced by the quartz-anthracite and quartz-bituminous-mixtures at either level of added quartz, nor did the mine dust have any inhibitory action on the quartz.

It would seem, then, that small amounts of quartz which do not produce fibrous lesions in the lungs when used alone, are capable of doing so when used in combination with coal dust. In a previous section of this work it was found that tridymite, which is a crystalline modification of pure silica, is very pathogenic when introduced into the lungs of rats, producing marked fibrosis rapidly, even in small quantities, and being about five times as fibrogenic as quartz. It seemed desirable, therefore, to study the action of inert dusts such as coal when mixed with a very small amount of a fibrogenic dust like tridymite, in order to determine whether the results would be similar to those obtained with quartz and coal mixtures. Both anthracite and bituminous dusts were used alone, and with tridymite; the same quantity of tridymite

was also used without any coal, to serve as a control, and to discover whether any lesions would result from the use of this small amount of pathogenic dust.

Experiments were set up as follows :-

Group I - 30 mg. anthracite coal.

Group II - 30 mg. anthracite coal + 1 mg. tridymite.

Group III - 30 mg. bituminous coal.

Group IV - 30 mg. bituminous coal + 1 mg. tridymite

Group V - 1 mg. tridymite

Description of Samples

Anthracite coal - A low ash fraction of anthracite coal from Llandebie Colliery, South Wales, was turbine-ground and a fraction below 10 μ separated by air elutriation (roller apparatus). Starting with this material, a fraction below 2 μ was prepared by centrifuging in water after adding sufficient teepol detergent to make the coal wettable. The material below 2 μ equivalent diameter was spun down by prolonged centrifuging, boiled with water to remove as much of the teepol as possible, and finally dried.

The particle size distribution of the dust and the chemical data are given in Table 1.

Bituminous coal - A sample of dust below 2 μ diameter was prepared by applying the above technique to a sample of bituminous coal from Haig Pit, Cumberland.

The size distribution of the dust and other data are in Table 1.

TABLE 1

ANALYSIS AND PARTICLE SIZE DISTRIBUTION OF ANTHRACITE AND BITUMINOUS COAL, AND TRIDYMITE USED IN ANIMAL EXPERIMENTS

Size in μ	Anthracite		Bituminous		Tridymite	
	Per Cent by No.	Per Cent by mass	Per Cent by No.	Per Cent by mass	Per Cent by No.	Per Cent by mass
0.32-0.45	7.55	0.08			12.5	0.7
0.45-0.64	17.30	0.53	4.4	0.37	22.4	3.4
0.64-0.9	20.71	1.75	18.7	4.26	29.4	12.3
0.9-1.3	32.89	7.73	49.4	31.2	27.3	31.8
1.3-1.8	9.26	6.24	23.9	43.4	6.2	20.7
1.8-2.6	9.26	17.47	3.3	16.76	1.6	15.0
2.6-3.6	1.46	7.88	0.3	3.99	0.6	16.1
3.6-5.1	0.78	11.71				
5.1-7.2	0.61	25.92				
7.2-10.2	0.17	20.68				
Specific surface m^2/gm	1.9		3.9		2.3	
Silica content (%)	1.4		1.05		99.3	
Silica Solubility (mg/100 ml.)	-		-		10.7	

Tridymite - This sample below 2 μ was the same as the one used in the earlier experiments in this work (Section II), and was prepared as follows :- Synthetic tridymite was prepared by Mr. Badger (North Thames Gas Board Laboratory) from Lochaline sand with a flux. The flux was dissolved, after heating, by prolonged boiling with hydrochloric acid. The resulting tridymite was very pure and a fraction below 2 μ was prepared at the Safety in Mines Research Establishment, Sheffield, by sedimentation in water after grinding the material in an agate mortar.

Table 1 shows the particle size distribution of the dust, together with other chemical data.

Preparation of Dust Suspensions for Injection

Anthracite and Bituminous Coal - Amounts of 0.75 g. anthracite and bituminous coal were weighed and transferred into screw-capped bottles and 24 ml. sterile physiological saline added to each. The suspensions were thoroughly shaken in a "Microid" flask-shaker for half and hour, autoclaved for 20 min. (15 lb. pressure) and 1 ml. sterile rat serum added to each bottle of suspension to help disperse and suspend the particles better. The final concentration of suspension was 30 mg. anthracite or bituminous coal per ml.

Anthracite plus Tridymite and Bituminous plus Tridymite Mixtures: 0.75 g. of anthracite and 0.75 g. bituminous coal were weighed and each was mixed intimately with 0.025 g. tridymite. The anthracite-tridymite and bituminous-tridymite mixtures were made up as before with 24 ml. sterile physiological saline and 1 ml.

sterile rat serum, so that the final concentrations were 30 mg. anthracite or bituminous coal and 1 mg. tridymite per ml. of suspension.

Tridymite - 25 mg. of tridymite were weighed and the suspension made up as previously described with 25 ml. sterile physiological saline, so that the concetration of dust was 1 mg. per ml. of suspension.

Animals

18 male rats were used in each of the five groups. They were black and white hooded rats of the Medical Research Council strain and were of average weight 250 g.

Experimental Technique

The animals were lightly anaesthetized with ether and the dust suspensions injected intratracheally via the mouth, following the technique of Kettle and Hilton (1932), modified by King et al (1953 (b)), a detailed description of which has been given earlier in this work.

Duration of Experiments

Several animals from the various groups died spontaneously due to intercurrent infection. Two animals from each group were killed at 90, 180, 240, 300 and 330 days; all surviving animals were killed at 350 days. Histological examination of the lungs was made in all cases.

Histo-pathological Technique

Routine post-mortem examinations were carried out on all animals which were killed, as well as on those which

died spontaneously. 10% Formol-saline was injected into the lungs before opening the thoracic cavity in animals which were killed, while in those found dead the lungs were distended with fixative after their removal from the thoracic cavity.

After preliminary fixation blocks were selected through the long axes of both lungs at the level of the hilum, to include the hilar lymph nodes, for histological sections. Blocks were embedded in paraffin and sectioned at 5 μ, as described in detail earlier. Serial sections were stained with haematoxylin and eosin, and by Gordon and Sweets' (1936) silver impregnation method; micro-incineration was also carried out to study the distribution of dust in the lesions.

HISTO-PATHOLOGICAL FINDINGS

The lungs of some of the animals which died spontaneously were found to be infected, there being evidence of bronchiectasis and broncho-pneumonic consolidation in most cases. Such lungs were not included among those used for the assessment of fibrosis produced by dust.

Sections from the lungs were examined microscopically and the pulmonary fibrosis seen in the most advanced lesions in each case was graded into one of five grades as described by Belt and King (1945), details of which have been given earlier. The pathological gradings so obtained, together with the number of days of survival of the animals and the mode of their death have been summarized in Table 2.

Gross Appearances of the Lungs

The macroscopic appearances of the lungs of animals

TABLE 2

SUMMARY OF PATHOLOGICAL CHANGES IN THE LUNGS OF RATS PRODUCED BY COAL AND TRIDYMITE ALONE AND IN COMBINATION

Days of Survival	Anthracite 30mg.		Anthracite 30 mg. + Tridymite 1mg.		Bituminous 30mg.		Bituminous 30 mg. + Tridymite 1 mg.		Tridymite 1mg.	
	Mode of death	Grade of Fibrosis	Mode of death	Grade of Fibrosis	Mode of death	Grade of Fibrosis	Mode of death	Grade of Fibrosis	Mode of death	Grade of Fibrosis
1-90	D(2)	-	D(2)	-			D(3)	-	D (1)	-
	K(2)*	0	K(2)+	1 Min	K(2)*	0	K(2)+	1	K (2)	0
91-180			D(4)	-	D(1)	-	D(1)	-		
	K(2)	0	K(2)	1	K(3)	0	K(2)	1	K(2)	0
181-240	D(1)	-	D(1)	-						
	K(3)	0	K(2)+	2	K(2)	0	K(2)	2 Min	K(2)	0
241-300			D(1)	-						
	K(2)	0	K(2)	2	K(2)	0	K(2)+	2	K(2)	0
301-350	K(8)	0	D(1)	-			D(1)	-		
			K(1)	2	K(8)	0	K(5)	2	K(9)	0

+ Sections from the lungs of these animals are reproduced in the figures

D = Died spontaneously; K = killed;

Min. = Minimal within the stated grade.

Numbers ( ) indicate the number of animals found dead or killed during that period.

injected with anthracite and bituminous coal were similar. The lungs of the animals injected with the anthracite-tridymite and bituminous-tridymite mixtures resembled each other very closely macroscopically. Hence they are described together.

Anthracite coal 30 mg; Bituminous coal 30 mg. - In both these groups, the lungs at 90 days showed collections of dust distributed diffusely throughout the surfaces. By 180 days these collections seemed to be denser, and there were no further changes either at 300 or 350 days.

The hilar lymph nodes were enlarged and black.

Anthracite coal + Tridymite; Bituminous coal + Tridymite - The lungs of animals killed at 90 days in each group showed diffuse deposits of coal dust on the surface. At 180 days the collections were larger and small white areas 2 - 3 mm. in diameter were seen associated with them. No other changes, beyond a few pleural adhesions, were visible macroscopically from this time till 350 days.

The hilar nodes were somewhat enlarged and black.

Tridymite 1 mg. - In a few cases, small, discrete, white areas were seen scattered over both lung surfaces from 90 days onwards, while in the majority of cases no macroscopic changes were noticed at any period.

The hilar lymph nodes were enlarged and firm in all cases.

Microscopic Appearances

The lesions produced in the lungs by anthracite and bituminous coal did not show any differences histologically.

The lesions produced by the mixtures of coal and tridymite were also similar, but they were more advanced than those due to the coal alone.

Anthracite coal 30 mg. - At 90 days the lungs showed some dust lying free in the alveoli, but most of it was collected together in the form of irregular nodules situated perivascularly. On reticulin staining, there was no evidence of fibrosis (Fig. 1). A mild degree of emphysema was present around the nodules and there was hyperplasia of the peribronchial lymphoid tissues with dust in them. From this time till 350 days there was no change in the lesions except for the dust collections becoming more compact. No fibrosis was seen at any stage.

The hilar lymph nodes revealed the presence of much dust from 90 days onwards without any fibrosis.

Anthracite Coal 30 mg. + Tridymite 1 mg. - The lungs examined at 90 days showed most of the dust collected into irregular, nodular lesions which were cellular and mainly perivascular. A few were subpleural. A mild degree of focal emphysema was present. The lesions, on reticulin staining, appeared to have within them a few loose reticulin fibres (grade 1 fibrosis, minimal. Fig. 2). Marked peribronchial fibrosis was seen. By 180 days the dust lesions were more compact and the reticulin fibres were thicker (grade 1 fibrosis). At 240 days there were many irregular, discrete nodules with thick and compact reticulin (grade 2 fibrosis. Fig.3), and by 300 days

the lesions had progressed very little, still being within the maximal limits of grade 2 fibrosis. No significant changes beyond those found at 300 days, were observed at 350 days.

The hilar lymph nodes showed mild reticulinosis at 300 days and 350 days.

Bituminous Coal 30 mg. - The lesions at 90 days were essentially the same as those produced by the same amount of anthracite coal in the lungs - irregular collections of dust without the presence of any fibrosis (Fig. 4), and with mild emphysema. The lesions remained so till 350 days.

There were collections of dust in the hilar lymph nodes, but no fibrosis.

Bituminous Coal 30 mg. + Tridymite 1 mg. - At 90 days a small amount of dust was seen lying free in the lung alveoli, but the greater part of it was in focal aggregations situated perivascularly. A loose network of fine reticulin fibrils was demonstrable within the lesions (grade 1 fibrosis. Fig. 5). There was marked peribronchial fibrosis and some emphysema. No further progress was seen till 240 days when the nodules appeared to be more compact with thick reticulin fibres (grade 2 fibrosis, minimal). The lesions remained within the limits of grade 2 fibrosis at 300 days (Fig. 6), and were no different at 350 days when the experiment was terminated.

Mild reticulinosis of the hilar lymph node was seen at 300 days and 350 days.

Tridymite 1 mg. - The lungs of several animals in this group were found, on histological examination, to be the seat of

spontaneous bronchiectatic lesions with chronic interstitial pneumonia. At 90 days a very occasional focal aggregation of dust cells was seen in the lungs, but there was no evidence of fibrosis. By 180 days some peribronchial fibrosis was found. There was no fibrosis in the few dust cell collections seen, although in areas where such lesions were associated with bronchiectatic changes some fibrosis with the development of collagen fibres was evident. Apart from this, no fibrosis was present in the lungs at 300 days or 350 days.

The hilar lymph node contained some dust at 90 days, and by 300 days, there were definite lesions which, on reticulin staining, revealed the presence of a loose network of thick reticulin fibres (grade 1 fibrosis maximal. Fig. 7), whereas the lungs at this same period revealed no lesions (Fig. 8). The lesions in the lymph nodes did not advance beyond the limits of grade 1 fibrosis at 350 days.

DISCUSSION

The results of these experiments confirm the earlier findings that anthracite and bituminous coal do not produce any lesions in the lungs of rats. The very pathogenic tridymite dust also failed to produce fibrosis when used in 1 mg. doses. The histological lesions in this case, however, were difficult to assess, since they were complicated by the presence of spontaneous pulmonary infection, when some of the lesions were

found to contain sufficient collagen to be classified as fibrosis of grade 3 severity. Nevertheless, in uncomplicated cases no lesions were produced in the lungs by this amount of tridymite, although in the hilar lymph nodes it produced grade 1 fibrosis by 300 days and 350 days. This is probably due to this small amount of dust being removed from the lungs to the lymph nodes without the occurrence of pulmonary fibrosis. Ray, King and Harrison (1951 (a)), obtained similar results using 2 mg. of quartz. When the same quantity of tridymite (1 mg.) was injected together with anthracite and bituminous coal in 30 mg. doses, definite pulmonary lesions were produced, there being reticulin fibrosis of grade 1 severity at 90 days, reaching an early grade 2 by 240 days in both groups. The lesions advanced only very slightly beyond this stage, remaining within the maximal limits of grade 2 at 300 and 350 days. It is likely that the inert coal retards the movement of tridymite in the lymphatics, causing it to be retained in the lungs where it exerts a toxic action, producing fibrosis. These results compare favourably with those of Belt and King (1945) who observed grade 4 fibrosis in 365 days, when a much higher percentage (20%) of quartz was used with mine dust, and of Ray, King and Harrison (1951 (c)) in which 2 mg. of quartz and 98 mg. of pure coal were found to produce grade 3 fibrosis. The much smaller amounts of tridymite (1 mg.) and coal (30 mg.) used in these experiments produced grade 2 fibrosis when injected in combination. The behaviour of anthracite and bituminous coal whether used with tridymite or alone appears to

be identical as far as pulmonary lesions are concerned. Moreover, coal does not act as an antidotal dust when used with free silica. On the other hand the two dusts appear to enhance the lesions produced by each other.

SUMMARY

The tissue reactions produced in the lungs of rats by small amounts of anthracite and bituminous coal when mixed with a smaller amount of tridymite were studied, along with the reaction to the same quantities of coal and tridymite alone. Anthracite and bituminous coal by themselves did not produce any lesions in the lungs or hilar lymph nodes up to 350 days. 1 mg. of tridymite produced grade 1 fibrosis in the hilar lymph nodes but no lesions in the lungs. The mixtures of anthracite coal and tridymite, and bituminous coal and tridymite, produced lesions of grade 2 fibrosis in the lungs. Presumably, small amounts of tridymite are eliminated easily from the lungs, but when introduced together with coal the movement of tridymite dust through the lymphatics is retarded, with consequent retention of tridymite in the lungs where fibrosis occurs.

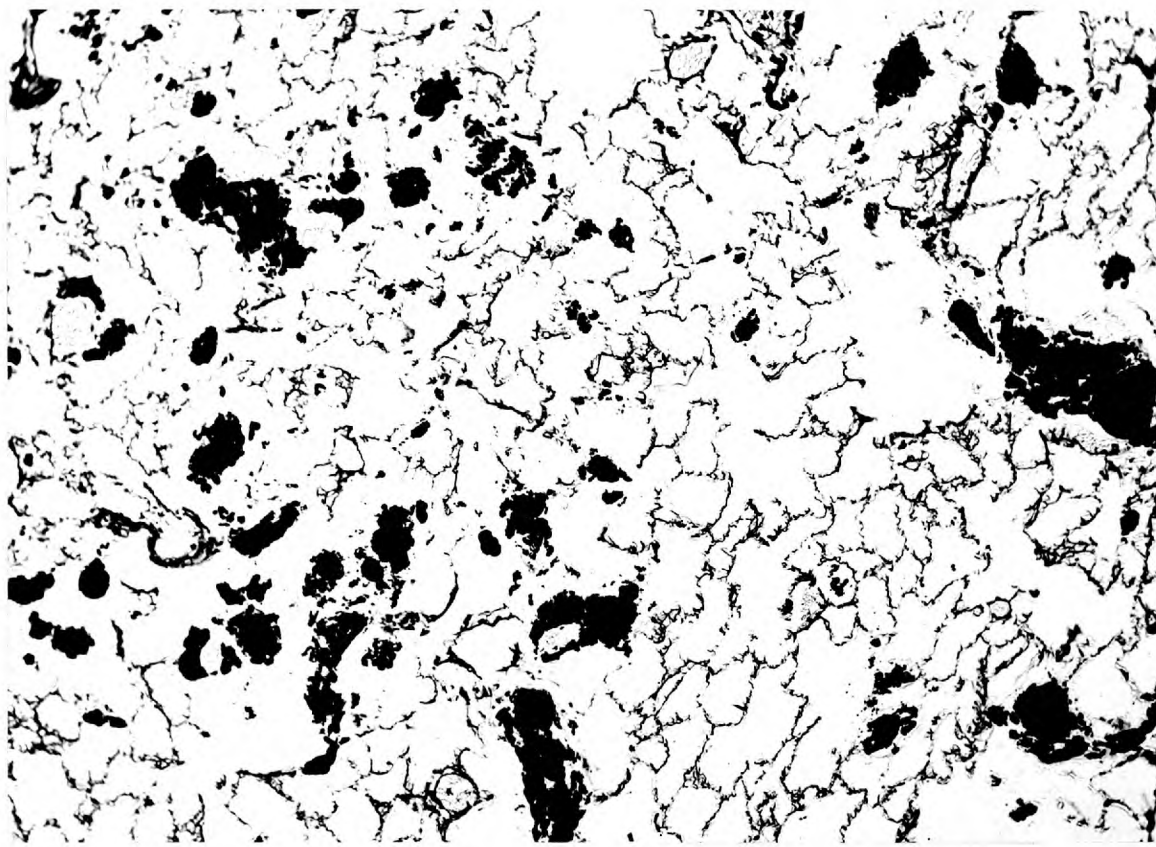


Fig. I. Rat lung, 90 days after the injection of 30 mg. anthracite coal. Dust gathered into compact nodules without any fibrosis. Silver impregnation. (X. 120).

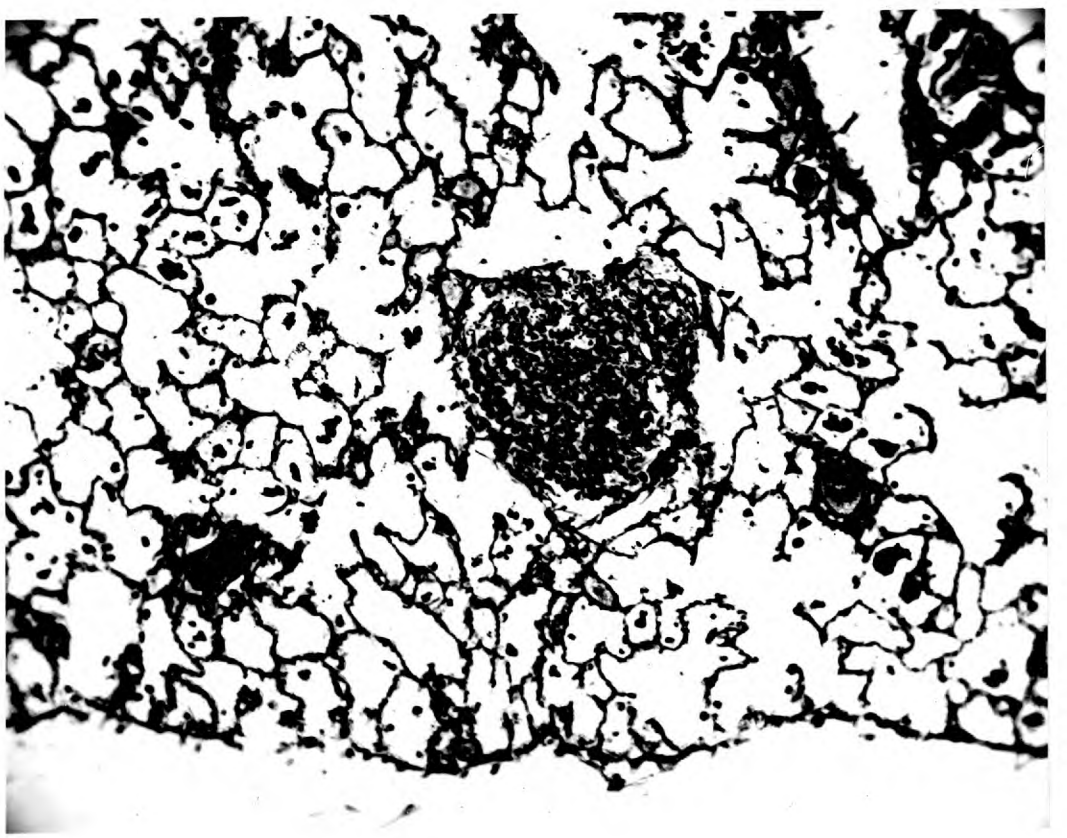


Fig. 2. Rat lung, 90 days after the injection of 30 mg. anthracite coal and 1 mg. tridymite. Shows nodular dust collections with a few fine reticulin fibres arranged loosely (grade I fibrosis, minimal). Silver impregnation. (X. 120).

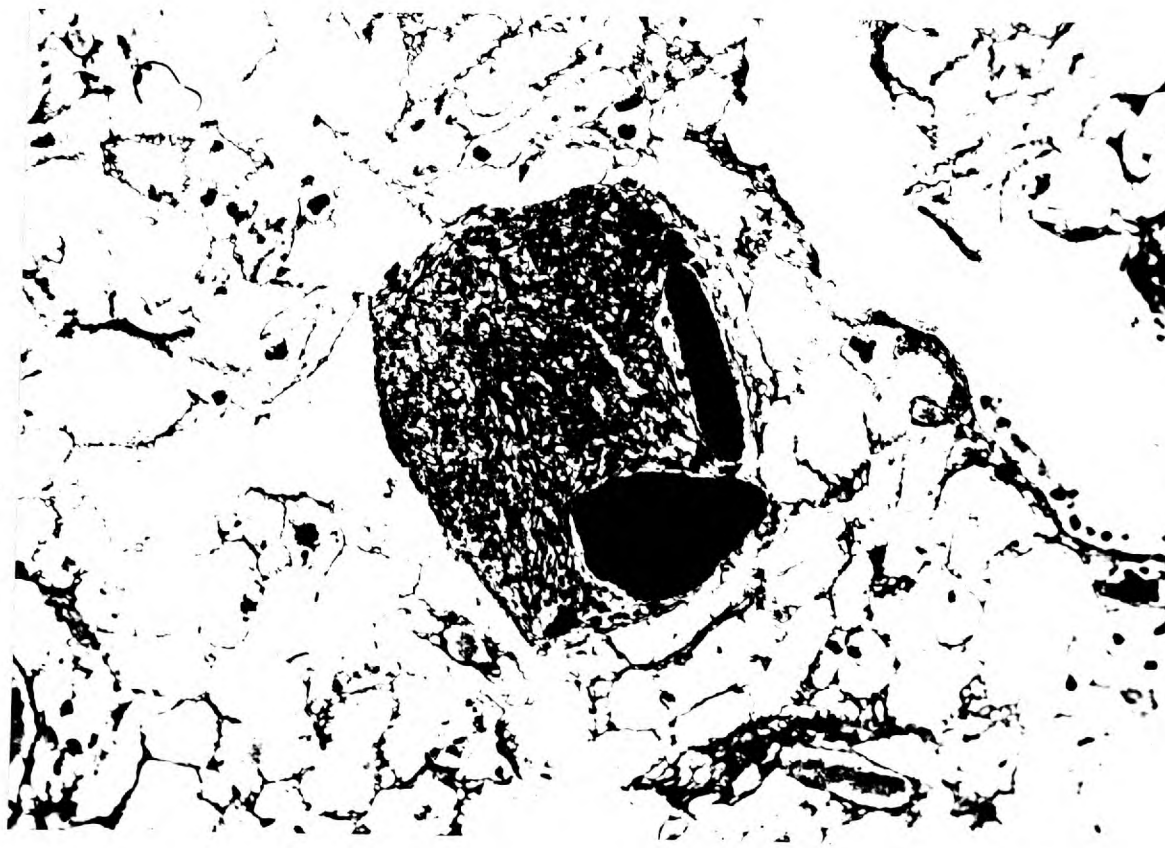


Fig. 3. Rat lung, 240 days after injection of 30 mg. anthracite coal and 1 mg. tridymite. Nodular lesions tightly packed with dust and thick reticulin fibres. (grade 2 fibrosis, minimal). Silver impregnation. (X.160).

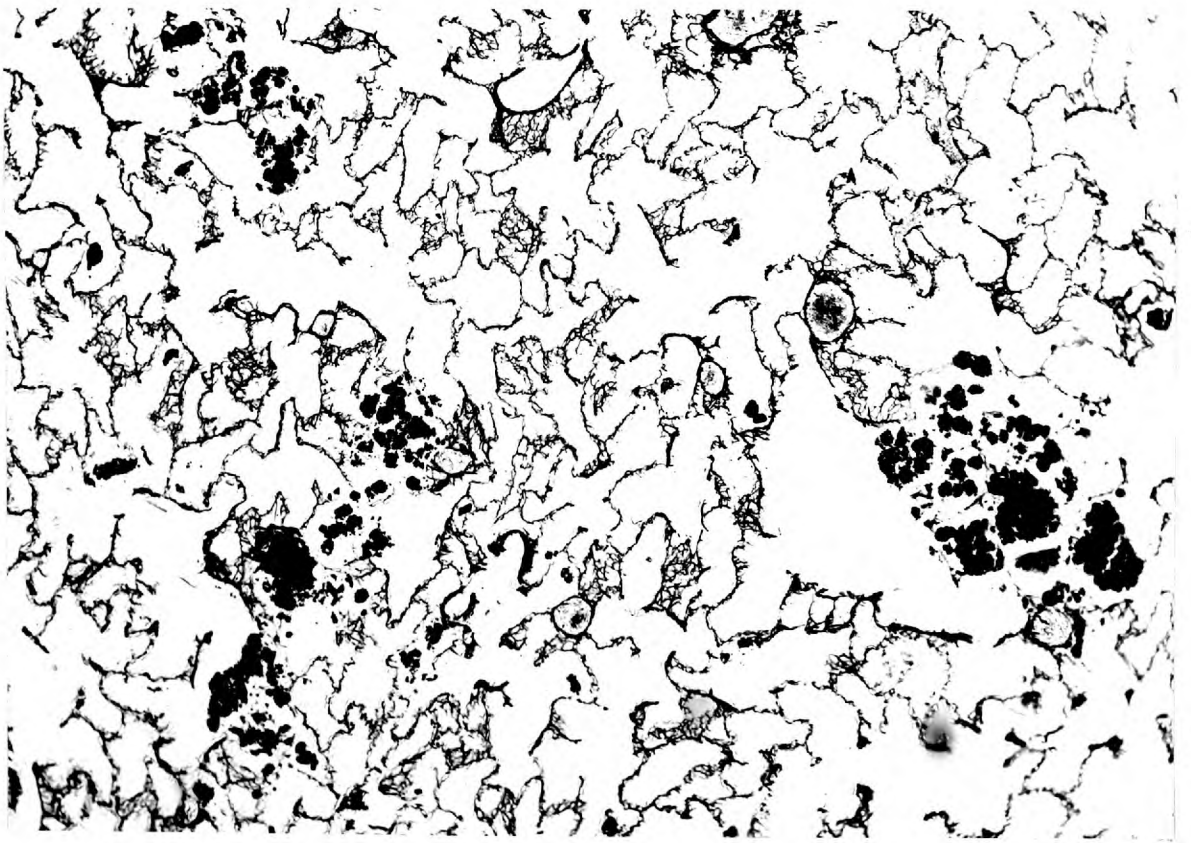


Fig. 4. Rat lung, 90 days after injection of 30 mg. bituminous coal. Focal accumulations of dust without any fibrosis. Silver impregnation. (X. 120).

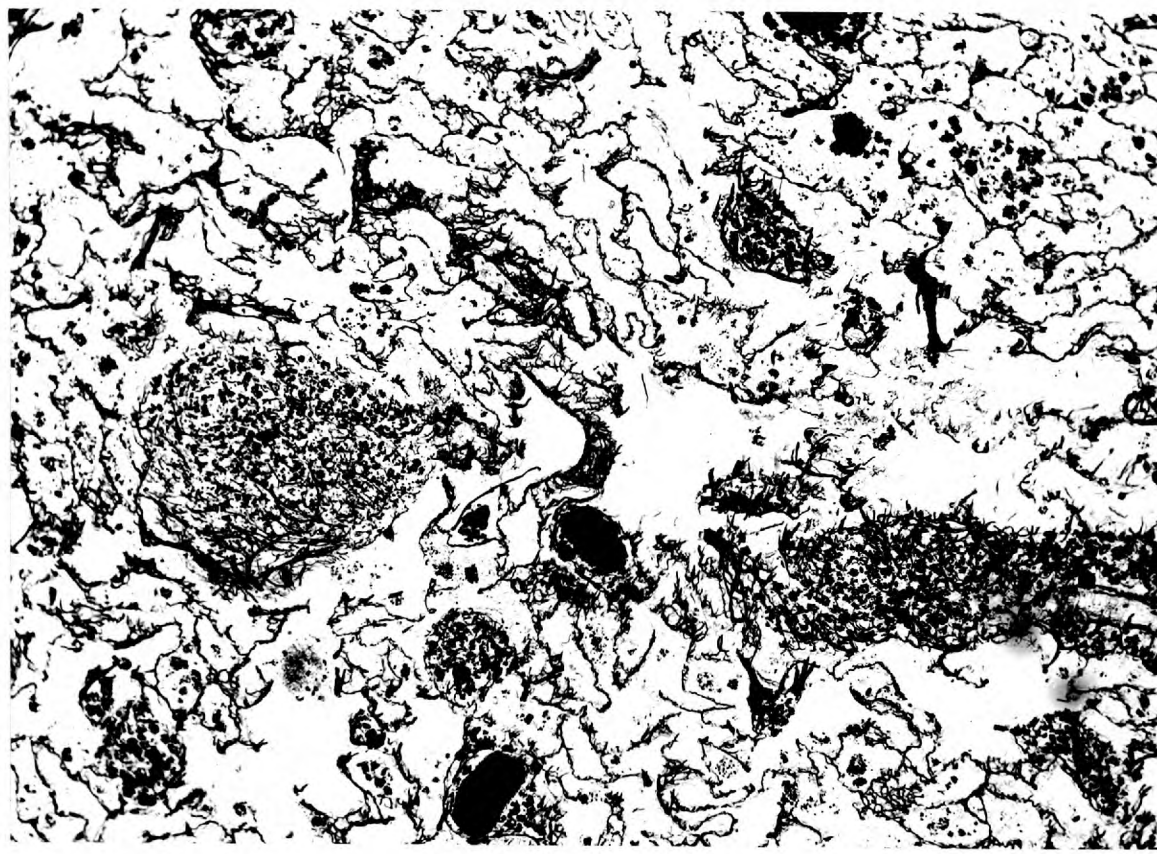


Fig. 5. Rat lung, 90 days after injection of 30 mg. bituminous coal and 1 mg. tridymite. Nodular lesions composed of dust with a loose network of reticulin fibres (grade 1 fibrosis). Silver impregnation. (X.120).

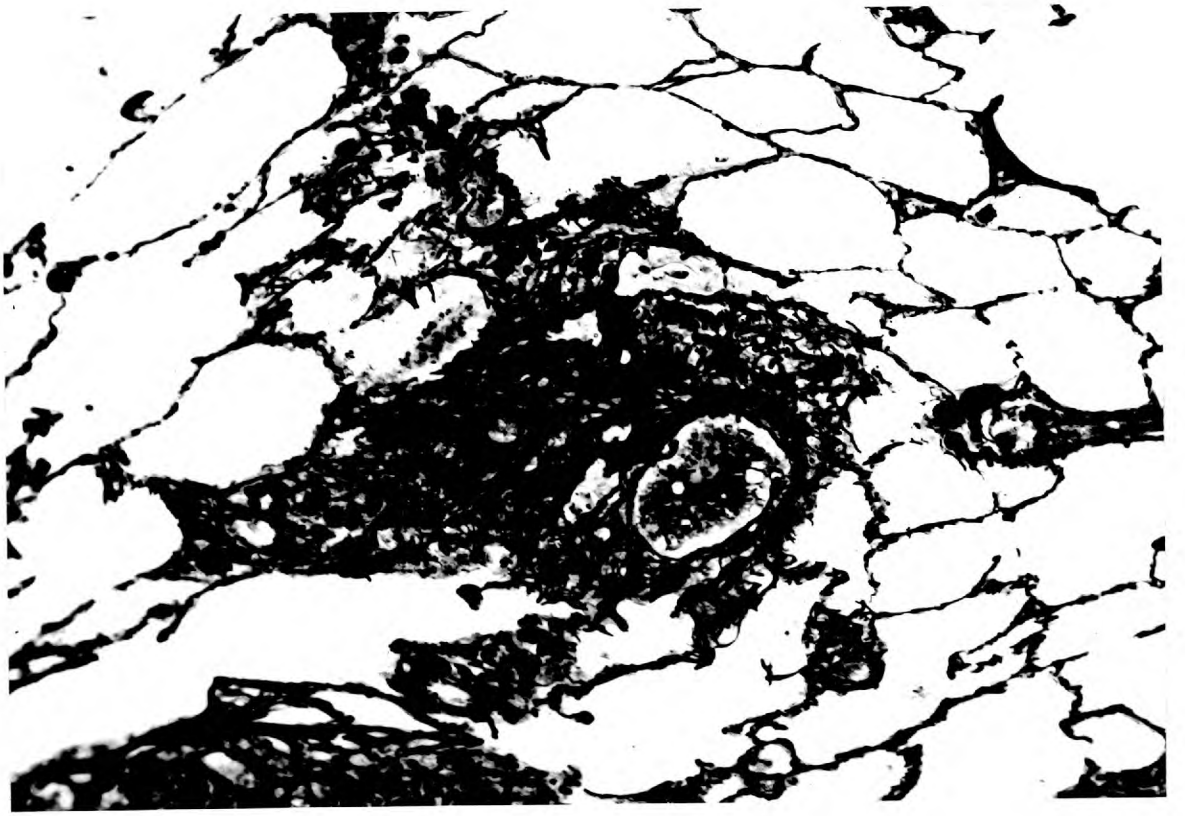


Fig. 6. Rat lung, 300 days after injection of 30 mg. bituminous coal and 1 mg. tridymite. Shows a dust lesion adjacent to a blood vessel, and containing thick and compact reticulin with some collagen (grade 2 fibrosis). Silver impregnation. (X. 160).

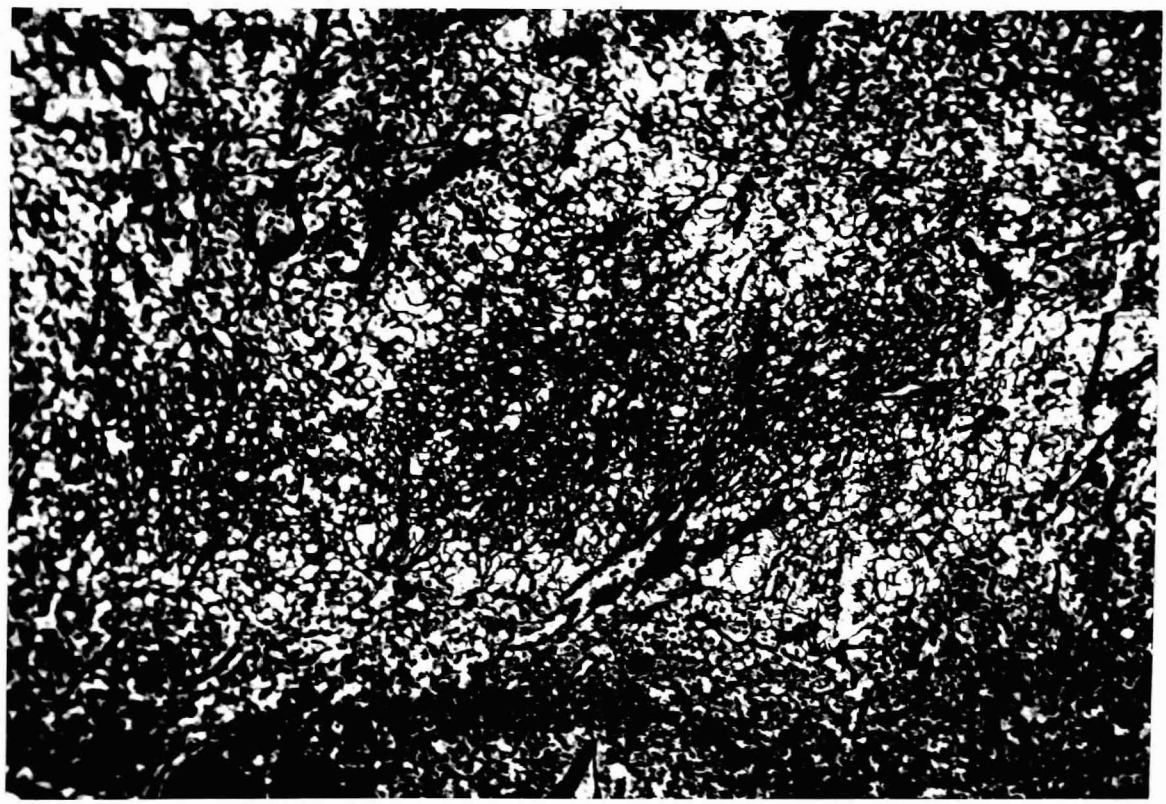


Fig. 7. Hilar lymph node of rat, 300 days after injection of 1 mg. tridymite. Lesions show a loose network of thick reticulin fibres (grade 1 fibrosis maximum). Silver impregnation (X. 160).

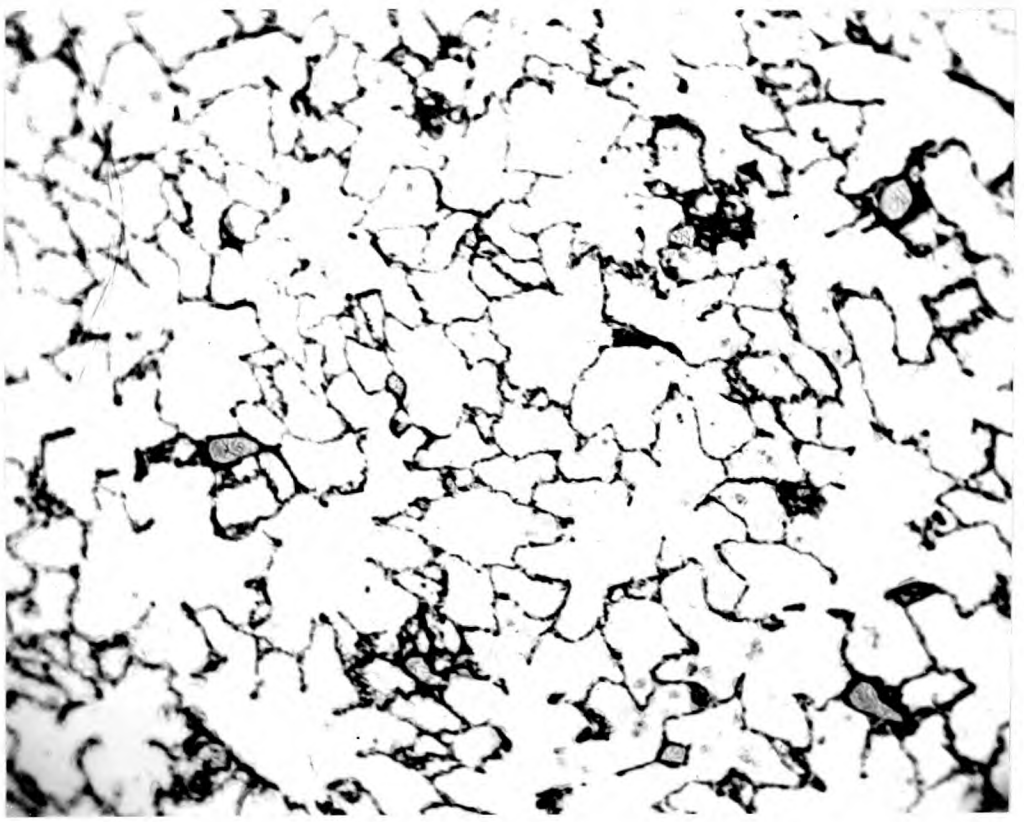


Fig. 8. Lung field from same rat as in Fig. 7.
There are no dust collections and there is no fibrosis.
Silver impregnation. (X. 120).

SECTION IV

SECTION IV

THE EFFECT OF CORTISONE ON ESTABLISHED SILICOTIC FIBROSIS IN THE LUNGS OF RATS

The inhibitory action of cortisone and A.C.T.H. on proliferating connective tissue is well-known. In human beings, alterations in cellular and fibrous tissue reactions due to cortisone have been reported by Freeman, Fershing, Wang and Smith (1950), while Cavallero, Sala, Amira and Borasi (1951) demonstrated similar changes in the livers of rats treated with carbon tetrachloride.

Several workers have investigated the effects of cortisone on the production of granulation tissue in animals by means of short-term experiments. Ragan, Howes, Plotz, Meyer and Blunt (1949) found that cortisone caused a delay in the formation of all the elements of connective tissue in experimentally-produced wounds in rabbits. Similar results were obtained in mice by Spain, Molomut and Haber (1950), whose experiments showed that cortisone inhibited the formation of granulation tissue when administered in the early stages of initiation of the repair stimulus, but did not have any effect on existing granulation tissue. Bangham (1951) concluded from his studies on the effect of cortisone on the rate of wound healing in animals, that it reduced the amount of granulation tissue formed in rats and rabbits, but had no effect in guinea-pigs. There was no qualitative difference between the granulation tissue of treated and untreated animals in the case of rats and rabbits.

Studies on the effects of cortisone on the phagocytic action of the reticulo-endothelial system have been carried out by Spain, Molomut and Haber (1950) by the introduction of carbon particles into the peritoneal cavity of mice, and by Policard and Tuchmann-Duplessis (1951) with silica particles in the peritoneum of rats. In both these experiments the migration of dust-laden phagocytes was found to be retarded by cortisone. Harrison, King, Dale and Sichel (1952) found a similar state of affairs in the lungs of rats after injection of quartz. Curran (1953), on the other hand, concluded from his experiments on the effect of cortisone on quartz nodules in the peritoneum of mice, that cortisone delayed the phagocytosis of free dust, this being due to the suppression of the cellular exudate.

The fibrogenic properties of quartz have lent themselves to the study of certain aspects of the action of cortisone. Magarey and Gough (1952(a)) studied the effect of cortisone on developing silicotic fibrous tissue produced by the introduction of finely powdered quartz into the peritoneum of mice, rats, rabbits and guinea-pigs. They found that cortisone prevented the development of fibrous silicotic nodules in mice, leaving collections of quartz free in the peritoneum; altered the fibrous tissue in the nodules both qualitatively and quantitatively in rats; in rabbits fibrosis in the nodules was diminished, but a more diffuse reaction was present, while it had no appreciable effect in guinea-pigs. Curran (1952) found that cortisone modified the reaction of the mouse peritoneum to quartz.

Phagocytosis was delayed and the dispersal of the dust was more marked, so that the dust collections were smaller. In spite of this, fibrogenesis was unaffected both in its time of onset and rate of progress.

In contrast to these experiments, which deal with the effects of cortisone on silicotic fibrous tissue in the peritoneum, is the work of Harrison, King, Dale and Sichel (1952), who investigated the action of cortisone on developing fibrous tissue in the lungs of rats produced by intratracheal injection of quartz particles. Their main findings were that cortisone interfered with the migration of dust-laden phagocytes in the lung, where they remained diffusely distributed within alveoli, instead of aggregating to form discrete nodules. Cortisone also modified the development of pulmonary silicotic nodules in amount and distribution, but did not significantly alter the maturation of fibrous tissue from reticulin to collagen.

In human beings, cortisone has been reported to exert beneficial effects in conditions characterized by long-standing fibrosis, such as scleroderma, and in certain so-called mesenchymal diseases, such as rheumatic fever, lupus erythematosus (Hench, Slocumb, Barnes, Smith/Polley and Kendall, 1949; Howard, 1949). In addition, Kennedy, Pare, Pump, Beck, Johnson, Epstein, Venning and Browne (1951) claim to have treated a case of pulmonary silicosis with some clinical improvement in the patient's condition, although this was not supported by the X-ray findings. These reports and the findings of certain workers that cortisone modified the development of quartz-induced fibrosis, led to

investigations of the effect of cortisone on established silicotic fibrosis. Curran (1952) showed that cortisone had no effect on existing silicotic lesions in the peritoneum of mice, while Magarey and Gough (1952(b)), working with rabbits, were unable to find any reduction of mature fibrous tissue in intraperitoneal silicotic nodules as a result of cortisone treatment. They were, however, able to find histological evidence which suggested that the administration of cortisone retarded the further proliferation of fibroblasts, with consequent retardation of concentric fibrosis in the nodules. Preformed collagen remained unaltered.

From the foregoing facts it is concluded that cortisone has a modifying effect on developing silicotic fibrosis in the peritoneum and lungs of experimental animals, while its action on established silicotic fibrosis in the peritoneum is less definite. It seemed desirable, therefore, to study the effect of cortisone on established silicotic fibrosis in the lungs of rats, with particular reference to the histological picture as well as to the collagen and silica content of the lungs.

Plan of Experiment

36 rats were given intratracheal injections of quartz dust (50 mg) in order to produce silicotic fibrosis in their lungs. A sufficient period of time (100 days) was allowed for the formation of fully-developed collagenous nodules, after which cortisone was administered to some of the animals. 3 animals having been lost by cannibalism, the

remaining 33 were divided into two groups; the first group of 11 animals served as controls and received no further treatment, while the 22 animals in the second group were given subcutaneous injections of cortisone acetate suspension over a period of 265 days. The amount given was 5 mg. three times a week. Animals from both groups were killed at regular intervals and careful histological examinations of the lungs carried out. Further, the collagen and silica content of the lungs and lymph nodes of the animals in the two groups were estimated chemically.

Description of Samples

The dust used was powdered quartz "Snowit II". The sample was prepared by mixing two previously prepared fractions from Belgian glass sand. 2/3 by weight of a fraction of nominal diameter 1-2μ, was mixed with 1/3 of a fraction 0.5-1μ in diameter. The resulting size distribution and the data obtained from chemical analyses are shown in Table 1.

Figure 1 shows the electron micrograph of the dust.

The cortisone acetate suspension used in this work was a preparation from Merck & Co. Inc.

Preparation of the Dust Suspension

2.5 g. of the quartz dust was weighed into a screw cap bottle and 50 ml sterile physiological saline added to it. The suspension was thoroughly shaken, autoclaved for 20 mins. at 15 lbs. pressure and was kept shaking in a microid flask-shaker till the time of injection. Thefinal concentration of dust was 50 mg./ml. of physiological saline, and was the amount injected into each animal.

TABLE 1

PARTICLE SIZE DISTRIBUTION AND ANALYSIS OF QUARTZ DUST USED FOR INTRATRACHEAL INJECTION

Size in μ	Per cent by No.	Per cent by mass
0.32 - 0.45	3.0	
0.45 - 0.64	18.9	1.5
0.64 - 0.9	38.2	7.9
0.9 - 1.30	16.8	9.6
1.3 - 1.8	12.3	20.2
1.8 - 2.6	9.5	43.7
2.6 - 3.6	1.3	17.1

Specific surface $m^2/g.$	1.8
SiO_2 (%)	98.7
Ignition loss (%)	0.17
Silica solubility (SiO_2 mg/100 ml.)	3.9

Animals

36 male, black and white hooded rats from the Medical Research Council strain were used. Their average weight was 250 g.

Experimental Prodedure

The animals were lightly anaesthetized with ether and the dust suspension injected intratracheally via the mouth, according to the technique of Kettle and Hilton (1932), modified by King et al (1953(b)), a detailed description of which will be found earlier in this thesis. There was no regurgitation of dust and no immediate post-operative deaths occurred.

Duration of Experiments

Cortisone injections were commenced 100 days after the injection of quartz in the group of test animals. They were continued for 265 days (5 mg. three times a week). The animals were weighed at weekly intervals and the weights of both groups recorded.

A pair of animals - one from each group - were killed at fortnightly intervals in the early stages of the experiment. Owing to a number of deaths occurring in the group of cortisone-treated animals, the intervals at which the killings were done had to be adjusted in order to extend the experiment over a sufficient period of time and to obtain equal days of survival in both groups. The experiment was concluded 365 days after the injection of quartz.

HISTO-PATHOLOGICAL TECHNIQUE

Routine post-mortems were carried out on the animals that were killed and on those found dead. Their lungs were removed and fixed in 10% formol saline as previously described. The tracheo-bronchial lymph nodes were dissected out, weighed and fixed separately. Blocks were taken along the long axes of both lungs through the hila, dehydrated and embedded in paraffin wax. Serial sections, 5μ in thickness, were taken from these blocks in all cases and stained by Gordon and Sweet's, (1936) silver impregnation method for reticulin, and with Ehrlich's haematoxylin and eosin. An unstained serial section was subjected to micro-incineration in each case, and then washed in concentrated HCl and dry-mounted.

Chemical Methods

Estimations of the collagen and SiO_2 content of the lungs and lymph nodes from the cortisone-treated and control rats were carried out. The lungs and lymph nodes were bulked separately into three groups as follows :- tissues from animals killed up to 190 days; from 191-330 days and from 331-365 days in each group.

Preparation of lungs and lymph nodes: The tracheo-bronchial lymph nodes were separated from the fixed lung tissues and the lungs and nodes dried, bulked (as above) and weighed. The remainder of the blocks from the lungs taken for sectioning were de-waxed and the dried tissue added to the respective groups. The bulked lungs were finally ground.

Estimation of Silica: A sample of material (containing about

10 mg. SiO_2) in a glass centrifuge tube was ashed for 12 hours at 320° and then for a further 12 hours at 480°. The ash was repeatedly extracted with warm 2N HCl on a boiling water bath, and the residue finally extracted by agitation with cold 2N HCl. The residue (SiO_2) was weighed after drying at 105°.

Estimation of Collagen: Fat was extracted with ether from a 100mg sample of dried ground lung in a graduated hard glass test tube. 5 ml. water were added to the mixture, autoclaved at 30 lb. pressure for 6 hours, water added to 10 ml. and the tube heated for 15 minutes on a water bath. The mixture was centrifuged and filtered, and to a known volume of filtrate was added an equal volume of 10% CCl_3COOH and the mixture filtered after standing for 1 hour. A measured volume (5 ml.) of filtrate was transferred to a beaker and evaporated to dryness on a water bath. The residue was then taken up in 2 ml. 6N HCl, washed with 4 ml. acid into a hard glass test tube, sealed and autoclaved for 4 hours at 40 lb. pressure. The contents of the tube were made up to 10 ml. with 6N HCl, shaken with charcoal, filtered and a sample of this hydrolysate, after neutralization with 6N NaOH and further dilution with a neutral solution of 6N NaOH and 6N HCl, was used for the estimation of hydroxyproline following the method of Neuman and Logan (1950). The collagen content was calculated from the figures obtained for hydroxyproline.

PATHOLOGICAL FINDINGS

The experimental animals received cortisone injections for varying periods extending up to 265 days. 21 days after the commencement of the injections, the animals showed signs of moulting and bare patches appeared at the site of the injections. These patches became larger as the experiment progressed. During the first 42 days, the cortisone-treated animals lost weight steadily. The protein content in their diet was then increased and a slight improvement was noticed. Towards the end of the experiment they lost weight considerably, particularly during the last 56 days. The effect of the cortisone on the average group weight is shown in the graph.

Amongst the 11 control animals there was one spontaneous death due to infection, while 9 animals from the cortisone-treated group died from infection. This is statistically significant and may be regarded as a result of cortisone treatment. The animals which died spontaneously, and whose lungs were found to be infected, were not used for histological comparison. Killings of animals from the two groups were adjusted as far as possible to obtain equal days of survival in both. The duration of the experiment, the days of survival of the animals, their mode of death and the pathological grading of the lesions produced in their lungs, as assessed according to Belt and King (1945), have been summarized in Table 2. The details of the histological grading of lesions have been described earlier.

TABLE 2

ASSESSMENT OF FIBROSIS IN THE LUNGS OF RATS INJECTED WITH QUARTZ DUST ALONE AND QUARTZ DUST FOLLOWED BY CORTISONE TREATMENT

Days of Survival	Control		Cortisone-treated	
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
122	D	-		
123	K	4	K	4
124 - 150	K	5	K	5
			D(4)	-
			D	5
151 - 200	D	5	D	5
			D(4)	-
201 - 230	K	5	K	5
231 - 300	K †	5	K †	5
			D	5
			D	-
301 - 330	K	5	K(2)	5
331 - 365	K(4)	5	K(4)	5

† Section from these animals are reproduced in the figures.

K = Killed; D = Dead; Numbers ( ) indicate the number of animals killed or found dead at that period.

The collagen content of the lungs from the control animals and cortisone-treated animals is shown in Table 3; the silica content of lungs and lymph nodes from the animals of the two groups is in Table 4.

Macroscopic Appearances of the Lungs

On macroscopic examination there was very little difference between the lungs of the control animals and those which had received cortisone.

Control quartz dust. The lesions in the lungs were similar to those found previously in cases of quartz-induced fibrosis. At 123 days both lungs showed firm, white areas of fibrosis, which appeared to be running together in certain areas. By 150 days there were large areas of confluent fibrosis in the right lung, while the fibrosis was mainly patchy in the left lung. At 230 days the greater parts of both lungs were replaced by white, fibrous areas which were confluent. From this period till the end of the experiment at 365 days, the naked-eye appearances of the lung were similar to those at 230 days, except for the fact that the amount of fibrosis in the lungs steadily increased. The lymph nodes were enlarged and firm from the first (123 days) and gradually enlarged further to several times normal as the experiment progressed.

Cortisone-treated animals: At 123 days there were firm, white areas of fibrosis in both lungs. They were mainly patchy, but showed a tendency to become confluent dorsally in some areas. The confluent areas had increased in size by 150 days and at 230 days most of the right lung showed areas of confluent fibrosis, while the left lung had a few discrete patches of

TABLE 3

COLLAGEN CONTENT OF LUNGS (BULKED)

Period of Time (Days)	No. of Rats in Group analyzed		LUNG COLLAGEN			
			CONTROL		CORTISONE	
	Control	Cortisone	mg/rat	% dry basis	mg/rat	% dry basis
190	3	5	190	16	130	11
330	3	5	260	19	190	10
365	3	4	300	23	300	16

TABLE 4

SiO_2 (mg/rat) in lungs, lymph glands and lungs + lymph glands

Period of Time (Days)	No. of Rats in Group		Weight of SiO_2 (mg.) in					
			LUNGS		LYMPH GLANDS		LUNG + GLANDS	
	Control	Cortisone	Control	Cortisone	Control	Cortisone	Control	Cortisone
190	3	5	28	28	4.3	2.7	32	31
330	3	5	19	23	6.5	4.0	26	27
365	3	4	21	26	8.4	3.2	29	29

fibrosis. After this, the fibrosis seemed to increase as judged by the confluent areas seen macroscopically, and by 365 days most of the lung tissue was replaced by fibrous areas. The tracheo-bronchial lymph nodes were slightly enlarged and firm; but, although they were larger than the lymph nodes of normal rats, were not as large as those found in the group of animals which had been injected with quartz dust without subsequent cortisone treatment.

Microscopic Appearances

The pulmonary lesions in the animals which had quartz dust only and those which had subsequent cortisone treatment revealed no significant differences as far as the maturity of the fibrosis, as denoted by the system of grading used, was concerned. But when paired lung sections from the two groups (animals killed at the same time) were examined with the aid of a hand lens, there was a definite difference in the area of lung tissue involved by the fibrous process. In the cortisone-treated animals, the area of lung involvement appeared to be less than in the controls.

Control quartz dust group: The earliest sections examined were at 123 days, when the lungs showed numerous nodular foci of fibrosis, typical of the lesions which quartz has been known to produce. The lesions were, for the most part, acellular at the centre, with some cellularity at the periphery. On reticulin staining, they were seen to be composed entirely of compact collagen fibres (Grade 4 fibrosis). A few nodules showed a tendency to become confluent, but the majority were discrete. The fibrous process involved approximately 1/3

of the left lung field and ½ of the right one. By 150 days the lesions were confluent, acellular and collagenous (Grade 5 fibrosis). Lung involvement was about½ the areas of both lung fields. At 230 days, the histological picture was essentially the same as at 150 days, with about 2/3 of the left lung field and ¾ of the right one replaced by confluent fibrotic lesions. From this period onwards the area of lung tissue involved increased, and at 300 days about ¾ of both lung fields were replaced by confluent, acellular, fully collagenous lesions (Grade 5 fibrosis. Figs. 2 and 3). A few patent alveoli and some new-formed blood vessels were seen within the confluent areas. There was no further change in the lesions from this period till the termination of the experiment at 365 days. The lungs of the four animals killed at this stage showed almost total replacement of normal lung tissue by fibrosis.

Cortisone-treated group: The histological appearances of the lungs at 123 days were similar to those in the control animals. There were numerous nodular lesions throughout the lungs, involving about ½ the area of the right lung field and a little over 1/3 of the left lung. The lesions were discrete, mainly acellular, with cellularity only at the periphery, and on reticulin staining were completely collagenous (Grade 4 fibrosis). At 150 days many of the lesions were confluent, acellular and fully collagenous (Grade 5), but the area of lung involved was about the same as at 123 days. The lesions at 230 days were no different to those at 150 days. By 300 days, 2/3 of the area of the right lung field and 1/3 of the left were

replaced by confluent, acellular, collagenous nodules (Grade 5 fibrosis. Figs. 4 and 5). An occasional discrete nodule was seen in some areas. Within the confluent fibrous lesions, a few patent alveoli were seen. At 365 days, the lesions were similar but the areas of lung involved were approximately ½-2/3 in one lung field and 2/3-3/4 of the other.

Results of Chemical Analyses

Collagen: The collagen content of bulked lungs from the animals which were injected with quartz dust alone was higher than that from the lungs of animals which were treated with cortisone subsequently. The difference was more marked in the earlier stages of the experiment (Table 3).

Silica - Lungs: There was less silica in the lungs of the control animals than in the cortisone-treated animals in the later stages of the experiment. The difference was not very outstanding but it was significant (Table 4).

Lymph Nodes: The tracheo-bronchial nodes from the control series of animals contained more silica than the nodes from the cortisone-treated animals at all stages.

The differences in collagen and silica content of lungs and lymph nodes between the two series of animals are summarized in Tables 3 and 4.

DISCUSSION

It has been found that cortisone has a modifying effect on developing silicotic nodules in the peritoneum of experimental animals (Curran, 1952; Magarey and Gough, 1952(a)),

as well as in their lungs (Harrison, King, Dale and Sichel, 1952). The effect of cortisone on established silicotic fibrosis in animals is rather different. Magarey and Gough (1952(b)) found that when cortisone was administered to rabbits with fully-developed intraperitoneal silicotic nodules there was no effect on existent fibrous tissue, although histologically there was some evidence of retardation of further concentric fibrosis in the nodules during the administration of cortisone.

In the experiments under discussion, cortisone was administered to rats 100 days after intratracheal injection of quartz, when silicotic nodules would have been fully developed. The histological lesions studied 123 days after the introduction of the dust - when one group of animals had had cortisone for 23 days - were very similar in both the control and cortisone-treated animals, the pulmonary fibrosis being assessed as grade 4, with fully collagenous nodules. At the end of the experiment, 265 days after the commencement of cortisone treatment, there was still no difference in the grade of fibrosis between the pulmonary lesions of the two groups, both exhibiting the maximum degree of fibrosis. The maturity of the fibrous tissue was in no way affected. Morphologically, the lesions in the control and cortisone-treated animals were identical; there were the same central collections of dust surrounded by dense collagen fibres. At the periphery of some lesions a few cells were seen, but this again was a common finding in the two groups. Magarey and Gough (1952(b)),

in their study of the effects of cortisone on intraperitoneal silicotic nodules in rabbits, found histologically that there was a difference between the lesions in the controls and cortisone-treated animals. The lesions in the controls were composed of a central collection of dust surrounded by a dense layer of compact collagen, outside which was a cellular fringe with many fibroblasts, suggestive of active fibrosis in progress. The lesions in the cortisone-treated animals, on the other hand, were similar in all respects except for the absence of the cellular fringe, which was replaced by diffuse, delicate, connective tissue with loosely-knit fibres and thin-walled blood vessels. Magarey and Gough are of the opinion that the absence of the cellular fringe may be an indication of the retardation of further concentric fibrosis occurring while cortisone was being administered. Cortisone treatment did not produce similar histological changes in quartz-induced pulmonary silicotic nodules in rats in these experiments.

Although there was no difference, histologically, in the maturity of the fibrous tissue in the silicotic nodules of control rats and those treated with cortisone, there seemed to be a slight variation in the actual amount of lung tissue involved when paired sections from the two groups were compared. The cortisone-treated animals showed somewhat less involvement of lung tissue by the fibrous process, as compared with untreated animals, particularly in the later stages of the experiment. This is supported by the chemical estimations of collagen in the lungs, which yielded higher values in the case

of untreated animals than in cortisone-treated ones. The histological and chemical findings are significant when viewed together, and suggest that although cortisone has no effect on the quality of fibrous tissue, or on its maturity, it may affect the quantity formed. Presumably, the silicotic nodules which were present in the lungs at the time the cortisone treatment was commenced were modified in the mode of their extension, without actual resolution of the fibrous tissue taking place. It would seem, therefore, that cortisone does not cause the regression or lysis of collagenous silicotic nodules produced by quartz in the lungs of animals. This has been the observation of other workers,too. Curran (1952) found that cortisone did not have any lytic effect on mature silicotic nodules in the mouse peritoneum, while Magarey and Gough (1952(b)) obtained similar results in rabbits.

The differences in silica recoveries from the lungs and lymph nodes of the cortisone-treated and control animals are significant. There was more silica in the lungs of the cortisone-treated animals as compared with those of the controls, while the picture was reversed in the tracheo-bronchial lymph nodes. This suggests that cortisone inhibits the transport of silica from the lungs to the lymph nodes, probably due to a retarded macrophage response or to dust particles not being readily phagocytosed. Cortisone has been found to retard the macrophage response in mice (Spain, Molomut and Haber, 1950) and rats (Policard and Tuchmann-Duplessis, 1951). Harrison et al (1952) observed in their studies that cortisone retarded

the migration of dust-laden phagocytes in the lungs to such an extent as to alter the usual pattern of quartz-induced fibrosis. Analyses of these lungs for silica showed that there was much more silica in them than in the lungs from their controls which had no cortisone treatment (Stacy and King, 1954).

From these results it is evident that cortisone has no beneficial effects on pulmonary silicosis in animals when administered after the formation of mature silicotic nodules. Harrison et al (1952) were unable to prevent the development of quartz-induced pulmonary fibrosis in rats by the administration of cortisone, even though the treatment was commenced almost with the introduction of the fibrogenic agent and pushed to the limit of tolerance.

It does not seem justifiable to apply these findings to silicosis in human beings, as different animals vary in their response to cortisone and it is therefore possible that cortisone may affect the pulmonary fibrosis of human silicosis in a manner different from that in rats. Nevertheless, it seems unlikely that cortisone would have any beneficial effect on human silicosis and this has been found to be so by Kennedy et al (1951) who were unable to detect any change in the lung fibrosis, radiologically, in a silicotic patient treated with cortisone. Cortisone certainly does not appear to have an appreciable effect on the mature fibrous lesions of silicosis in animals, whether it be in their peritoneum (Curran, 1952; Magarey and Gough, 1952(b)) or lungs.

SUMMARY

The effect of cortisone on established silicotic fibrosis in the lungs of rats was studied by the intratracheal injection of 50 mg. of quartz dust in rats, followed 100 days later by the administration of 5 mg. cortisone acetate three times a week for 265 days.

Cortisone did not alter the pattern of the fibrosis or bring about any regression of existing silicotic nodules. Mature collagenous lesions were present in the control and cortisone-treated animals at all stages of the experiment. The actual amount of lung tissue involved by the fibrotic process was slightly less in the cortisone-treated animals, when paired histological preparations from the two groups were compared. This was borne out by chemical estimations of collagen, which was found in smaller amounts in the lungs of cortisone-treated animals. There was more silica in the lungs of these (treated) animals than in the controls, while the lymph nodes contained less silica.

It is concluded that cortisone does not have any appreciable effect on established silicosis as far as the grade of fibrosis is concerned, although a slight reduction in the actual amount of fibrous tissue may be found. Cortisone retards the phagocytic transport of dust from the lungs to the lymph nodes.

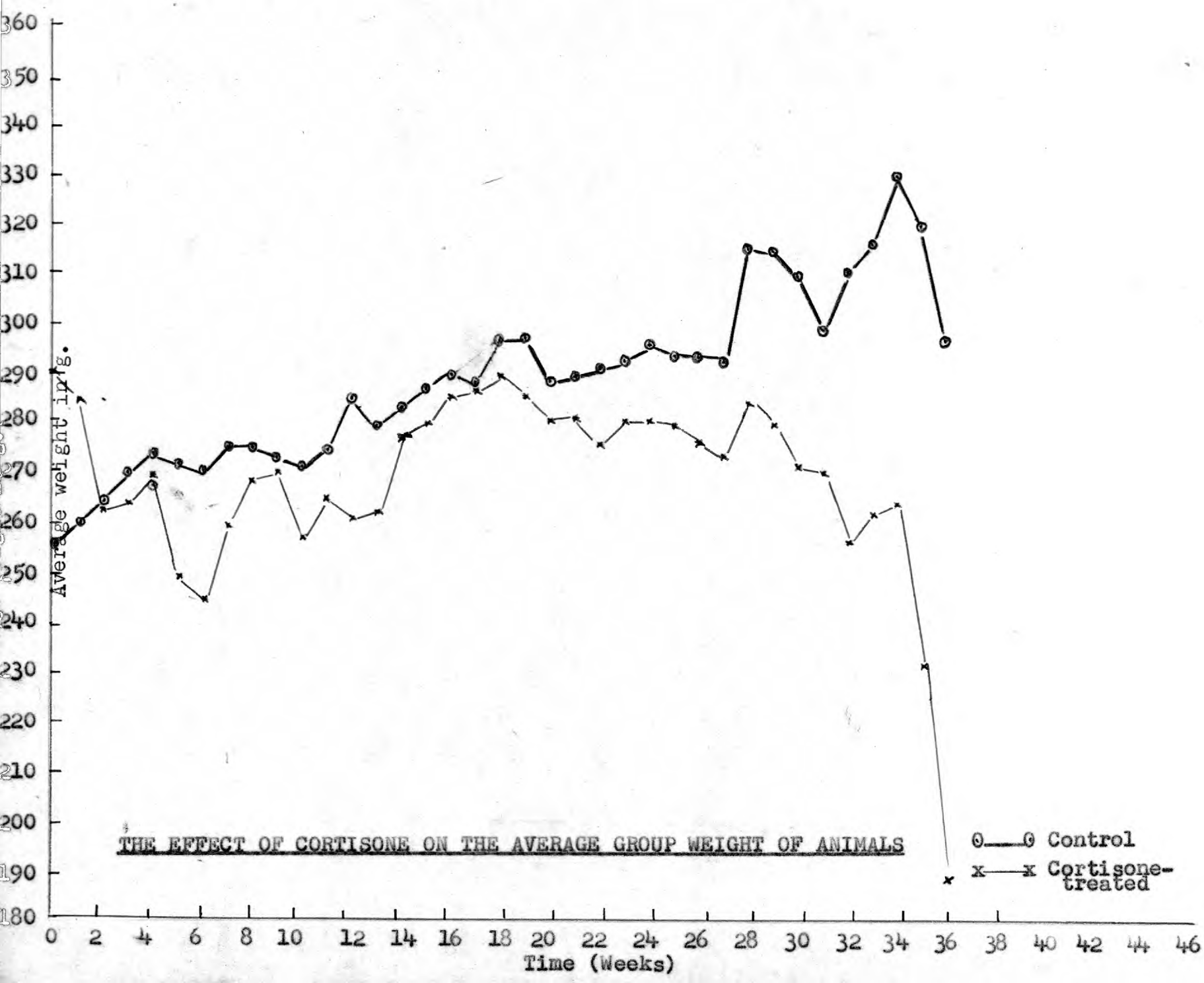

Average weight in gms.
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Time (Weeks)
THE EFFECT OF CORTISONE ON THE AVERAGE GROUP WEIGHT OF ANIMALS
0—0 Control
x—x Cortisone-treated



Fig. I. Electron micrograph of quartz dust (Snowit).
< 2 /u (1 /u = 6mm.) (X. 6000).



Fig. 2. Rat lung, 300 days after the injection of 50 mg. of quartz dust. Shows confluent silicotic nodules composed of compact collagen (grade 5 fibrosis). Silver impregnation. (X. 48).

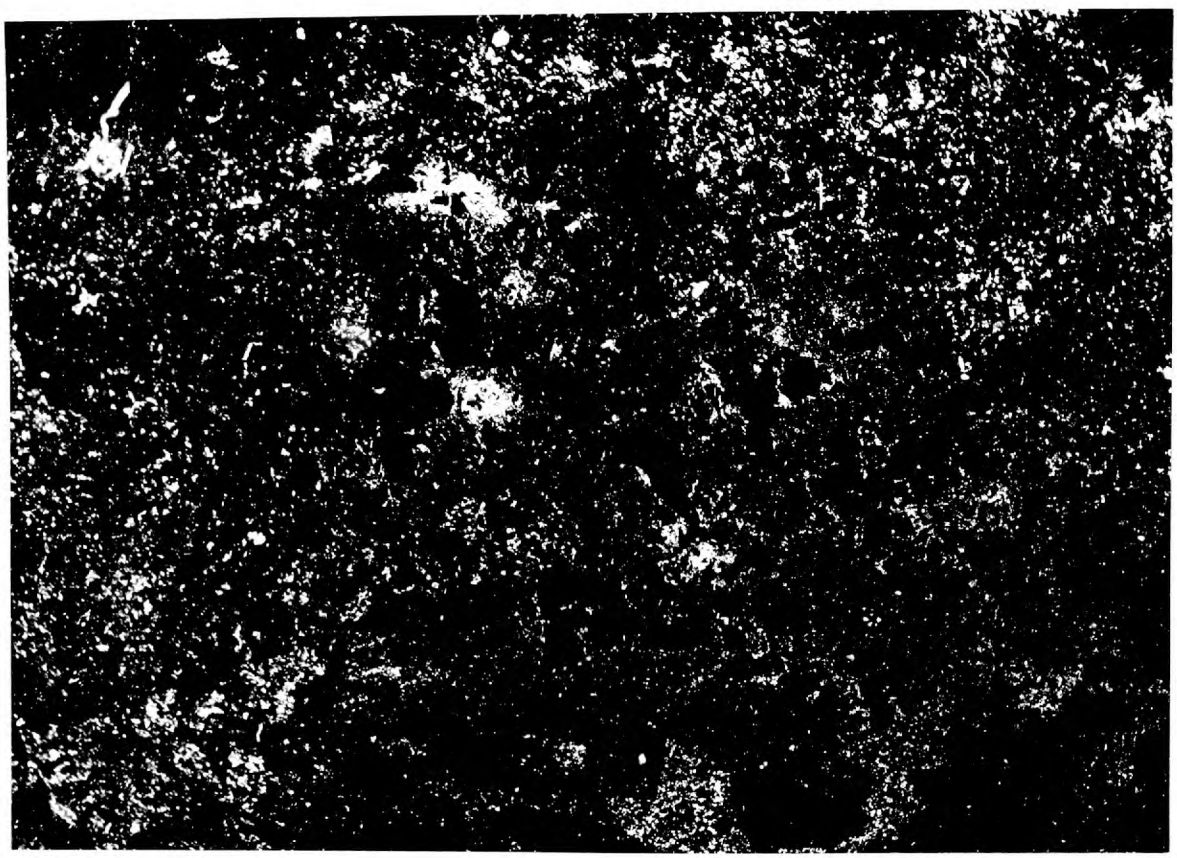


Fig. 3. Serial section to Fig. 2, showing the same area after micro-incineration. Dense collections of dust in the lesions. Dark ground illumination. (X. 48).

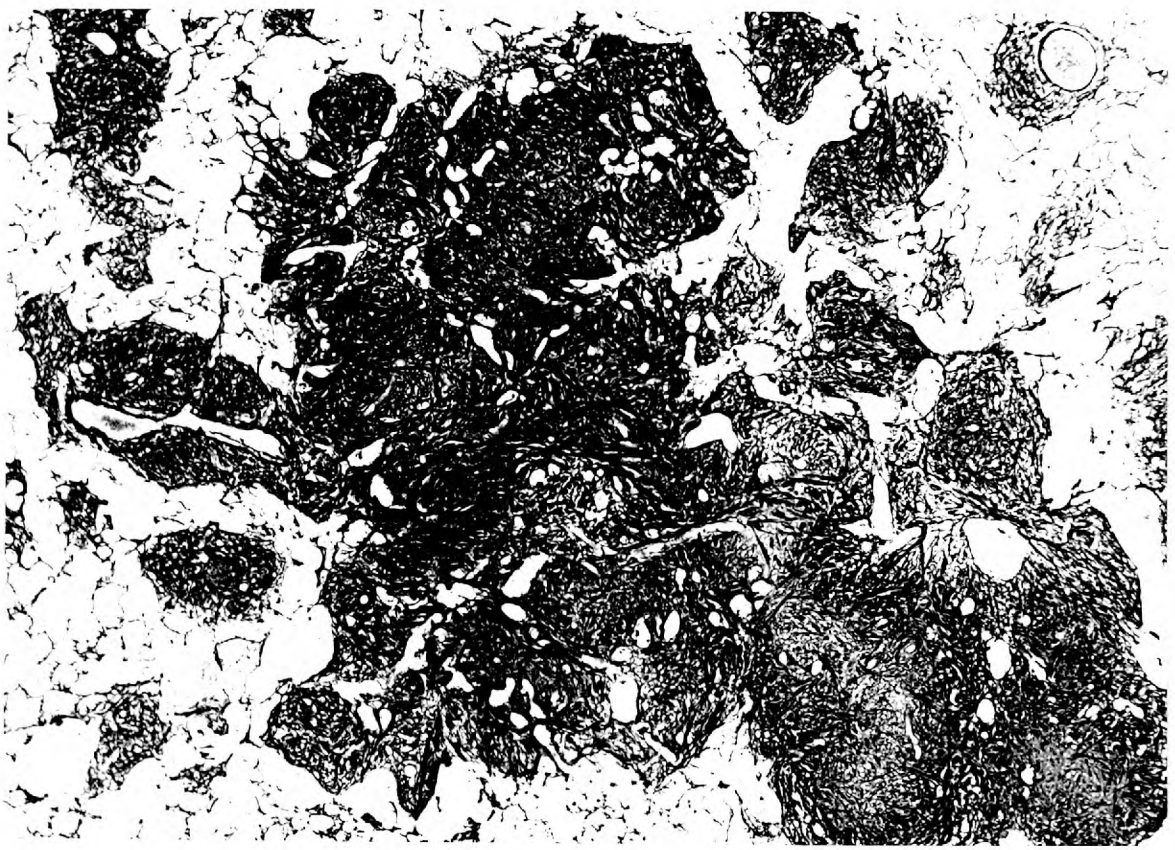


Fig. 4. Rat lung, 300 days after the injection of 50 mg. of quartz, followed by subcutaneous injections of cortisone (begun at 100 days and continued thrice weekly). Confluent, collagenous lesions (grade 5 fibrosis). Silver impregnation. (X. 48).

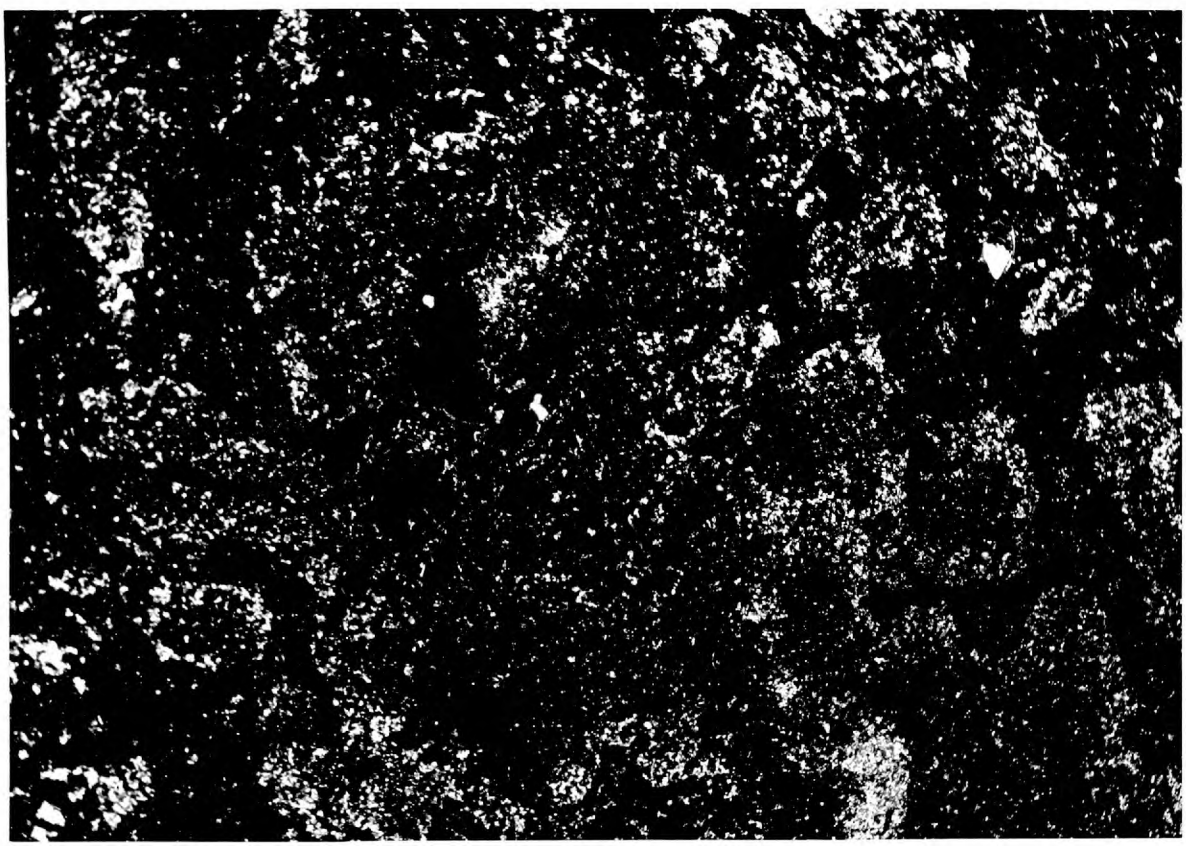


Fig. 5. Serial section to Fig. 4. Micro-incinerated preparation showing the distribution of dust in the lesions. (X. 48).

SECTION V

SECTION V

THE INFLUENCE OF COAL, MINE DUST AND KAOLIN ON AVIRULENT TUBERCULOSIS INFECTION PRODUCED BY DEAD ORGANISMS (B.C.G.) IN THE LUNGS OF RATS AND GUINEA-PIGS

The close association between silicosis and tubercular infection has long been recognized by many workers all over the world. In South Africa, Mavrogordato (1926) concluded that infection played an important part in the development of the disease, while Sutherland, Strachan and Simson (1930), writing about the condition in the Rand, stated that "if a silicotic patient did not die of some other disease or by accident, he ultimately died of tuberculosis." Policard (1933) was of the opinion that practically all cases of silicosis were complicated by tuberculosis:- "En fait, dans l'immense majorité des cas, sinon dans tous, le poumon silicotique est toujours aussi un poumon tuberculeux." Kettle and Archer (1933) observed that infective silicosis was more common in Great Britain than simple silicosis. By infective silicosis was meant "the modification in a dusty lung of the reaction of a chronic low grade infection, frequently tuberculosis, in the direction of increased fibrosis", a definition first used by Simson, Strachan and Irvine (1931) to describe the condition as it occurred in the Rand.

One of the great problems in pneumoconiosis is the relation of dust to the tubercle bacillus. Clinical evidence supports the view that silica has a specific action on the growth of the bacillus. The effect of silica on tuberculosis in experimental animals has been widely studied. Gye and

Kettle (1922) showed that tubercle bacilli proliferated readily in necrotic lesions produced in mice by the inoculation of silica into their interstitial tissues. Later, Kettle (1927) proved that the proliferation of the bacilli was not dependent on the presence of necrotic tissue, but on the actual presence of silica in the lesion. Other workers found that in experimental animals, attenuated or dead tubercle bacilli produced proliferating lesions in the presence of silica (Gardner, 1929; Cummins, 1940; Vorwald and Delahant 1938; Vorwald, Pratt, Dworski and Delahant, 1950). Experiments conducted by Gardner (1930) on guinea-pigs, showed that healed tubercular foci which had been produced originally by a particular strain of tubercle bacillus of low virulence - the R1 strain - were reactivated as a result of exposing the animals to silica dust inhalation. This capacity for new growth on the part of the organisms was not due to an increase in their virulence, since subinoculation from these animals did not produce anything other than the lesions characteristic of the strain. Policard (1939) and Sen (1939) also demonstrated the reactivation of latent tuberculous infections in animals in the presence of silica.

Kettle (1932) found experimentally, that living tubercle bacilli introduced into the general circulation of mice, proliferated in the interstitial lesions previously produced in these animals by inoculating them with silica, mine dust, shale and kaolin, while no growth of bacilli was seen in lesions produced by inert substances such as iron, carbon and aluminium. He was of the opinion that all the experimental evidence showed

that by a combination of dust and infection it was possible to produce a degree of fibrosis which could not be obtained from the action of either factor alone in the same period of time (Kettle, 1934). To prove this point, he introduced into the lungs of guinea-pigs, an infective agent in the form of a suspension of killed tubercle bacilli from an avirulent strain (B.C.G.) with and without kaolin dust. The advantage of using dead organisms was that the usual active infection which results from the presence of live organisms was replaced by a transient one which, while merely accelerating the pneumoconiotic process would not dominate the histological picture. From the results of these experiments he concluded that the combination of dust and an infective agent (killed avirulent tubercle bacilli, in this case) was capable of producing more fibrosis than either the dust or the infective agent alone.

Although it was known that crystalline silica was capable of enhancing the growth of tubercle bacilli it was not till recently that the role of other dusts, such as coal, granite, asbestos, in tubercular infection received attention. Several workers have reported a high incidence of tuberculosis among coal-miners whose lungs show the condition known as progressive massive fibrosis (Cummins, 1931; Belt and Ferris, 1942; Rogers, 1946; Gough, 1947(a); Fletcher, 1948). Tubercle bacilli have been demonstrated in the lesions in 38.5% of cases of progressive massive fibrosis by James (1948), Davies and Mann (1949), and Mann (1951) showed the relation between tuberculosis and the massive fibrosis of coal pneumoconiosis

radiologically. In spite of the fact that the organism has not been demonstrated in all cases of progressive massive fibrosis, the general concensus of opinion among workers in this field is that the tubercle bacillus is the chief causative agent. Experimentally, Cummins (1940) injected coal dust and quartz with and without dead tubercle bacilli intratracheally in rabbits and found that the reactions produced in the lungs by the combined action of dust and organisms were more extensive than those produced by dust alone.

The results of biological experiments only barely support the clinical and statistical evidence that the susceptibility to pulmonary tuberculosis is increased among workers in industries involving the inhalation of certain dusts. On the basis of these facts it was decided to conduct further animal experiments in order to study the effect of tubercle bacilli on dust in the lungs. Since coal dust and mine dust are of great importance from the social and industrial points of view, it seemed desirable to use these dusts in experiments. Kaolin dust was also included in order to compare the results with those obtained by Kettle in his experiments in 1934.

Experiments Planned

In order to study the effect of various dusts on tuberculous infection in the lungs of animals over a long period of time, it was thought better to use a species of animal which is known to be more resistant to this infection, rather than one which is more susceptible. Since rats are highly resistant to tuberculosis, and since they are the animals most commonly used in pneumoconiosis research, it was decided to use them.

In order to compare the results with Kettle's work (1934), a parallel series of experiments was set up with guinea-pigs.

Six groups of rats and six groups of guinea-pigs were injected with the following :- (1) B.C.G. (heat-killed); (2) Coal-mine dust; (3) Coal-mine dust + B.C.G. (4) Anthracite coal + B.C.G. (5) Kaolin; (6) Kaolin + B.C.G.

The results of experiments conducted by various workers, as well as some reported earlier in this work (Section I), have revealed that anthracite coal does not produce any permanent lesions in the lungs of experimental animals, even after long periods of time, grade 1 fibrosis rarely being attained. Therefore it was deemed unnecessary to set up a control experiment with anthracite coal alone, since the pulmonary reaction produced by it would be minimal, and no fibrosis would follow its introduction into the lungs.

DESCRIPTION OF SAMPLES OF DUST AND ORGANISMS

Mycobacterium tuberculosis - B.C.G. The strain of B.C.G. was kindly supplied by Dr. H.J. Benstead, Central Public Health Laboratory, Colindale. It was from the standard Copenhagen strain, 12 days' culture on Lowenstein-Jensen's medium.

Anthracite coal: This sample was from South Wales and was turbine ground and kindly prepared by Dr. B.M. Wright. Its ash content was 2.4% and its size distribution is given in Table 2.

"Mine-dust" Because of the difficulty of obtaining sufficient air-borne coal-mine dust it was decided to prepare a synthetic mixture of powdered coal and shale which would closely

TABLE 1

Analysis of Shale Dust

Ammonford B

	Per cent
SiO_2	60.42
Al_2O_3	20.79
Fe_2O_3	0.36
FeO	3.18
MnO	trace
MgO	1.16
CaO	0.53
BaO	-
Na_2O	0.90
K_2O	3.35
TiO_2	1.04
P_2O_5	0.12
CO_2	1.38
SO_3	-
S	0.08
F	trace
$H_2O + 105^o$	3.76
$H_2O - 105^o$	1.40
C	1.46
Cr_2O_3	-
	99.93
Less O for S	0.04
	99.89

TABLE 2

SIZE DISTRIBUTION AND ANALYSIS OF DUSTS USED FOR INJECTIONS

Samples	Anthracite		Coal-mine Dust (80% Anthracite + 20% Shale)		Kaolin	
Size in μ	Per Cent		Per Cent		Per Cent	
	By No.	By Mass	By No.	By Mass	By No.	By Mass
0.16 - 0.23					3	0.08
0.23 - 0.32					18.9	1.32
0.32 - 0.45			19.2	0.6	38.2	7.9
0.45 - 0.64			16.7	1.6	16.8	9.8
0.64 - 0.90	37.7	1.0	25.9	6.6	12.3	20.5
0.90 - 1.30	14.7	1.1	24.7	17.4	9.5	43.6
1.30 - 1.80	11.7	2.4	8.7	17.5	1.3	16.8
1.80 - 2.60	16.9	9.6	3.4	19.3		
2.60 - 3.60	9.9	15.9	0.9	14.6		
3.60 - 5.10	6.5	29.4	0.5	22.5		
5.10 - 7.20	0.6	33.3				
7.20 - 10.20	0.2	7.3				

TABLE 2 (Ctd.)

Samples	Anthracite	Coal-mine Dust (80% Anthracite + 20% Shale)		Kaolin
Particles counted	390	863		640
Estimated surface area $m^2/g.$	1.2	1.8		
Silica Solubility (mg/100 ml.)	0.2	0.85		
Ash Content (%)	2.4	22		
Composition (%)		Coal	78	
		Kaolin	5	
		Mica	10	
		Quartz	6	
		Carbonates	1	

approximate coal-mine dust in composition. The sample contained 80% anthracite coal (turbine-ground as above) + 20% shale. The shale (Ammonford B) was likewise from a South Wales anthracite mine, from a seam overlying the coal measure. It was powdered in a rubber-lined tumble mill (King, 1945), and was analysed by Geochemical Laboratories, Ltd. (Table 1). By x-ray analysis it was found to consist of 31% quartz, 51% mica, 6% kaolin and 7% carbonates.

Kaolin: The sample of kaolin was obtained from British Drug Houses, and was labelled "Society of Leather Trades Chemists' Specification". It was largely crystalline, but contained a considerable amount of amorphous material; and consisted of practically pure aluminium silicate. This was the same sample as that used by Kettle in his experiments on infective silicosis in 1932 and 1934.

The size distribution and analysis of these dusts are given in Table 2.

Preparation of Suspension for Injection

Myco.tuberculosis. B.C.G. The strain of B.C.G. was sub-cultured on glycerine nutrient agar medium in 8 oz. flat medicine bottles. In order to obtain good growth, the organism was first transferred to a sterile Grifith's tube and a few ml. of sterile saline added. By grinding this very gently, a thick suspension of the organism was made. A few drops of the suspension were transferred, by means of a sterile Pasteur pipette to each of 12 glycerine agar bottles and spread over the surface of the medium with a glass spreader. These inoculated

bottles were incubated at 37°C for 15 days, when sufficient growth was found to have taken place. The culture mass from each of these bottles was transferred to a previously weighed Grifith's tube. The weight of the growth was determined by re-weighing the Grifith's tube. Large flakes of growth were broken up and the contents transferred to a 4 oz. screw cap bottle, sufficient physiological saline being then added to bring the concentration of the growth to 10 mg/ml. The suspension was shaken for 15 minutes in a mechanical shaker, after a few glass beads had been added to it to facilitate the process. By this means a fine granular suspension of the organisms was obtained. The bacilli were killed by auto-claving for 20 mins. at 15 lb. pressure. (Sterility tests were carried out on Lowenstein-Jensen medium to ensure the effectiveness of autoclaving). The bottle was labelled "Stock B.C.G. Suspension (10 mg/ml)." Just before the injections were made this suspension was added to the various dust suspensions, or sterile physiological saline, in the case of the controls, in such volumes as to make the final concentration of B.C.G. 2 mg/ml of suspension when introduced into the lungs of the experimental animals.

Dust Suspensions:

2.5 g. of each of the dusts - anthracite coal, mine dust and kaolin (washed in normal saline) - were weighed into 4 oz. screw cap bottles. Cream-free milk (about 3%) was added to the coal and mine dusts to help keep the particles in suspension. Sterile physiological saline was then added

to the bottles so that the suspension contained 100 mg. of dust per ml. The bottles were then sterilized by autoclaving (20 min. at 15 lb. pressure).

To those suspensions which were to be injected in combination with killed B.C.G., the previously sterilized B.C.G. suspension was added and intimately mixed immediately before the injections were made. The volume of B.C.G. suspension added was calculated to a concentration of 2 mg. B.C.G./ml. of final mixture.

The composition of the various suspensions used in the experiments was as follows :-

(1) B.C.G. 5 ml. of stock B.C.G. suspension + 20 ml. sterile physiological saline (2 mg. B.C.G./ml.)

(2) Mine dust - 2.5 g. of dust + 24 ml. of physiological saline + 1 ml. of milk (100 mg/ml).

(3) Mine dust + B.C.G. - 2.5 g. of dust + 19 ml. of physiological saline + 1 ml. of milk + 5 ml. of stock B.C.G. suspension (100 mg. dust + 2 mg. B.C.G./ml.)

(4) Anthracite coal + B.C.G.- 2.5 g. of dust + 19 ml. of physiological saline + 1 ml. of milk + 5 ml. of stock B.C.G. suspension (100 mg. dust + 2 mg. B.C.G./ml.)

(5) Kaolin - 2.5 g. of kaolin + 25 ml. of physiological saline (100 mg. kaolin/ml.)

(6) Kaolin + B.C.G.- 2.5 g. of kaolin + 20 ml. of physiological saline + 5 ml. of stock B.C.G. suspension (100 mg. kaolin + 2 mg. B.C.G./ml.)

Thus the individual suspensions contained 100 mg. of the particular dust and/or 2 mg. of B.C.G. per ml.

The dose of dust and organisms injected varied in rats and guinea-pigs, depending on their body weight, and was as follows :-

Body weight of animal in g.	Amount of Dust in mg.	Amount of B.C.G. in mg.	Volume of Suspension (ml.)
200	50	1.0	0.5
300	75	1.5	0.75
400	100	2.0	1.0
500	125	2.5	1.25
600	150	3.0	1.50
700	175	3.5	1.75
800 and over	200	4.0	2.0

Animals

Twenty rats were used in each of the six groups (B.C.G., mine dust, mine dust + B.C.G., anthracite + B.C.G., Kaolin, Kaolin + B.C.G.). They were all of the male sex and were from the black and white hooded variety of the Medical Research Council strain. Their average weight was 300 g.

Eight guinea-pigs, four male and four female, were used in each of the six groups of experiments. Their body weights varied from 300 g. - 800 g.; however, they were equally distributed in all the groups.

Experimental Procedure

The method of introduction of the suspensions was by the intratracheal route via the mouth (Kettle and Hilton, 1932; modified by King et al, 1953 (b)). The animals were lightly anaesthetized with ether and the injections made as described in an earlier section of this work. There was no regurgitation

of dust. No immediate post-operative deaths occurred in any of the groups.

Duration of Experiments

The experimental animals were killed at monthly intervals in the case of rats and the experiments extended up to a period of 500 days.

In the case of guinea-pigs the animals were killed at longer intervals to enable the experimental period to be prolonged for a sufficient number of days comparable with that in rats. In spite of careful planning, some animals died of intercurrent infections, and the killings had to be modified still further. These experiments were extended up to 365 days only in most cases.

Histo-pathological technique

Routine post-mortems were carried out on all the animals which were killed at regular intervals, as well as on any that were found dead. The lungs were fixed in 10% formol-saline and blocks taken along their long axes through the hilum to include the hilar lymph glands. The blocks were then treated with fresh fixative, dehydrated, impregnated with paraffin wax, embedded and sectioned at 5u.

Serial sections were stained as usual by Gordon and Sweet's silver impregnation, with haematoxylin and eosin, and in addition with Van Gieson's stain. In addition, the sections from the lungs of animals which had been injected with B.C.G. were stained with Ziehl-Neelsen's carbol-fuchsin, and also with phenol auramine and the sections examined under fluorescent light in order to study the distribution of the organisms in the lesions.

PATHOLOGICAL FINDINGS

Sections from the lungs were studied microscopically, and the fibrosis seen in the most advanced lesions in each case was graded according to Belt and King (1945). The five grades of fibrosis recognized have already been described in detail earlier.

The pathological gradings obtained, together with the number of days of survival of the animals and their mode of death, have been summarized in Tables 3 and 4.

The macroscopic and microscopic appearances in the two species of animals were very similar, except in the case of the animals which had been given kaolin + killed B.C.G., where, histologically, the reaction in rats seemed to be slightly more marked than that in guinea-pigs.

Macroscopic Appearances in Rats and Guinea-pigs

Macroscopically there was no difference between the lungs of rats and guinea-pigs in the various series, and they are described together. The lesions appeared somewhat larger in the groups of animals which received dust and organisms than in those which had the dust or the organisms alone.

B.C.G.- Evenly distributed minute, discrete, whitish lesions, 1-2 mm. in diameter, were seen in the lungs in the early stages, from 30 days onwards. They were present at 240 days, and were slightly larger, being about 4 mm. in diameter. In the later stages, the lesions diminished in size and number and when the experiments were concluded at 500 days they were still present but scanty.

TABLE 3

ASSESSMENT OF FIBROSIS IN SECTIONS OF LUNGS OF RATS AFTER INTRATRACHEAL INJECTION OF DIFFERENT DUSTS, ORGANISMS OR BOTH

Days of Survival	B.C.G.		Coal-mine Dust		Coal-mine dust + B.C.G.		Coal + B.C.G.		Kaolin		Kaol
	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death
30	K†	1 min	K†	0	K†	1	K†	1	K†	1 min	K†
					D	-					
60	K	1 min	K	0	K	1	K	1	K	1	K
90	K	1 min	K	0	K	1	K	1	K	1	K
			D	-							
120	K	1 min	K	0	K	1	K	1	K	1	K
150	K†	1 min	K	0	K	1	K	1	K	1	K
			D	-							
180	K	1 min	K†	0	K†	2 min	K	1 max	K†	1	K†
210	K(2)	1 min	K(2)	0	K(2)	2 min	K(2)	1 max	K(2)	1	K(2)
240	K	1 min	K	0	D	-	D	-			
					K	2 min	K†	2 max	K	1	K
270	K(2)	1 min	K(2)	0	K(2)	2 min	K(2)	2 max	K(2)	1	K(2)
300	K†	1 min	K	0	K	2 min	K	2	K	1	K
	D	-									
330	K	1 min	K	0	K	2 min	K	2	K	1	K
365	K	1 min	K	0	K	2 max	K	2	K	1	K
400	K	1 min	K	0	K	1	K	1	K	1	K
							D	-	D(2)	-	
450	K	1 min	K	0	K	1	K	1	K	1	K
477					D†	1 min					
500	K(2)	1 min	K†	1	-	-	K†	1 min	K(3)	1	K(2)
			D	-	-	-	K	1 min	K†	1	K†

† Sections from these animals are reproduced in figures

K - Killed; D - Dead; Max - Maximal; Min - minimal within the indicated group.

Numbers ( ) refer to the number of animals killed or found dead.

TABLE 4

ASSESSMENT OF FIBROSIS IN SECTIONS OF LUNGS OF GUINEA-PIGS AFTER INTRATRACHEAL INJECTION OF DIFFERENT DUSTS, ORGANISMS OR BOTH

	B.C.G.		Coal-mine dust		Coal-mine dust + B.C.G.		Coal + B.C.G.		Kaolin		Kaolin + B.C.G.	
Days of Survival	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis	Mode of Death	Grade of Fibrosis
30	K‡	1 min	K‡	0	K‡	1	K‡	1	K‡	1	K‡	1 max
			D	-							D	-
90	K	1 min	K	0	K	1	K	1	K	1	K	1 max
120	K	1 min	K	0	K	1 max	K	1	K	1	K	1 max
180	K‡	1 min	K	0	K	2 min	K	2 min	K	1	K‡	2
240	K	1 min	K‡	0	K‡	2 max	K+	2 max	K+	1	K	2
300	K	1 min	K	0	K	2	K	2 min	K(2)	1	K	2
							D	-				
365	K‡	1 min	K‡	0	K‡	1	K‡	1	K‡	1	K‡	1
500	K	0			K	1						

‡ Sections from these animals are reproduced in the figures

K = Killed; D = Dead; Max = Maximal; min = minimal within the indicated grade of fibrosis.

Numbers ( ) indicate the numbers of animals killed or found dead.

Mine dust - In both rats and guinea-pigs' lungs the dust was distributed diffusely. In some cases, dense collections of dust were seen on the dorsal aspects of the lungs.

Mine dust + B.C.G. - On the lung surfaces there were collections of dust, around which in the early stages were greyish lesions about 1-2 mm. in diameter. These lesions increased in size during the course of the experiment and persisted till its end - 500 days - being intimately associated with the collections of dust all the time.

Anthracite coal + B.C.G. - The macroscopic appearances in this group of experiments in both rats and guinea-pigs were very similar to the appearances found in the lungs of animals receiving mine dust + B.C.G.

Kaolin - Small, whitish collections of dust were seen distributed diffusely throughout the lungs of both rats and guinea-pigs. There was no change in the macroscopic appearances till the end of the experiment at 500 days in rats and 365 days in guinea-pigs.

Kaolin + B.C.G. - In the early stages (from 30 days onwards) there were white areas over the lung surfaces which were larger than those seen in the previous group. From about 210 days, there were definite firm white areas on the lungs, similar to patches of fibrosis. Later still, towards the termination of the experiment, the appearances were remarkably like those seen in the previous experiment (kaolin alone) except that the dust collections were larger.

The tracheo-bronchial lymph nodes were enlarged to several times the normal in all these groups. In the animals which received mine dust, mine dust + B.C.G. and anthracite

coal + B.C.G., the nodes were, in addition, black in colour.

Microscopic Appearances

The histological lesions in the lungs of rats and guinea-pigs in the various groups of experiments were very similar in the type of reaction. Mine dust did not produce any fibrosis in either rats or guinea-pigs; B.C.G. alone and kaolin alone produced mild fibrosis (Grade 1); while mine dust + B.C.G., anthracite + B.C.G. and kaolin + B.C.G. produced well-marked fibrosis (Grades 2 and 3 respectively in rats and Grade 2 in guinea-pigs). The lesions reached a maximum at about the same period in all cases and then decreased in severity towards the end of the experiments. The demonstration of acid-fast organisms in the case of guinea-pigs was difficult.

Rats

B.C.G. - The sections examined at 30 days showed many nodular lesions, about 100-200μ in diameter, throughout the lung fields. They were composed mainly of mononuclear cells with some bacilli in the centre. On reticulin staining there appeared to be a loose network of fine reticulin fibres within these nodular lesions (Grade 1 fibrosis. Fig. 1). Thereafter, the lesions showed a slight increase in size (200-300μ) but there was no evidence of increase in reticulin up to 150 days (Fig. 2). In the sections examined after this period there appeared to be a diminution in the size of the lesions (100-150μ). The fibrosis still remained the same as at first and at 300 days was Grade 1 (Fig.3.) From this time onwards the lesions seemed to be reduced, both in size and in number, and at 500 days only an occasional small cellular lesion could be seen. Acid-fast

bacilli were, however, demonstrable in such lesions, although they were reduced in number as compared with the earlier stages.

Coal-mine dust: In the early stages, the dust appeared to be distributed diffusely throughout both lungs. At 30 days there were plenty of dust particles lying free within the alveoli. In some areas, dust cells were collected into small aggregations, but there was no evidence of fibrosis (Fig. 4). Between this time and 180 days, the dust cells began to collect into definite nodules, leaving the lung fields clear. At 180 days, the dust cell collections had enlarged to a certain extent, without the appearance of any fibrosis (Fig. 5). Between 180 days and the termination of the experiment at 500 days, the dust appeared to become more tightly packed together into focal aggregations with just enough reticulin fibres around them to hold the dust together, but no further fibrosis (Fig. 6).

Coal-mine dust + B.C.G. - At 30 days there were some dust cells lying free within the lung alveoli, while others were aggregated to form nodular lesions. These lesions were composed of dust cells together with many epitheloid cells and some acid-fast organisms, and seemed to be due to the combined action of the dust and organisms (Fig. 7). Within such lesions were loosely arranged fine reticulin fibres (Grade 1 fibrosis; Fig. 8). By 120 days the lesions had increased in size, but there was very little increase in fibrosis, the lesions remaining within the maximum limits of Grade 1 fibrosis. At 180 days, they appeared to have advanced and the reticulin fibres were thicker and becoming compact (Grade 2 fibrosis, minimal. Fig. 9). No

further progress was noted in the lesions till 365 days, when the reticulin appeared to be very thick and compact (Grade 2 fibrosis, maximal. Fig. 10). After this, the lesions gradually diminished in number and severity. The nodules were smaller and the reticulin fibres, which had been thick and compact earlier, were finer and loosely arranged and, at the end of the experiment (477 days) only a few small nodules were seen. At this stage a few collections of dust with mild thickening of the alveolar walls were seen, but no definite fibrosis (Fig. 11).

With fluorescent light the microscopic preparations examined for organisms showed about 8 in an area 2 cm. x $\frac{3}{4}$ cm. of lung at the period when the fibrosis was at its maximum, and 2 only in the same area approximately at 500 days.

Anthracite coal + B.C.G. - The lesions produced by coal and B.C.G. in the lungs of rats were very similar to those produced by coal-mine dust and B.C.G. In the early stages (30 days) there were cellular lesions composed mainly of epitheloid cells, dust cells and acid-fast organisms (Fig. 12). Within these lesions there was a slight increase of reticulin fibres (Grade 1 fibrosis, minimal. Fig. 13). The lesions remained unchanged till 150 days, when there was thickening of the reticulin fibres in them and the fibrosis reached a fully developed Grade 1. By 240 days, the cellular lesions were larger, more numerous and showed thick and compact reticulin (Grade 2 fibrosis, maximal. Fig. 14). There was no appreciable change from this time till 400 days. At this stage the lesions were somewhat smaller and less numerous, and at 500 days there were only scattered lesions

with a few reticulin fibres (Grade 1 fibrosis. Fig. 15).

Examination of sections stained with auramine revealed, under fluorescent light, about 8 bacilli in an area 2 x ½ cm. of lung tissue at 180 days, and only 3 bacilli in an area 3 x 1 cm. at 500 days.

Kaolin - This dust produced a diffuse exudative reaction in the lungs at 30 days. There were numerous giant cells containing dust particles (Fig. 16). In certain areas the cells were collected together but did not form compact nodules. A few fine reticulin fibres were seen within these collections (Grade 1 fibrosis, minimal. Fig. 17). The lesions did not appear to progress and at 180 days the fibrosis still remained at Grade 1 minimal (Figs. 18 and 19). No significant differences were evident in the lesions from this time till the termination of the experiment at 500 days, when the fibrosis was still Grade 1 minimal (Fig. 20).

Kaolin + B.C.G. - The histological lesions in the lungs, resulting from the introduction of kaolin dust and B.C.G., were very different from the lesions produced by the dust alone. At 30 days there was the same diffuse infiltration of the lungs, with large numbers of giant cells containing engulfed dust particles and collected together in some areas to form nodules, within which were acid-fast organisms and loose reticulin fibres (Grade 1 fibrosis. Fig. 21). With the advance of time, however, the lesions showed definite increase of reticulin in them. There was generalized thickening of the alveolar walls, and at 180 days portions of the lung showed some consolidation due to dust-laden cells. Some bacilli were demonstrable in

these areas, and there were many thick compact bundles of collagen fibres (Grade 3 fibrosis. Fig. 22). The fibrosis remained at this stage till 270 days, when the lesions began to show signs of regressing, and at 500 days there were only a few nodular lesions present together with mononuclear cells distributed throughout the lungs and some alveolar fibrosis in the form of a reticular network (Fig. 23).

Auramine-stained sections examined with fluorescent light revealed about 7 bacilli in an area 2 cm. x ½ cm. of lung tissue at the period when the fibrosis was most marked, and only about 3 bacilli in an area 3 cm. x 1 cm. at the end of the experiment.

Guinea-pigs

The histological lesions produced by dust, organisms and the combined action of the two in the lungs of guinea-pigs were similar to those produced in the lungs of rats by the same agents. The different degrees of susceptibility of the two animal species to the infecting organism did not seem to affect the lesions.

B.C.G. - Histological examination of the lungs at 30 days revealed numerous cellular lesions about 100-200μ in diameter and composed mainly of mononuclears and epitheloid cells. A few fine, loosely arranged reticulin fibres were seen in some of the lesions (Grade 1 fibrosis, minimal; Fig. 24). Within the next six months the lesions enlarged in size to some extent (200-350μ), and at 180 days, apart from this change, there was no visible increase in the amount of reticulin fibres within

the lesions (Fig. 25). From this time onwards the lesions diminished in size (100-200u) and at 365 days were seen as small cellular areas with a few reticulin fibres (Fig.26). They persisted even up to 500 days although they were few in number and small.

Coal-mine dust - Numerous dust particles were seen distributed diffusely throughout the lung fields within the alveoli and in the interstitial tissue at 30 days (Fig. 27). By 150 days these dust cells seemed to be aggregating to form irregular nodules and at 240 days there were many small collections, while some dust cells still remained free in the alveoli. There was no development of reticulin fibres within such nodules (Fig. 28). At 365 days the dust collections appeared compact but there was no fibrosis at all (Fig. 29).

Coal-mine dust + B.C.G. - The lungs examined at 30 days showed small nodular lesions composed of dust cells and epitheloid cells together with a loose network of fine reticulin fibres in them (Grade 1 fibrosis. Fig. 30). The lesions seemed to progress and at 180 days the reticulin fibres appeared thicker. By 240 days the nodules had enlarged and the reticulin fibres were thick and compact, with a small amount of collagen (Grade 2 fibrosis. Fig. 31). From this time till 300 days there was no progress, and after that the lesions seemed to regress; by 365 days there were only a few cellular collections with scanty reticulin fibres (Grade 1 fibrosis, subminimal. Fig. 32), and at 500 days the picture was no different.

Anthracite coal + B.C.G. - Histologically the lesions were similar to those seen in the previous group. At 30 days some dust was seen lying within the alveoli, while in some places there were small focal accumulations of dust cells and epitheloid cells in the form of nodules (Fig. 33). On reticulin staining the presence of a loose network of reticulin fibres within the nodules was seen (Grade 1 fibrosis. Fig. 34). At 180 days there were many lesions in the lungs, some of them associated with reticulin fibres which were increasing in thickness (Grade 2 fibrosis, minimal). By 240 days the nodules were larger and the reticulin fibres compact, with some collagen (Grade 2 fibrosis, maximal. Fig. 35). After this the lesions gradually diminished in size and at 365 days there were only a few with some reticulin fibres in them (Fig. 36).

Kaolin - There appeared to be a marked exudative reaction in the lungs as a result of the introduction of kaolin. At 30 days a large number of foreign-body giant cells containing dust particles were seen distributed throughout the lung fields (Fig. 37). A loose reticulin network was demonstrable in some of these areas, as well as among localized collections of giant cells (Grade 1 fibrosis, minimal, Fig. 38). From this period up to 240 days, there was very little change in the lesions, with the exception of a slight increase in reticulin fibres (Fig. 39). The lesions were, on the whole, diffuse, and remained so from the commencement of the experiment to its termination at 365 days (Grade 1 fibrosis. Fig. 40).

Kaolin + B.C.G. - At 30 days there were large portions of the lung packed with giant cells containing dust particles. In certain areas there were nodular lesions composed of these giant cells, mononuclears and epitheloid cells. Some fine strands of reticulin were seen in the affected parts of the lung (Grade 1 fibrosis. Fig. 41). During the next five months the lesions advanced in severity and the histological appearances were markedly different in every respect from those produced by kaolin alone at the same period of time. Areas of necrosis were seen in some of the lesions and parts of the lung were solid with dust cells. By 180 days there was coarse reticular fibrosis of the alveolar walls in the affected regions and thick compact reticulin, together with some collagen within the actual lesions (Grade 2 fibrosis, maximum. Fig.42), whereas in the lesions produced by kaolin alone at this same period there was nothing more than a fine network of reticulin (Grade 1 fibrosis). Thereafter, the lesions decreased in severity and at 365 days only a few fine reticulin fibres remained in the lesions, together with some mild alveolar fibrosis (Fig. 43). The lesions produced by kaolin and dead B.C.G. were in marked contrast to those produced by the dust alone, where the lesions showed no apparent difference from the commencement of the experiment to its termination, remaining all the time within the limits of grade 1 fibrosis.

DISCUSSION

Clinical and statistical evidence suggest that silica has a specific effect on the growth of the tubercle bacillus.

Similar evidence is available that the tubercle bacillus flourishes in the lesions found in the lungs of coal-miners. Experimental work, however, has supported these findings only to a limited extent. Since both organisms and dust produce similar types of fibrotic lesions, the effect of one on the other is difficult to evaluate.

Kettle (1932) studied the interstitial reactions caused by various dusts and their influence on tuberculous infection by injecting suspensions of silica, mine dust, kaolin, shale and asbestos subcutaneously in mice, followed by intravenous injection of suspensions of living tubercle bacilli, and found that tubercle bacilli proliferated in the lesions produced by these dusts. Later, he injected kaolin and avirulent dead tubercle bacilli, together and alone, into the lungs of guinea-pigs and was able to show that the lesions produced by the combined action of dust and organisms were more severe than those produced by either factor alone in the same period of time (Kettle, 1934). Experiments on similar lines were conducted by Cummins (1940), who injected anthracite coal dust and dead tubercle bacilli into the lungs of rabbits and found that the lesions produced by them were more extensive than those produced by dust alone.

In the present series of experiments, anthracite coal dust, mine dust and kaolin have been injected into the lungs of rats and guinea-pigs, with and without dead tubercle bacilli from a relatively avirulent strain (B.C.G.), and the effect of dust on the organisms studied. The purpose in using organisms of low virulence which had been previously

killed, was in order to ensure that the reaction produced by them would not be so acute as to obscure the lesions produced in the lungs by the dust.

Histologically, the lesions produced by anthracite coal, mine dust, kaolin and organisms by themselves and in combination, were similar in the two species of animals in spite of their different degrees of susceptibility to tuberculous infection. B.C.G. by itself produced only very mild lesions in the lungs of both rats and guinea-pigs. There were a few fine reticulin fibres in the lesions, these being evident in the early stages of the experiment and persisting throughout, although in much smaller numbers by 500 days when the experiment was terminated. Mine dust did not produce any permanent pulmonary lesions in either rats or guinea-pigs. The same has been found to be true with regard to coal dust (Section I of this work). Kaolin produced a diffuse exudative reaction and mild fibrosis with a few fine reticulin fibres (Grade 1 fibrosis). However, when these same substances, which by themselves produced only very mild reactions in the lungs, were injected in combination, a very different histological picture ensued. Anthracite coal and B.C.G. when injected together produced marked lesions with a general histological picture of Grade 2 fibrosis, some lesions containing collagen fibres. The maximum fibrosis was present at 240 days in both rats and guinea-pigs. After this the lesions appeared to regress and by 500 days in rats, and 365 days in guinea-pigs, only a few were seen with mild reticulinosis. Mine dust and B.C.G. produced lesions of the same intensity as

anthracite and B.C.G. The reaction was similar except that in the case of rats the maximum fibrosis was present at 365 days, after which the lesions showed the same tendency to regress. In guinea-pigs the maximum fibrosis was at 240 days. This slight difference in the time at which the maximum degree of fibrosis was found in the lungs of rats with mine dust and B.C.G. and anthracite coal and B.C.G. is very probably a mere experimental variation which is not statistically significant. Kaolin and B.C.G. produced an even more marked reaction in the lungs of rats than did the other dusts when injected with organisms. At 180 days the maximum fibrosis was present and there were large areas with much collagen (Grade 3 fibrosis) in rats, while in guinea-pigs the lesions were slightly less advanced, being within the maximal limits of Grade 2 fibrosis. Thereafter, in both species of animals the lesions began to regress and by the end of the experiment (500 days in rats and 365 days in guinea-pigs), there remained only a mild alveolar fibrosis as evidence of the earlier severe lesions.

It is apparent from the foregoing facts that the influence of coal, mine dust and kaolin on avirulent tuberculous infection produced by dead B.C.G. in the lungs of rats and guinea-pigs, is such that the lesions are aggravated by them. The combined action of any one of these dusts and dead B.C.G. produced more marked lesions in the lungs than any of these substances alone in the same period of time. For instance, coal dust and mine dust did not produce any permanent pulmonary lesions by themselves, and kaolin and B.C.G. alone produced mild

reticulinosis only, while the combination of coal dust or mine dust with B.C.G. produced definite fibrotic lesions (Grade 2) and kaolin and B.C.G. together produced collagenous lesions of Grade 3 severity. Thus there is no doubt that dust and dead tubercle bacilli influence each other in such a way that one enhances the lesions produced by the other.

A striking feature in all the experiments where dust and organisms were injected simultaneously, is the regularity with which the severe reactions present in the lungs at one stage gradually disappeared during the later stages of the experiment. In all cases the lesions became more pronounced during the early period of the experiment and the maximum fibrosis was noted between 180-240 days, after which the lesions showed signs of regression till, at the termination of the experiment at 365 or 500 days, no fibrosis was evident. It would seem from these findings that fibrous tissue is capable of undergoing partial resolution spontaneously in certain cases. Collagen fibres which were plentiful at the peak period when the fibrosis was at the maximum were no longer found in any of the lesions examined in the later stages of the experiment. The fact that this was repeatedly found in every experiment involving the use of dust and organisms together is of significance and cannot be dismissed as a chance occurrence. Although it is not possible to offer a wholly satisfactory explanation for the partial resolution of the lesions produced by the combined action of dust and organisms, the study of the nature of the stimulus producing these lesions may yield some information. It is presumably due to the disappearance of the

stimulus which, in this case, may have occurred in one of two ways. Since the stimulus concerned was in the form of dead bacilli, it is unlikely that its action would have been continuous, as it might have been with living organisms, which have been found to flourish in dust lesions, so supplying the continued stimulus necessary for the progression of the fibrotic lesions. The stimulus afforded by dead organisms, on the other hand, would have acted at one stage when it was introduced into the tissues, persisted for a while, and then have ceased to act. Another possibility is that the disappearance of the stimulus by the removal or dissolution of the dead bacilli from the lungs may be responsible for the partial resolution of the lesions. There is some evidence that the bacilli are being removed from the lungs during the later stages of the experiment. Examination of lesions for organisms (under fluorescent light) during the period when the fibrosis was maximum, and again when partial resolution had taken place, revealed a definite difference in the number present. Even at the time when the lesions were severe bacilli were scanty, being only about 6-10 in the whole lung field examined (approximately 3 sq. cm.), but towards the end of the experiments, when partial resolution had taken place, the numbers were still further reduced, being about 2-4 in a lung field of approximately the same area as before. This is convincing evidence that bacilli are disappearing from the lungs, but only very slowly, and agrees with Kettle's (1934) findings in part. Thus it is likely that, with the disappearance of the bacilli, the stimulus to the further progress of the lesions is withdrawn, and partial resolution takes place.

These findings are in agreement with those of Kettle (1934) up to a point. He found that kaolin and dead B.C.G. by themselves did not produce any permanent lesions in the lungs of guinea-pigs, but when they were injected together produced collagenous lesions very similar to the infective silicotic nodules seen in human beings. The maximum fibrosis was found at 112 days and was still present at 141 days. No descriptions are available of the lesions found beyond this period. It is possible that if his experiments with dust and organisms had been continued for a longer period, results similar to those found in the present series of experiments may have been obtained, i.e. partial resolution of lesions. With regard to his studies on the action of dead B.C.G. alone, he found that the organisms "disappeared altogether in the later stages of the experiment", but no definite time is mentioned. In these experiments, however, the organisms were demonstrable even as late as 500 days after injection, in the case of rats, although they were very scanty, and would probably have disappeared entirely soon after. In the case of guinea-pigs it was very difficult to demonstrate the organisms at any stage.

Cummins (1940) studied the action of anthracite coal and slate dust with dead tubercle bacilli on the lungs of rabbits. He found that the combined action of dust and organisms produced more "extensive lesions" than dust alone. His experiments, again, were conducted up to a period of 180 days only, and it is just possible that had they been continued longer, the lesions may have resolved partially as in the series under discussion.

These experiments definitely prove that the combined action of dust and tubercle bacilli (dead) of low virulence on the lungs of rats and guinea-pigs produces more severe lesions than either the dust or the organisms alone in the same period of time. The lesions reach the maximum degree of fibrosis and after some time partial resolution takes place. These findings are in agreement with those of Kettle (1934) and Cummins (1940), who also found that dust and organisms together produced more reaction in the lungs than either factor alone.

SUMMARY

The influence of coal, mine dust and kaolin on avirulent tuberculous infection produced by dead B.C.G. in the lungs of rats and guinea-pigs has been studied by the intra-tracheal injection of these substances alone and in combination. The reactions produced by the combinations of these substances were similar in the two species of animals and did not seem to have been affected by their different degrees of susceptibility to tuberculosis.

B.C.G. alone produced mild lesions in rats and guinea-pigs; they appeared at 30 days and persisted till 500 days, although at this stage they were scanty and smaller in size. Mine dust did not produce any pulmonary fibrosis in as long a period of time as 500 days in rats and 365 days in guinea-pigs. Kaolin produced minimal fibrotic lesions (Grade 1) at 30 days and these did not progress any further, even up to 500 days.

Mine dust and B.C.G. produced definite fibrosis which reached its maximum (Grade 2) at 365 days in rats and 240 days in guinea-pigs; thereafter, the lesions underwent partial resolution. Anthracite coal and B.C.G. produced lesions essentially similar to the foregoing, which reached the maximum severity (Grade 2) between 180-240 days in both species and then resolved partially. With kaolin and B.C.G. the reaction was more marked in rats than in guinea-pigs. In both, the maximum fibrosis (Grade 3 and Grade 2 respectively) was reached at 180 days, the lesions diminishing in severity beyond that period.

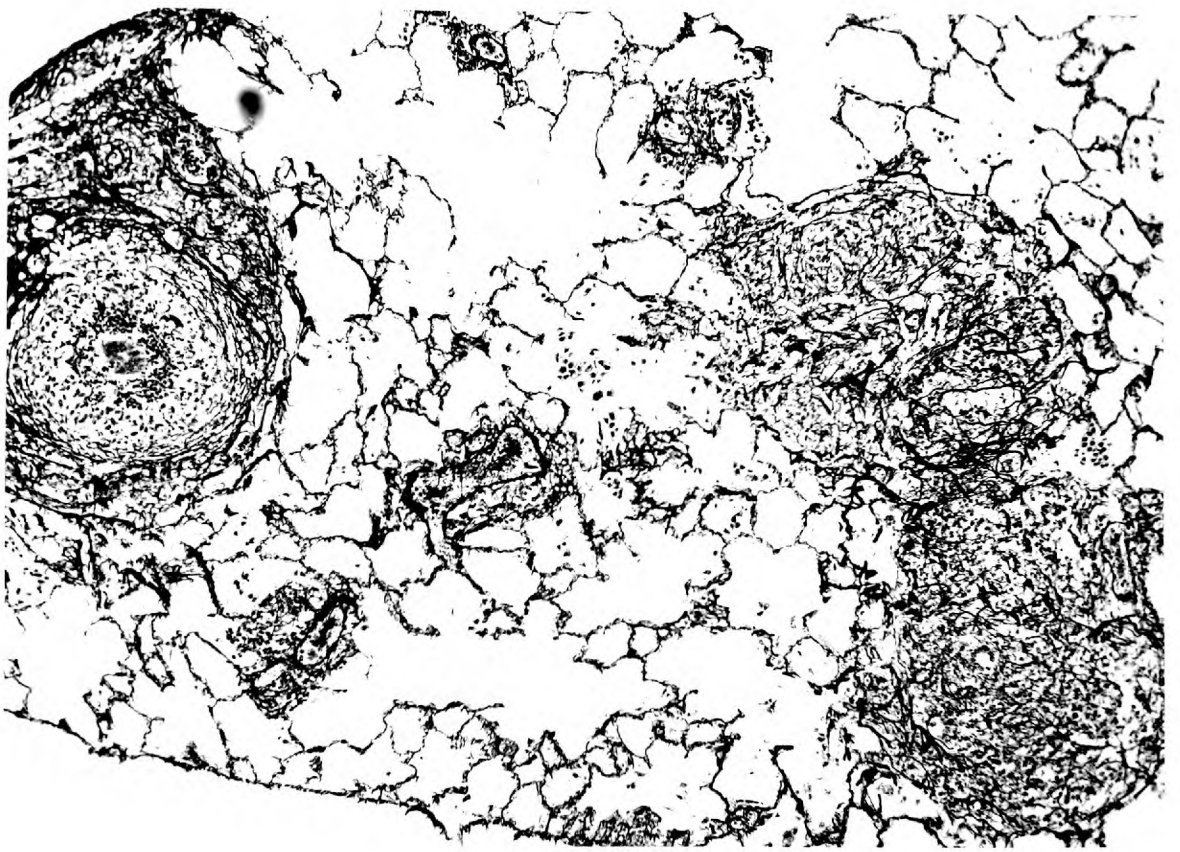


Fig. I. Rat lung, 30 days after the injection of 1.5 mg. of dead B.C.G. Nodular lesions with a loose network of reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 100.)

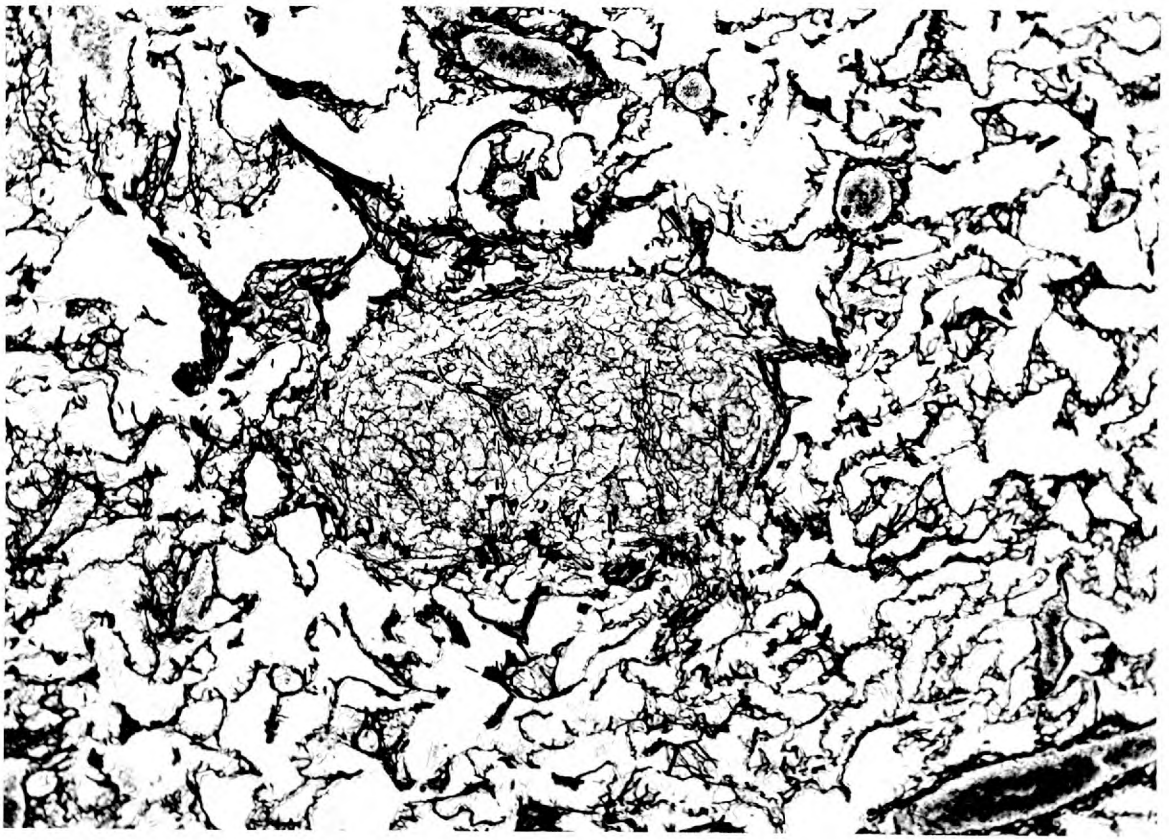


Fig. 2. Rat lung, 150 days after the injection of 1.5 mg. of dead B.C.G. Nodular lesion with loosely woven reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 130).

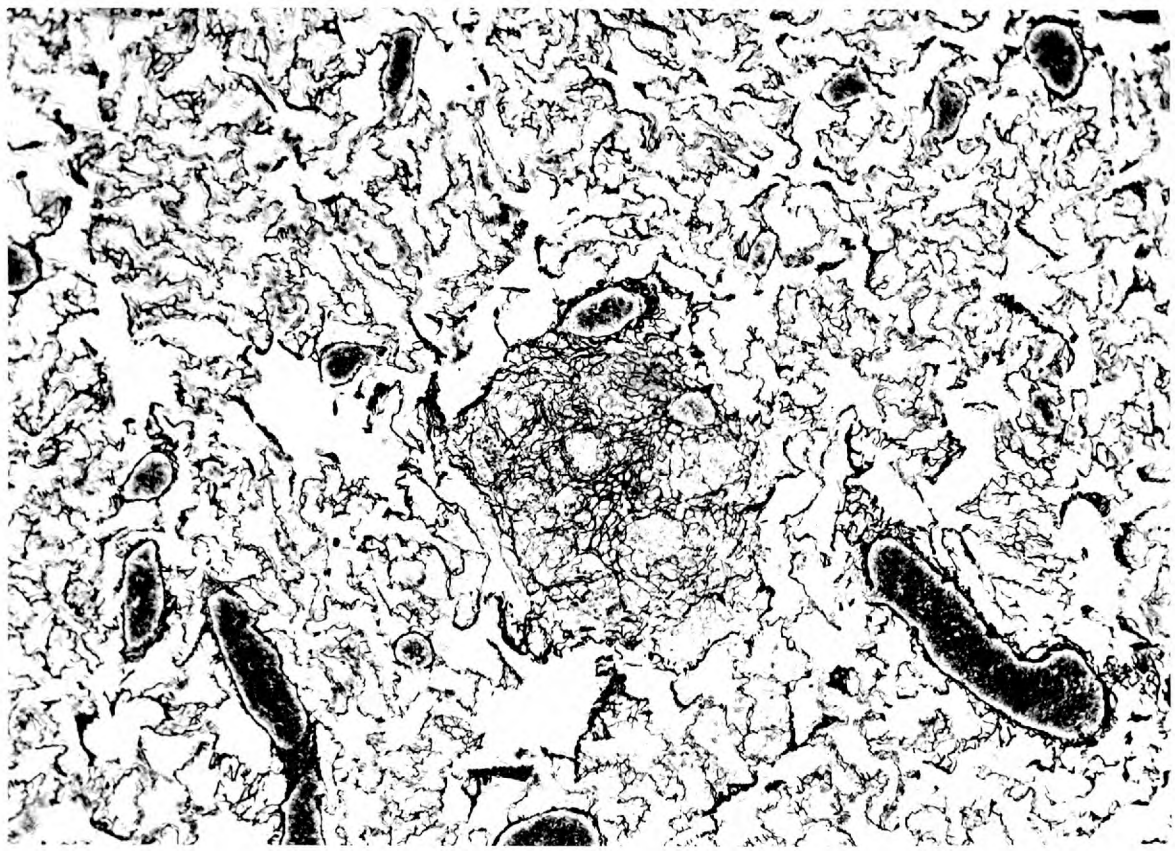


Fig. 3. Rat lung, 300 days after the injection of 1.5 mg. of dead B.C.G. Small nodular lesion with a loose reticulin network (grade 1 fibrosis). Silver impregnation. (X. 130).

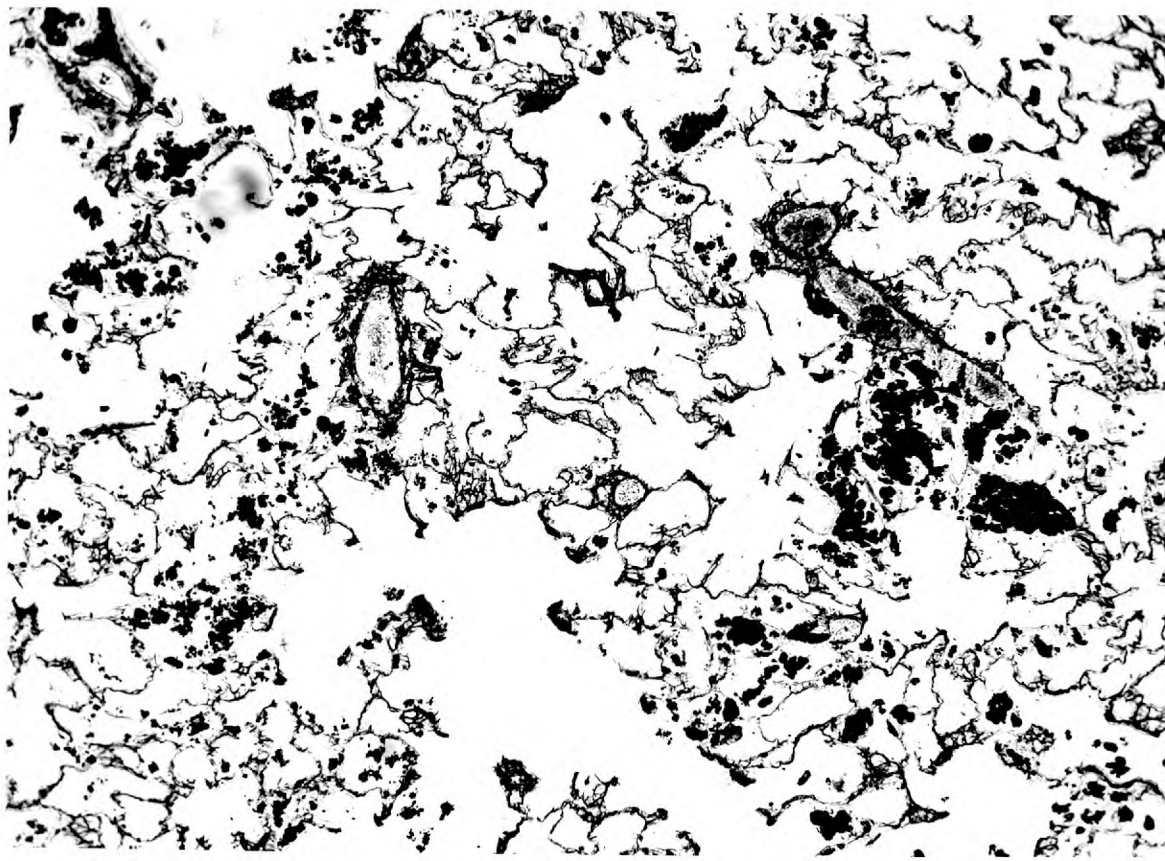


Fig. 4. Rat lung, 30 days after the injection of 75 mg. of coal-mine dust. Diffuse distribution of dust particles with an occ sional small aggregation. No fibrosis. Silver impregnation. (X. 100).

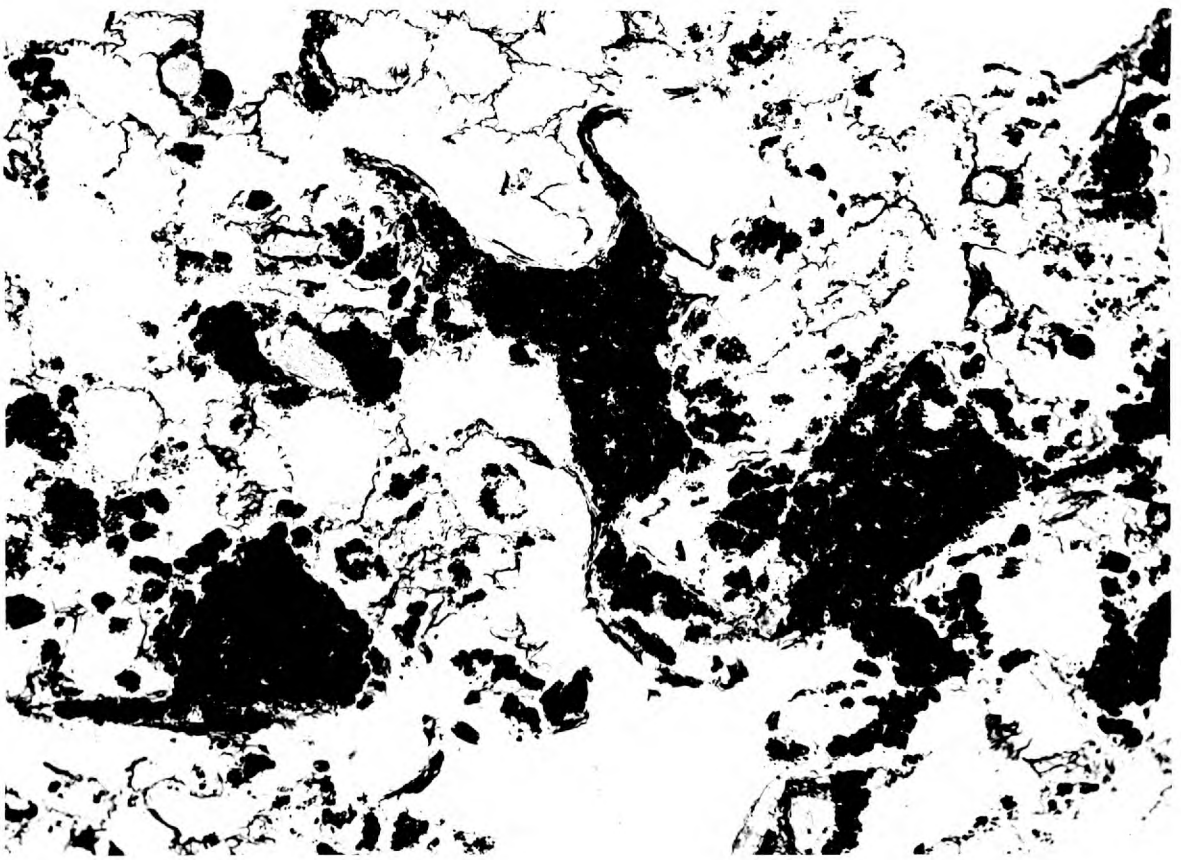


Fig. 5. Rat lung, 180 days after the injection of 75 mg. of coal-mine dust. Dust collected into dense aggregations without any fibrosis. Silver impregnation. (X. 130).

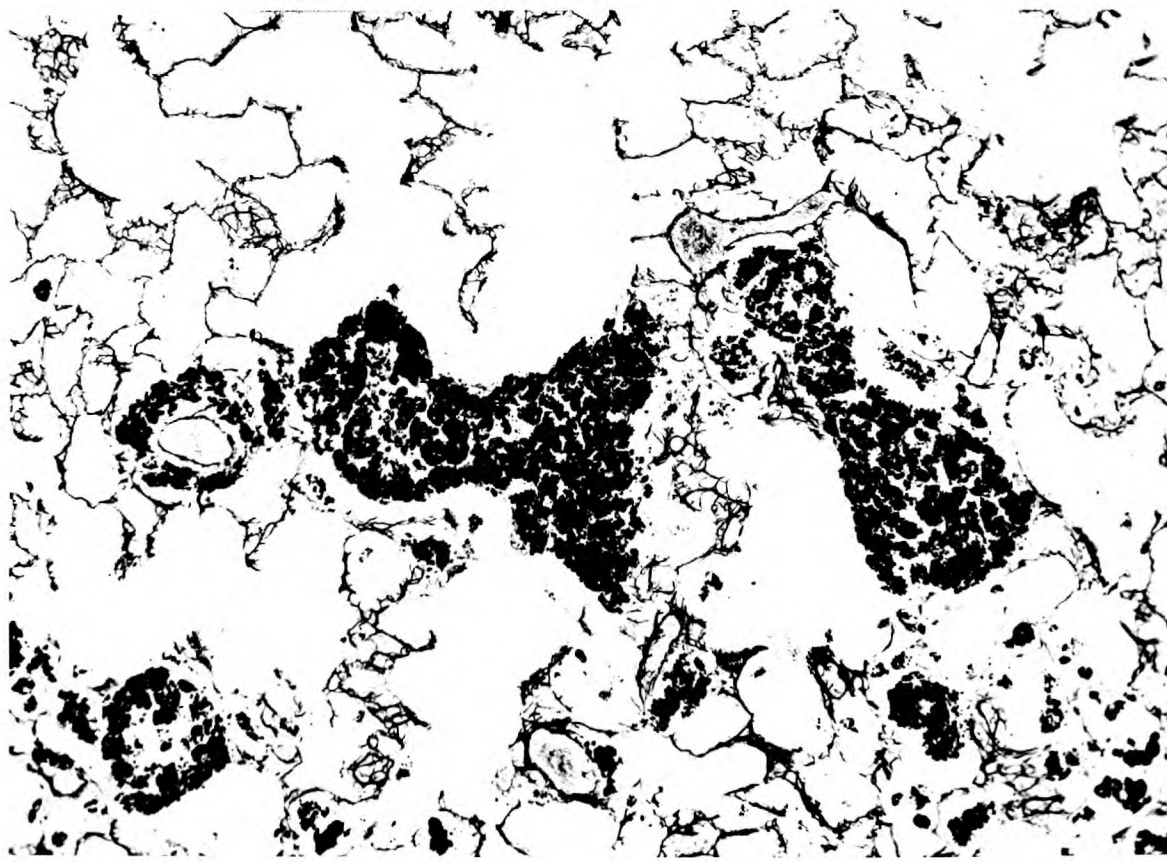


Fig. 6. Rat lung, 500 days after the injection of 75 mg. of coal-mine dust. Dust tightly packed together into focal aggregations with a few reticulin fibres around them, but no fibrosis. Silver impregnation. (X. 130).

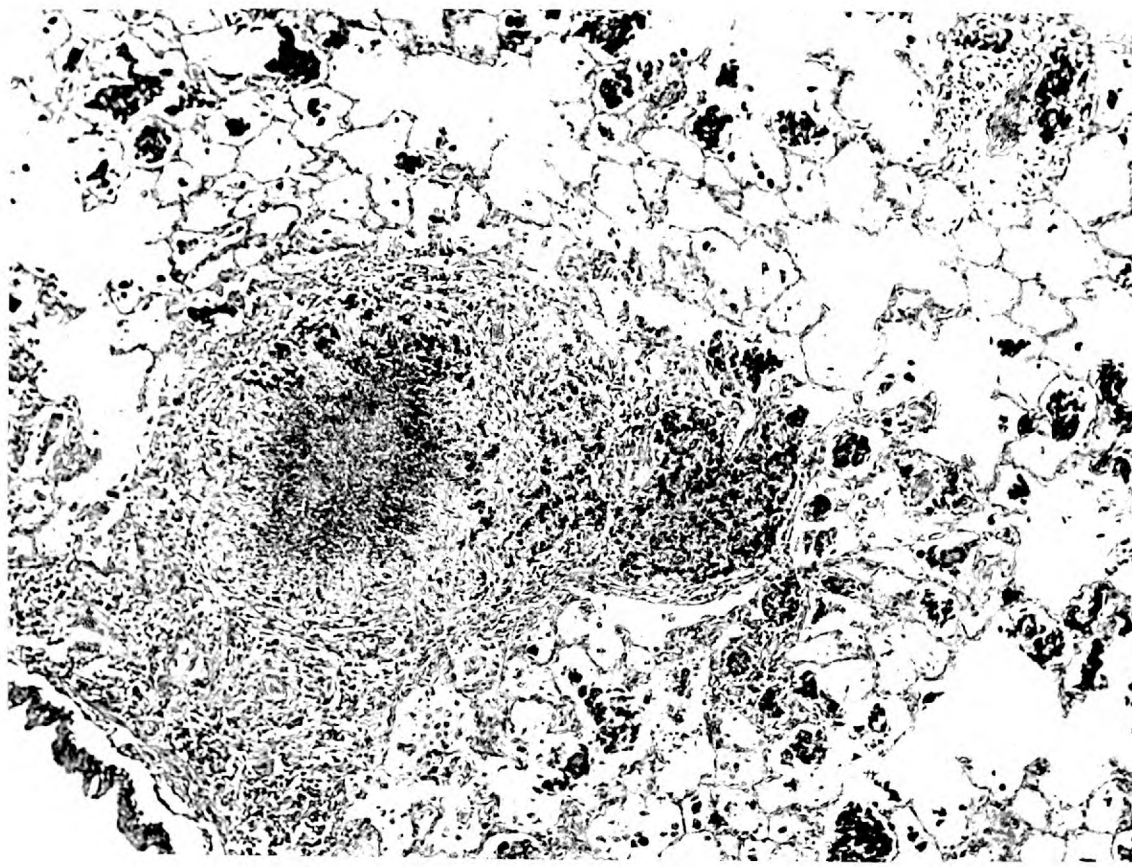


Fig. 7. Rat lung, 30 days after the injection of 75 mg. of coal-mine dust and 1.5 mg. of dead B.C.G. Nodular lesion composed of dust cells and epitheliod cells, due to the combined action of dust and organisms. Haematoxylin and eosin. (X. 90).

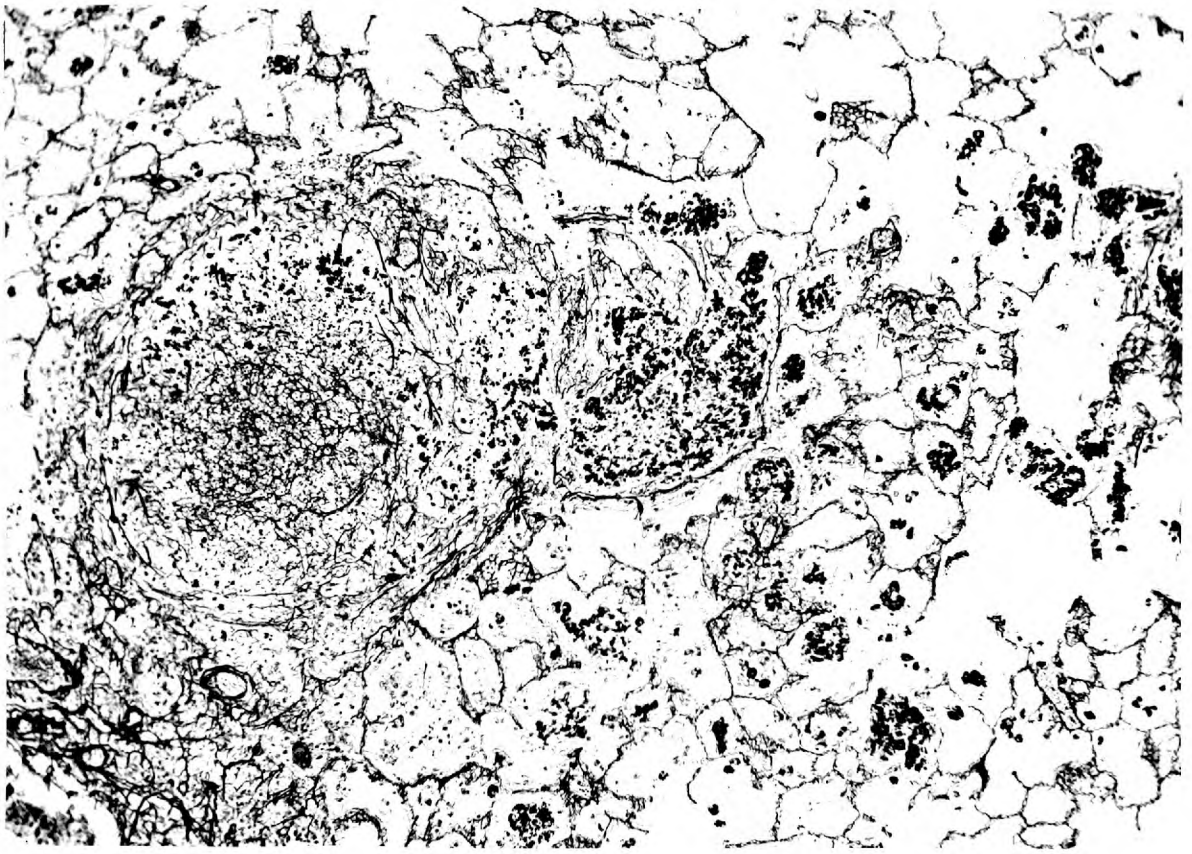


Fig. 8. Rat lung, 30 days after the injection of 75 mg. of coal-mine dust and 1.5 mg. of dead B.C.G. Same area as in Fig. 7. Combined dust and infective lesion with loose network of fine reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 100)

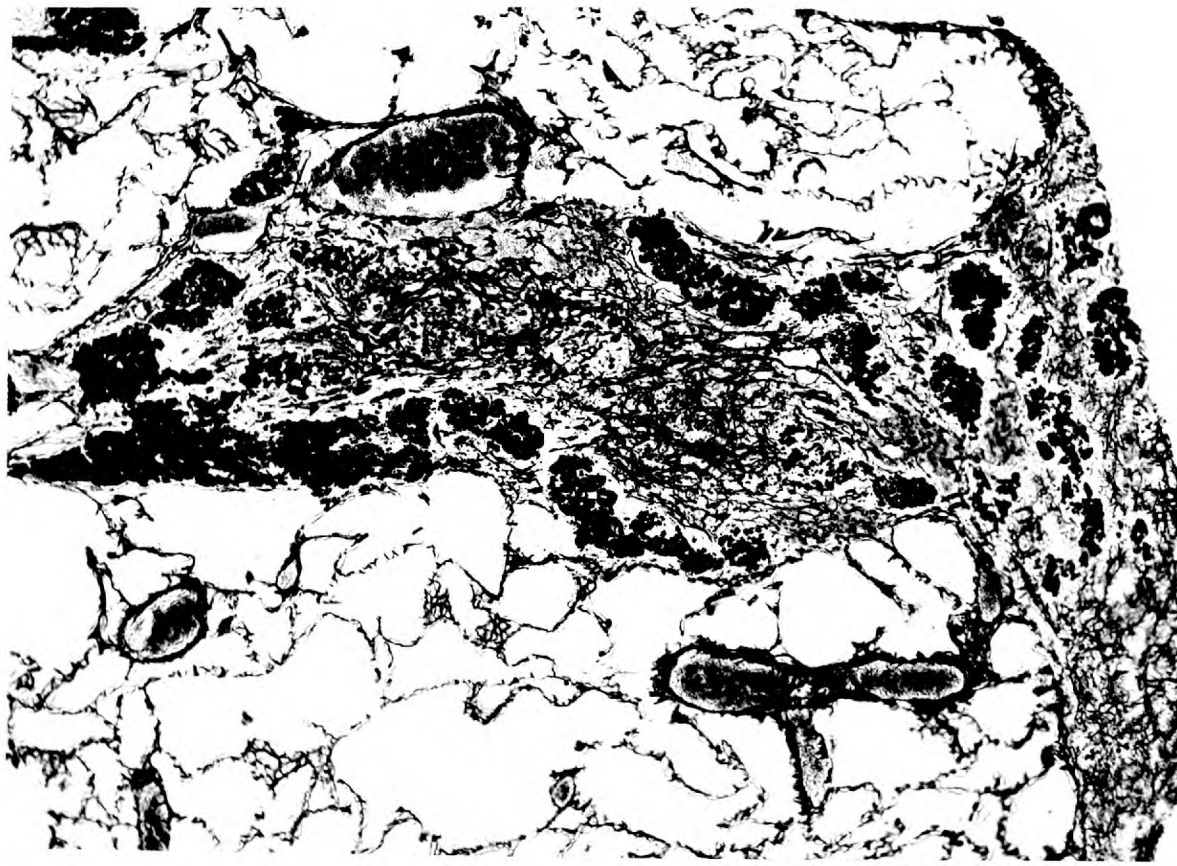


Fig. 9. Rat lung, 180 days after the injection of 75 mg. of coal-mine dust and 1.5 mg. of dead B.C.G. Combined dust and infective lesion with thick reticulin fibres (grade 2 fibrosis, minimal). Silver impregnation. (X.130).

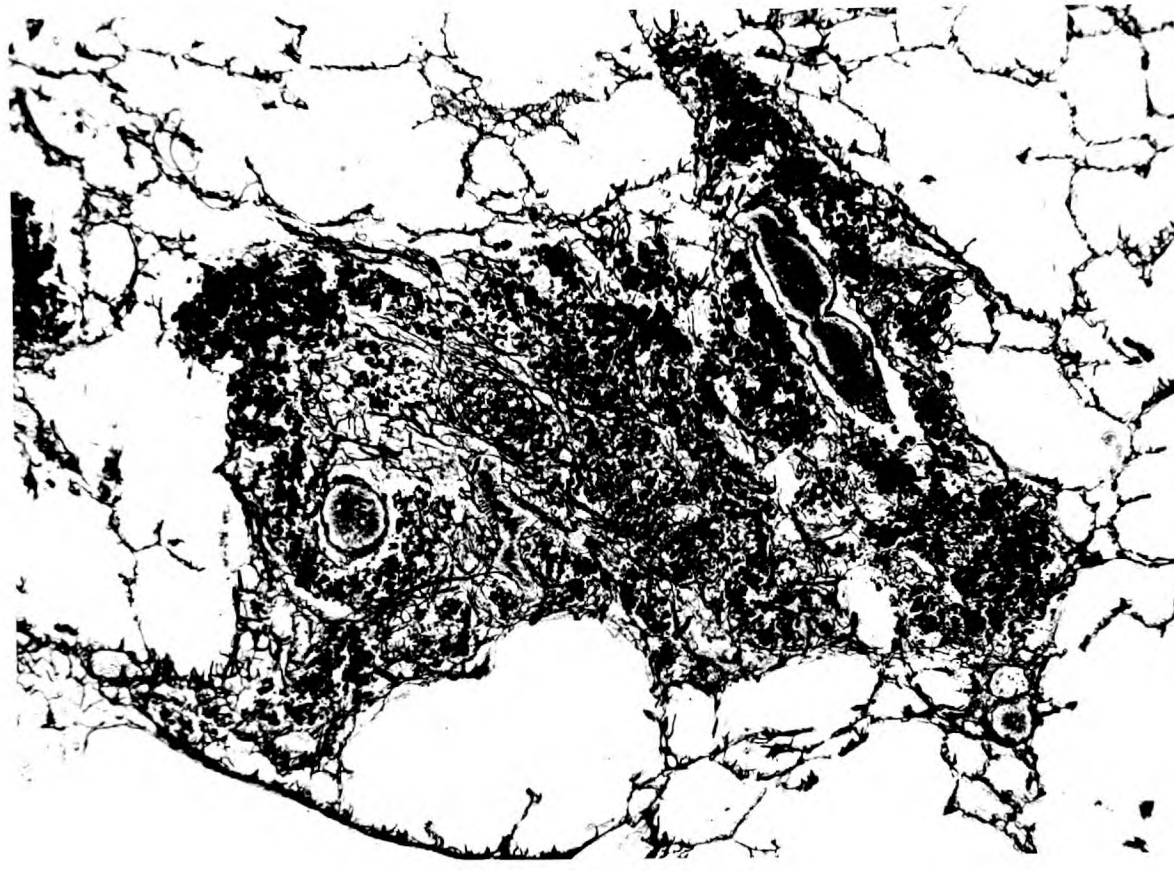


Fig. 10. Rat lung, 365 days after the injection of 75 mg. of coal-mine dust and 1.5 mg. of dead B.C.G. Combined dust and infective lesion with thick and compact reticulin fibres (grade 2 fibrosis, maximal). Silver impregnation. (X. 130).

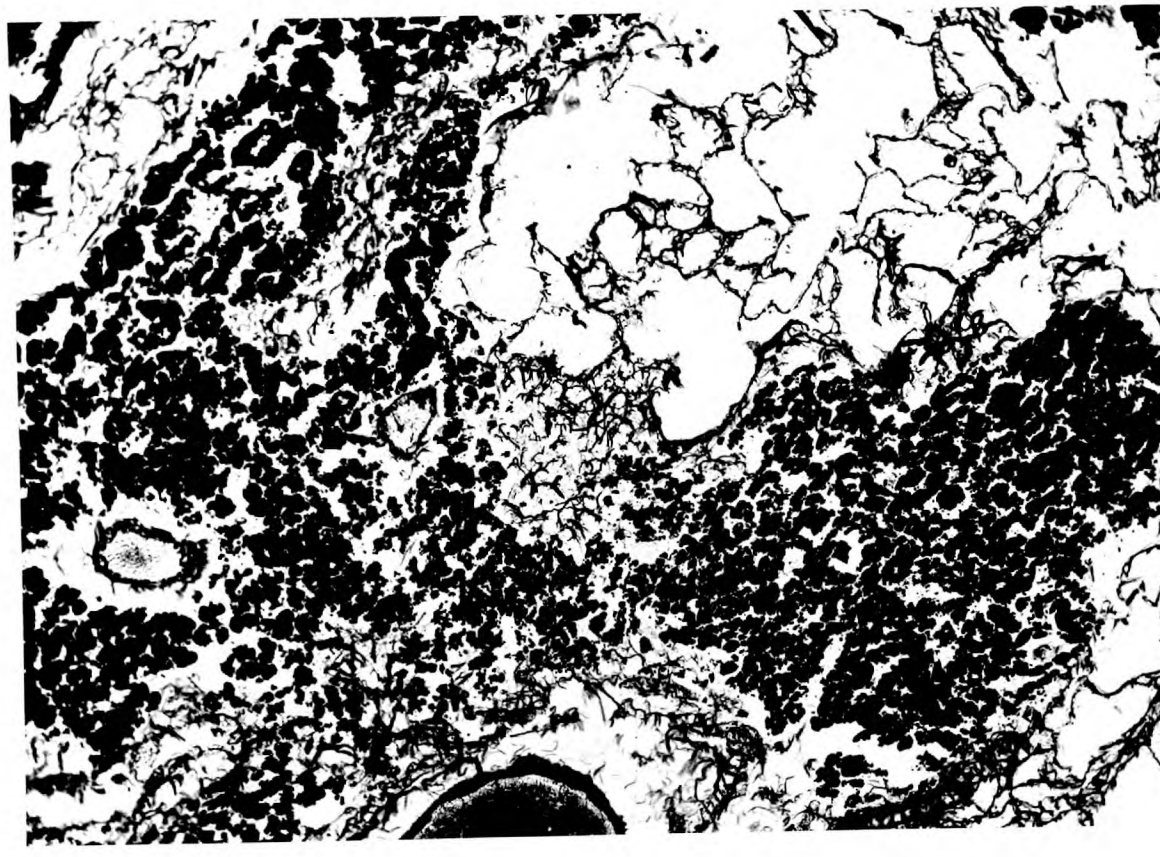


Fig. 11. Rat lung, 477 days after the injection of 75 mg. of coal-mine dust and 1.5 mg. of dead B.C.G. Collections of dust with mild thickening of the surrounding alveolar walls, but no fibrosis. Silver impregnation. (X. 130).

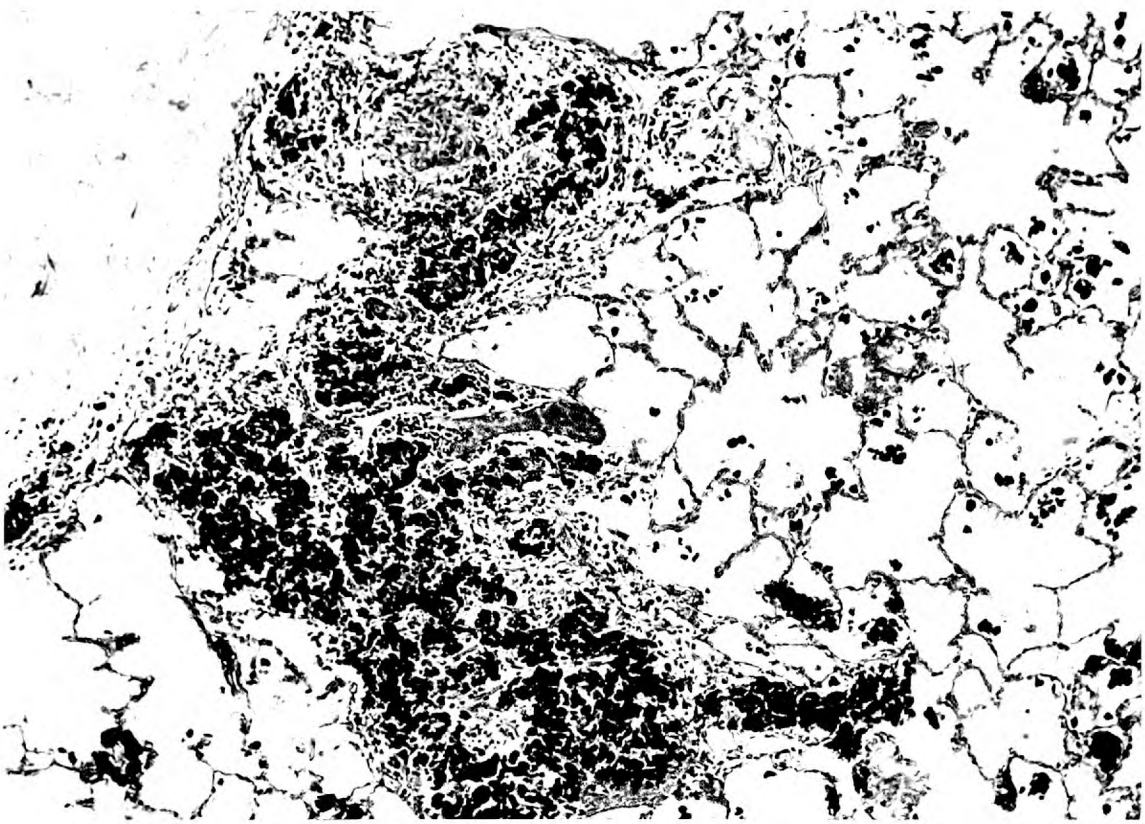


Fig. 12. Rat lung, 30 days after the injection of 75 mg. of anthracite coal and 1.5 mg. of dead B.C.G. Nodular lesions composed mainly of dust cells and epitheloid cells due to the combined action of dust and organisms. Haematoxylin and eosin. (X. 100).

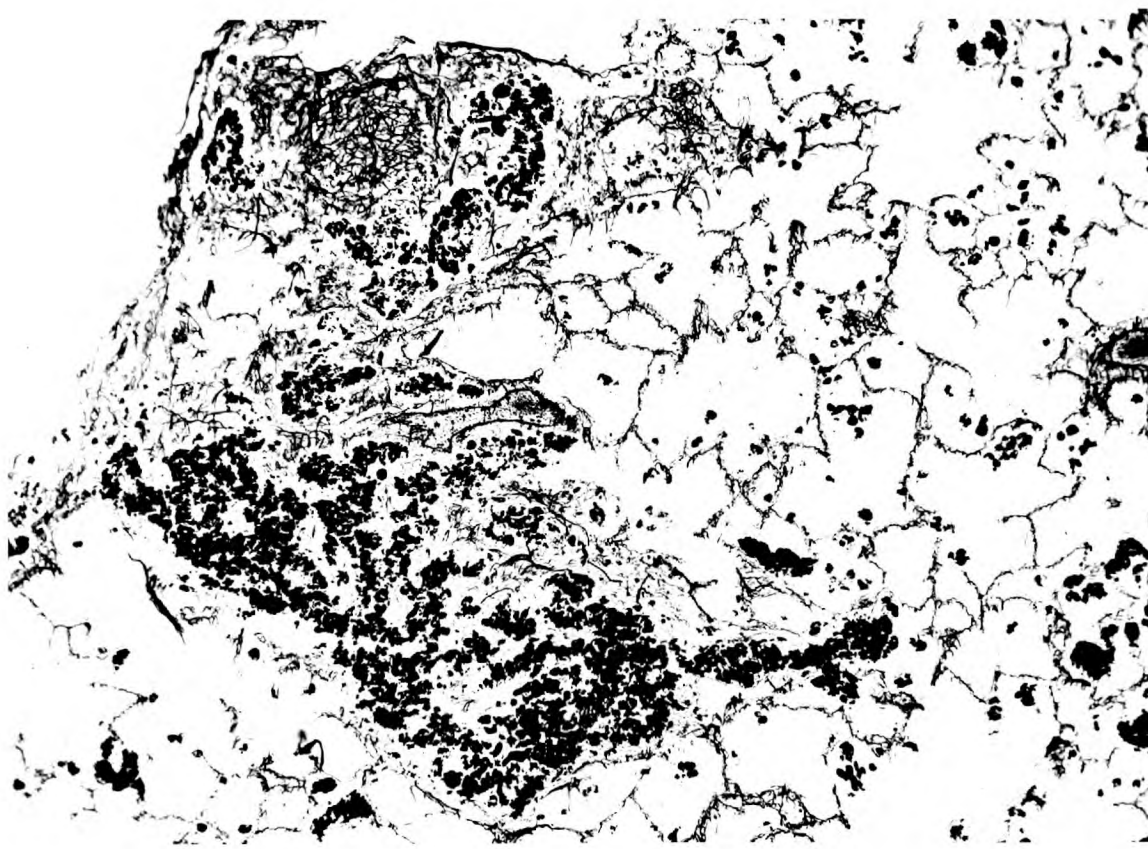


Fig. 13. Rat lung, 30 days after the injection of 75 mg. of anthracite coal and 1.5 mg. of dead B.C.G. Same area as in Fig. 12. Combined dust and infective lesions with a few fine reticulin fibres within (grade 1 fibrosis, minimal). Silver impregnation. (X. 100).

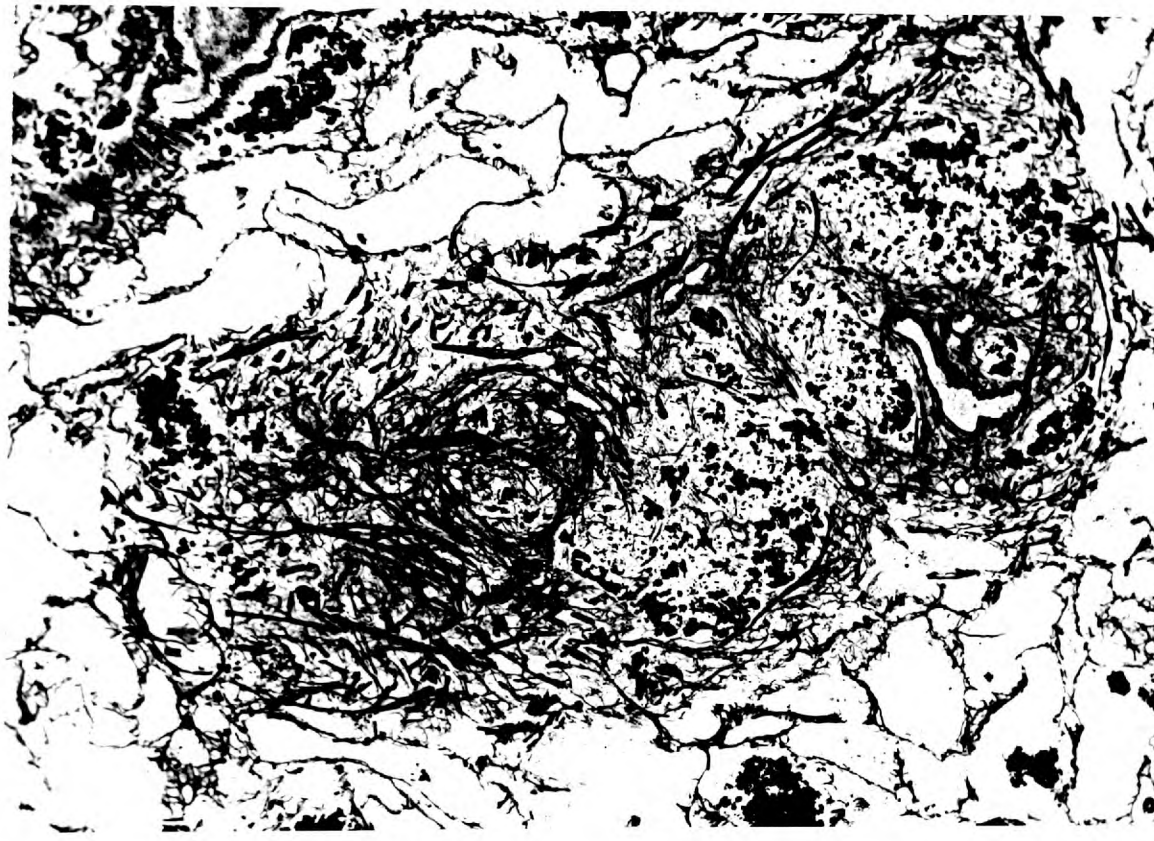


Fig. 14. Rat lung, 240 days after the injection of 75 mg. of anthracite coal and 1.5 mg. of dead B.C.G. Nodular lesions due to dust and organisms, and showing thick and compact reticulin within (grade 2 fibrosis, maximal). Silver impregnation. (X. 130.).

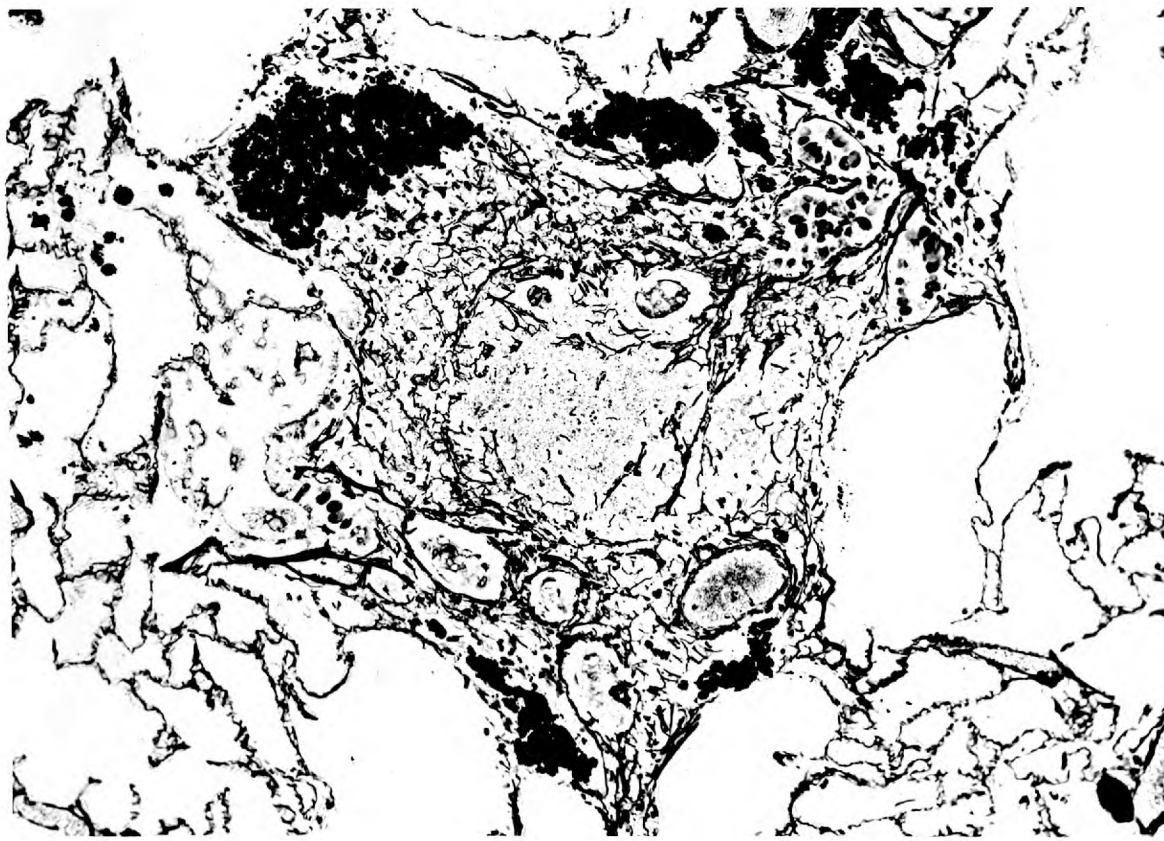


Fig. 15. Rat lung, 500 days after the injection of 75 mg. of anthracite coal and 1.5 mg. of dead B.C.G. Combined dust and infective lesion, much less severe than at 240 days, showing a few fine reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 130).

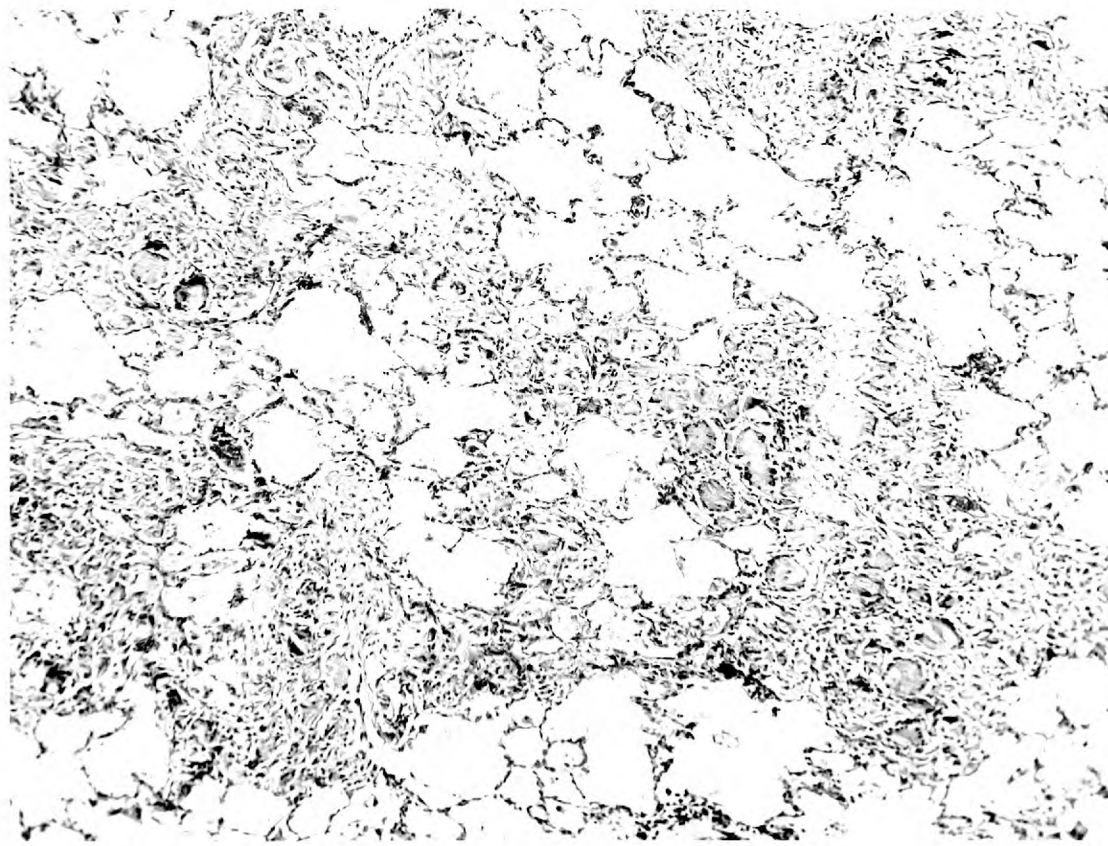


Fig. 16. Rat lung, 30 days after the injection of 75 mg. of Kaolin dust. Diffuse exudative reaction with numerous giant cells containing dust particles and showing commencing aggregation. Haematoxylin and eosin. (X. 90).

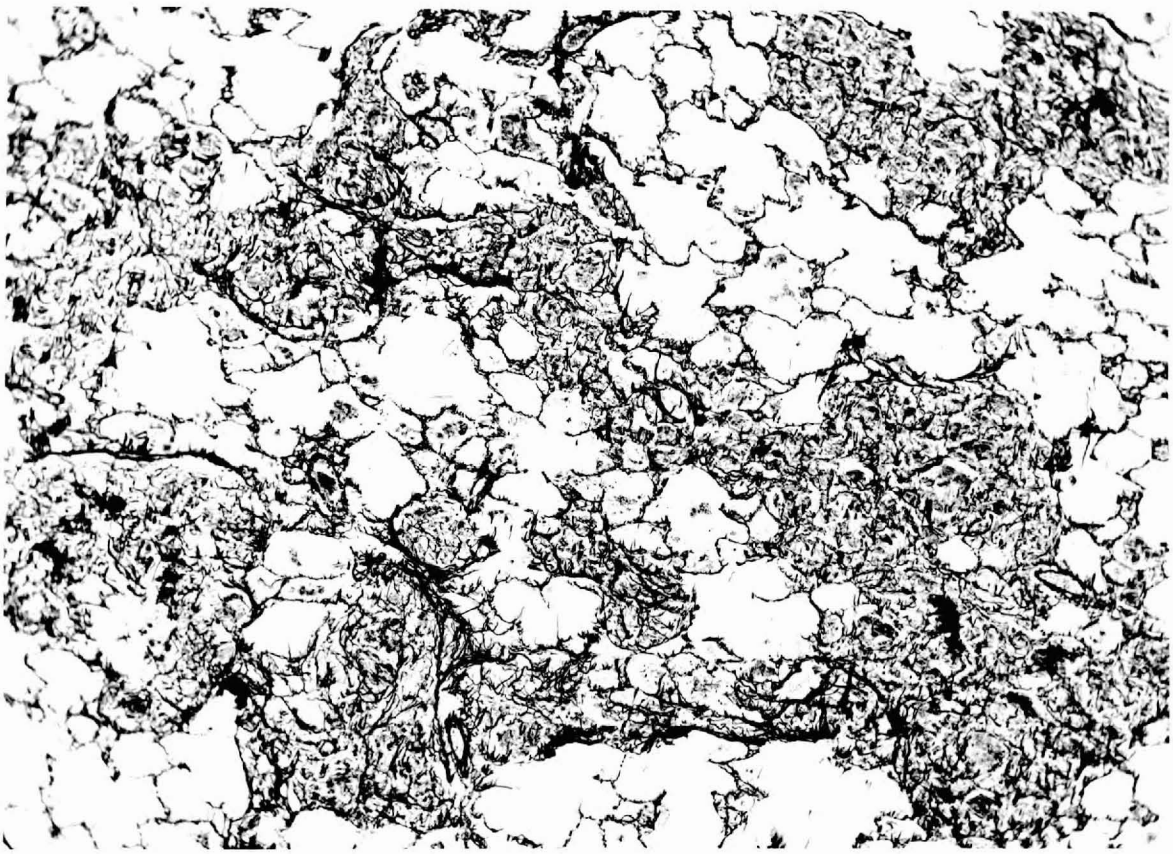


Fig. 17. Rat lung, 30 days after the injection of 75 mg. of Kaolin dust. Same area as Fig. 15. A few fine reticulin fibres are seen within the aggregations (grade 1 fibrosis, minimal). Silver impregnation. (X. 90).

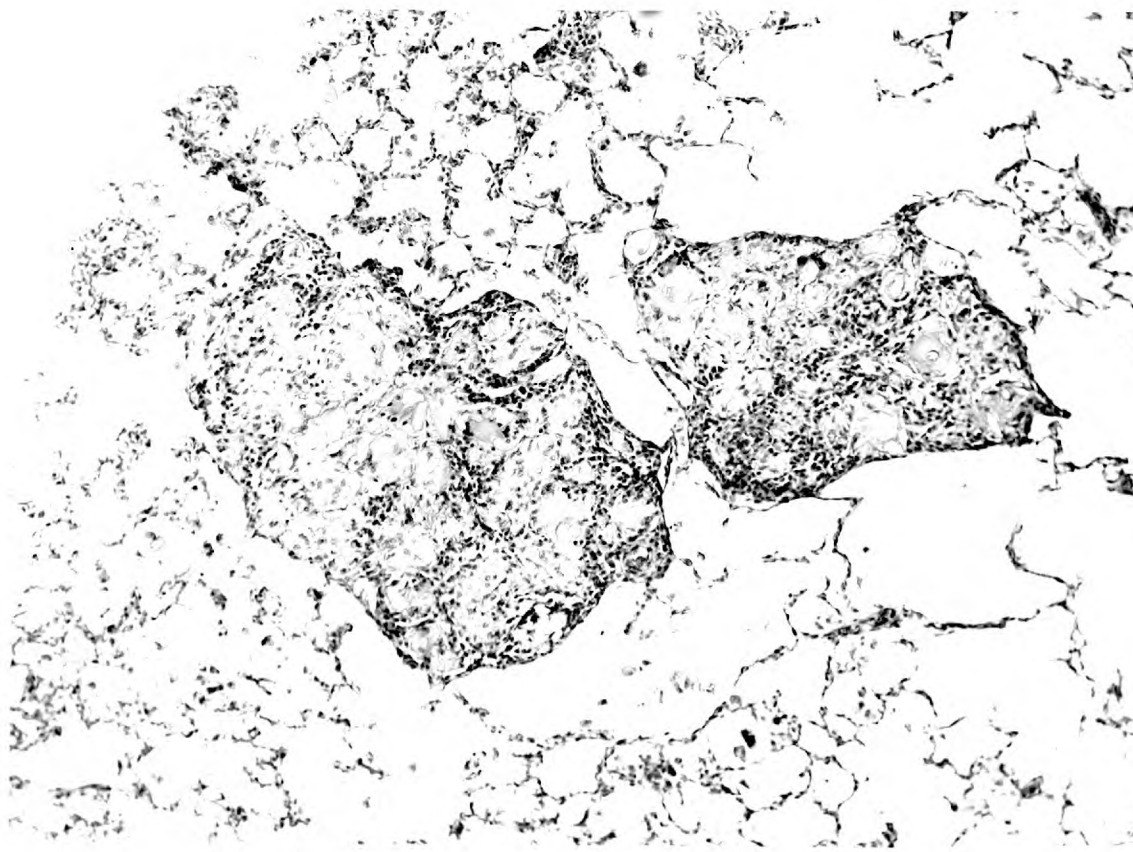


Fig. 18. Rat lung, 180 days after the injection of 75 mg. of Kaolin dust. Nodular collections of giant cells containing dust particles within them. Haematoxylin and eosin. (X. 115).

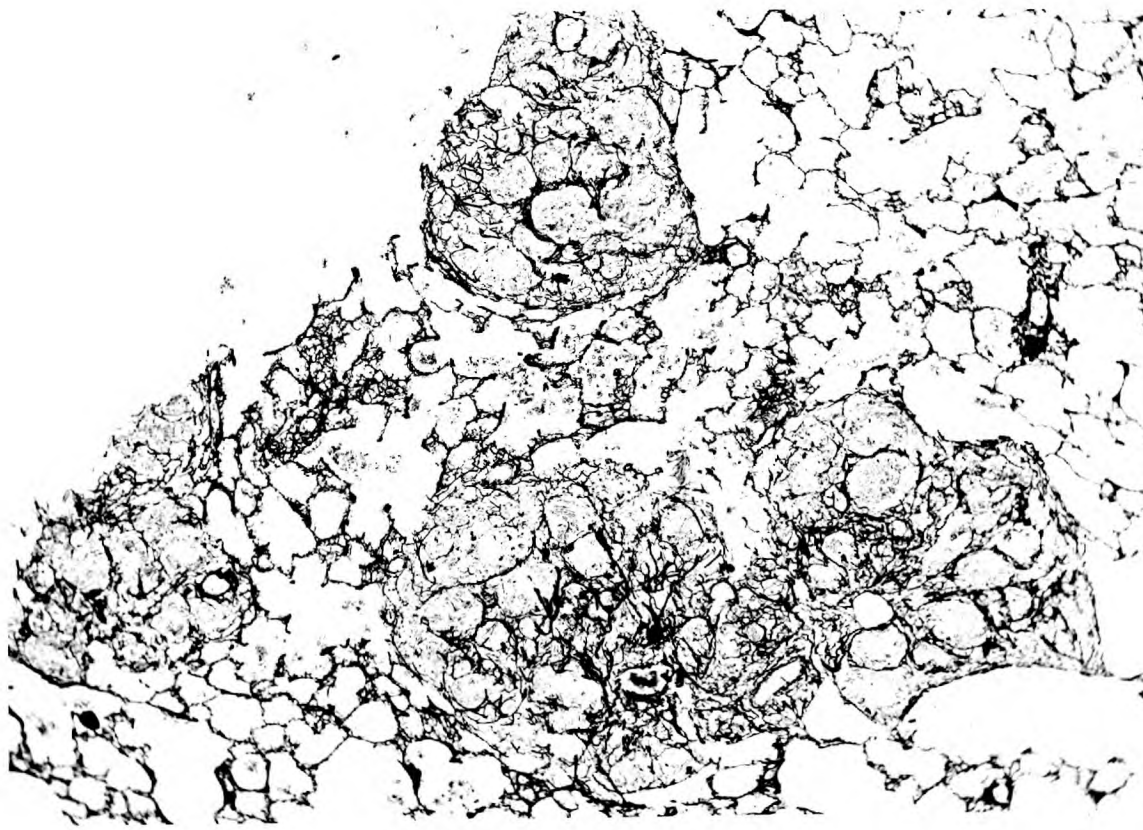


Fig. 19. Rat lung, 180 days after the injection of 75 mg. of Kaolin dust. Same area as Fig. 18. Few reticulin fibres arranged loosely within the nodules (grade 1 fibrosis, minimal). Silver impregnation. (X. 90).

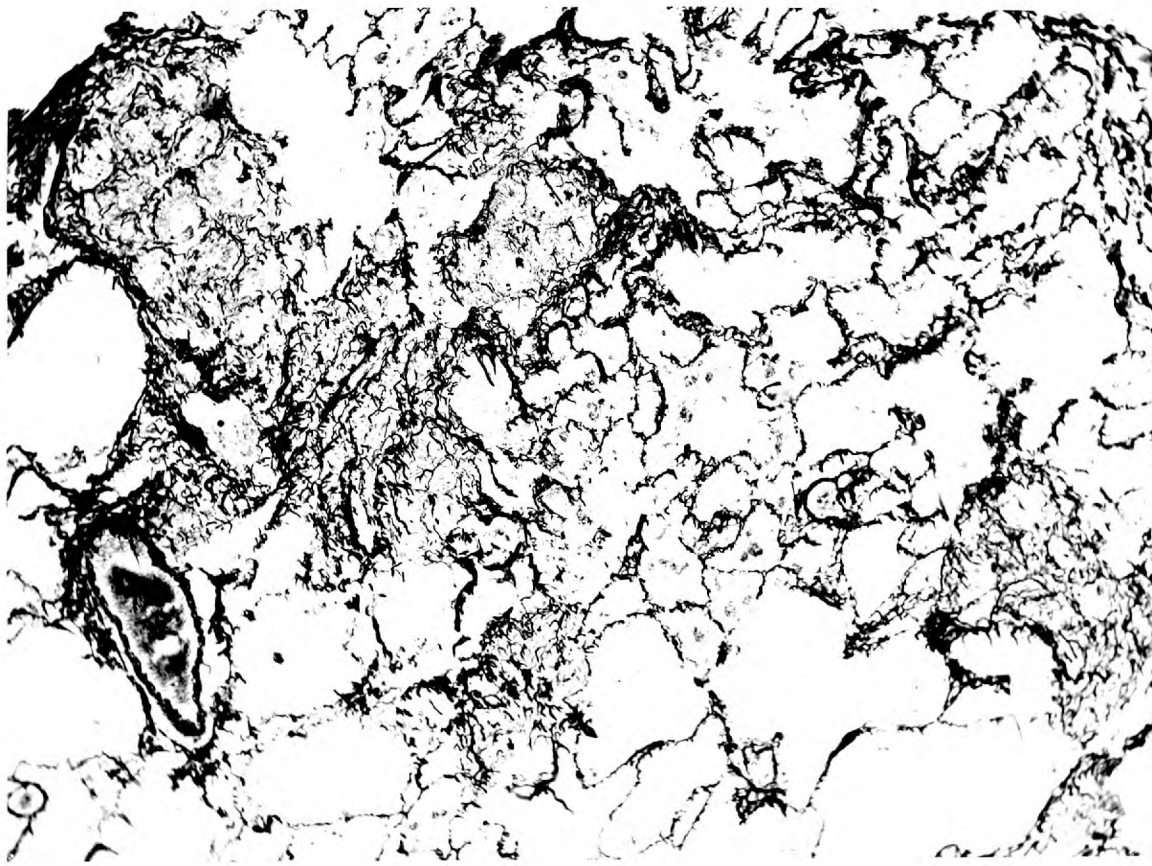


Fig. 20. Rat lung, 500 days after the injection of 75 mg. of Kaolin dust. Nodular lesions with loose reticulin network (grade 1 fibrosis, minimal). Silver impregnation. (X. 100).

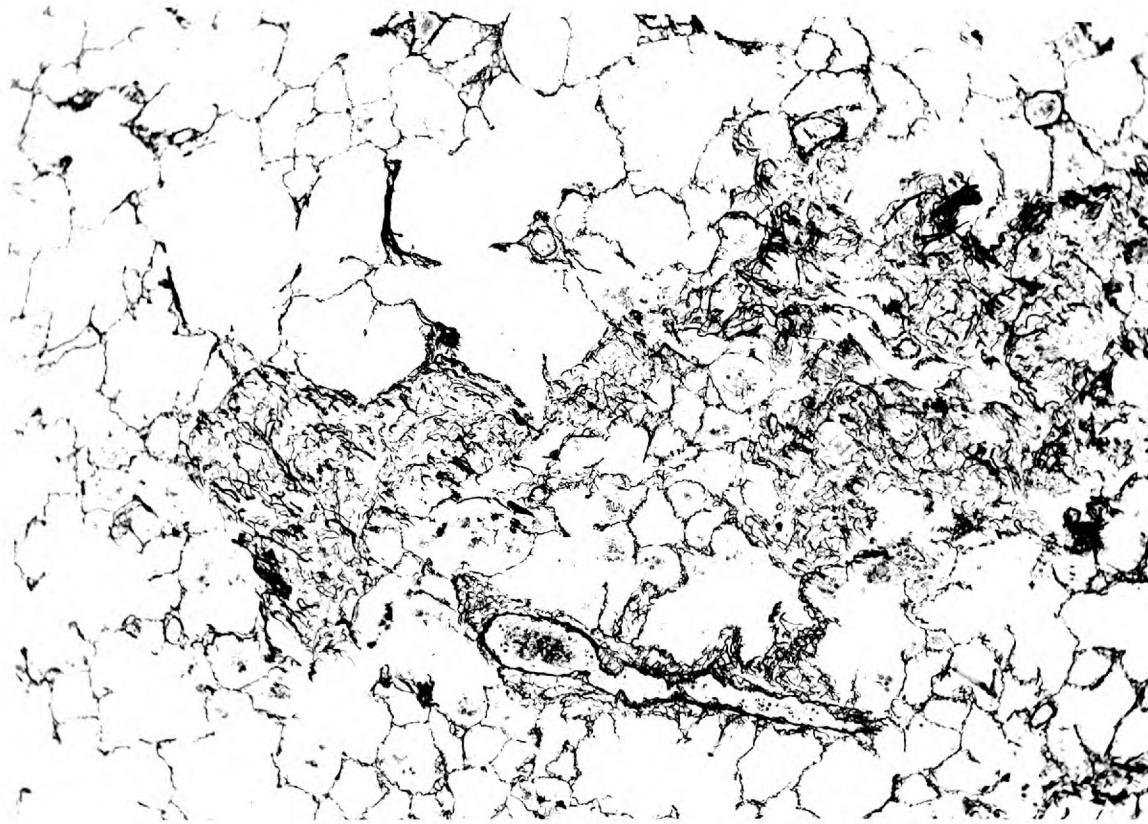


Fig. 21. Rat lung, 30 days after the injection of 75 mg. of Kaolin dust and 1.5 mg. of dead B.C.G. Combined dust and infective lesions with a loose network of reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 100).

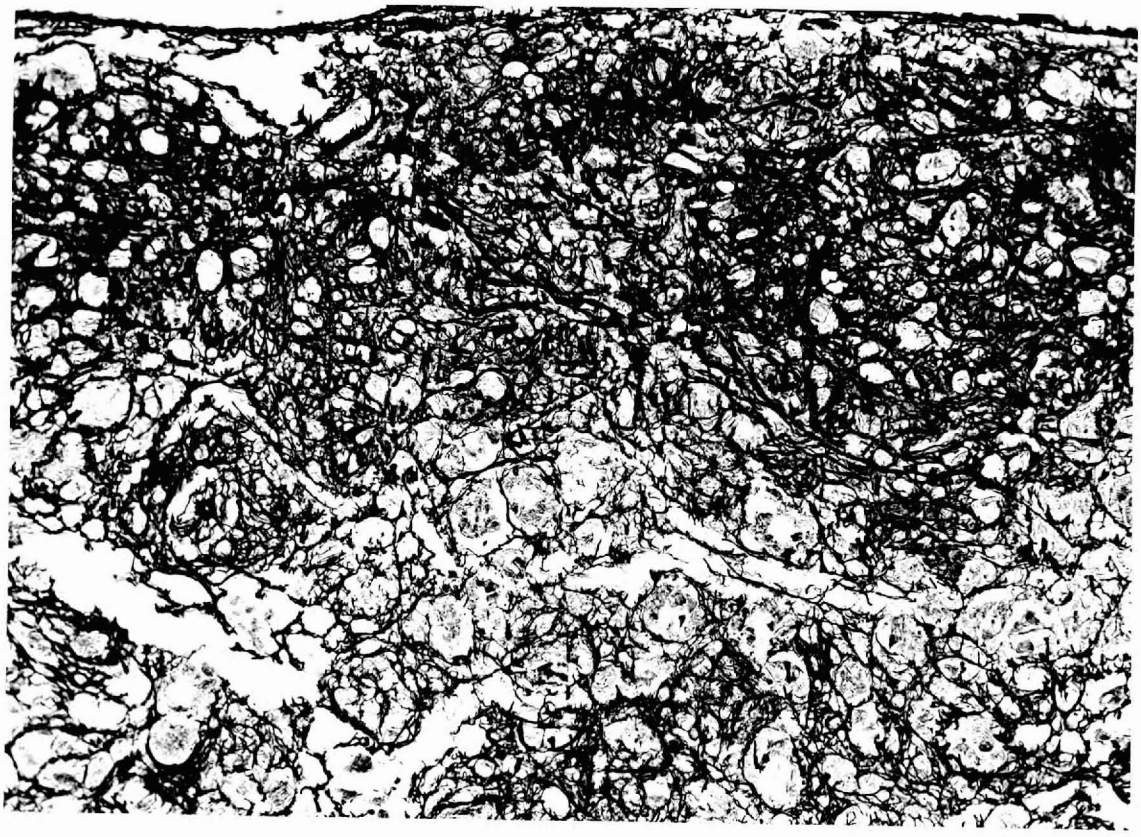


Fig. 22. Rat lung, 180 days after the injection of 75 mg. of Kaolin dust and 1.5 mg. of dead B.C.G. Diffuse lesions due to the combined action of dust and organisms, with thick and compact collagen in them (grade 3 fibrosis). Generalised thickening of alveolar walls in the neighbourhood of the lesions. Silver impregnation (X. 115).

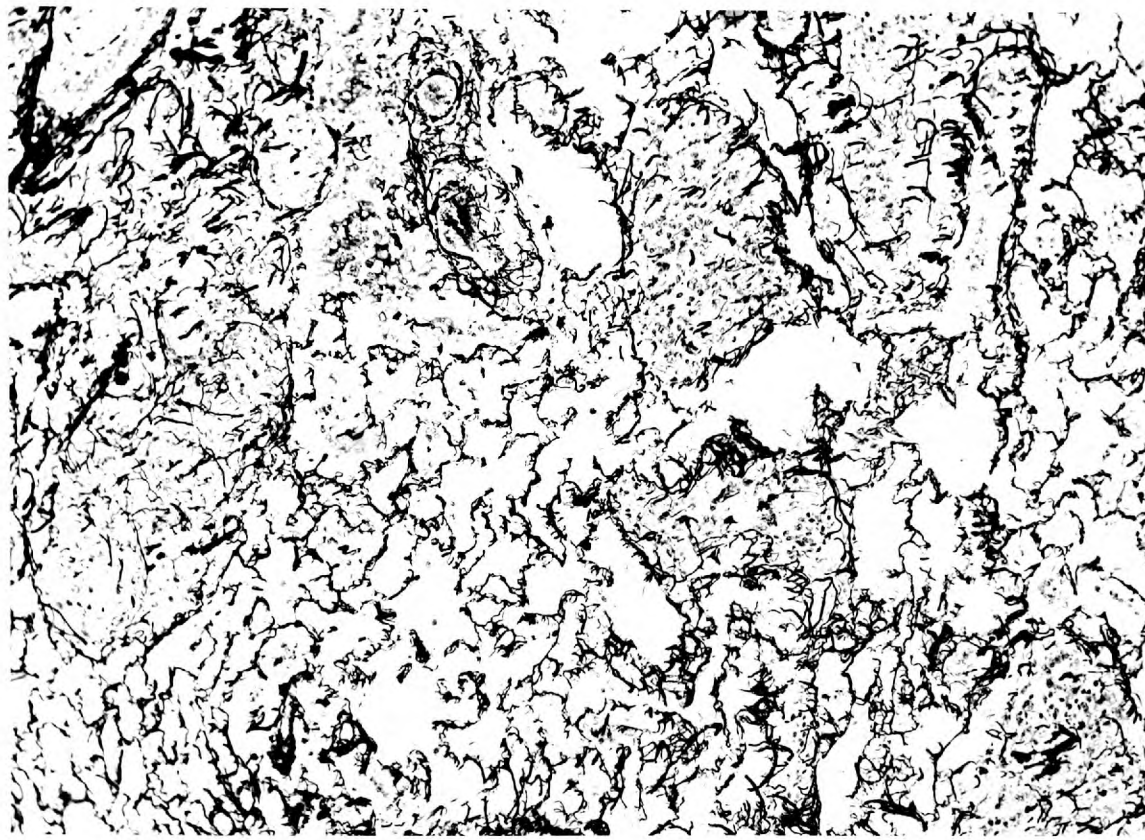


Fig. 23. Rat lung, 500 days after the injection of 75 mg. of Kaolin dust and 1.5 mg. of dead B.C.G. Few reticulin fibres within the nodules and some alveolar fibrosis in the form of a reticular network. Reaction much milder than at 180 days. Silver impregnation. (X. 120).

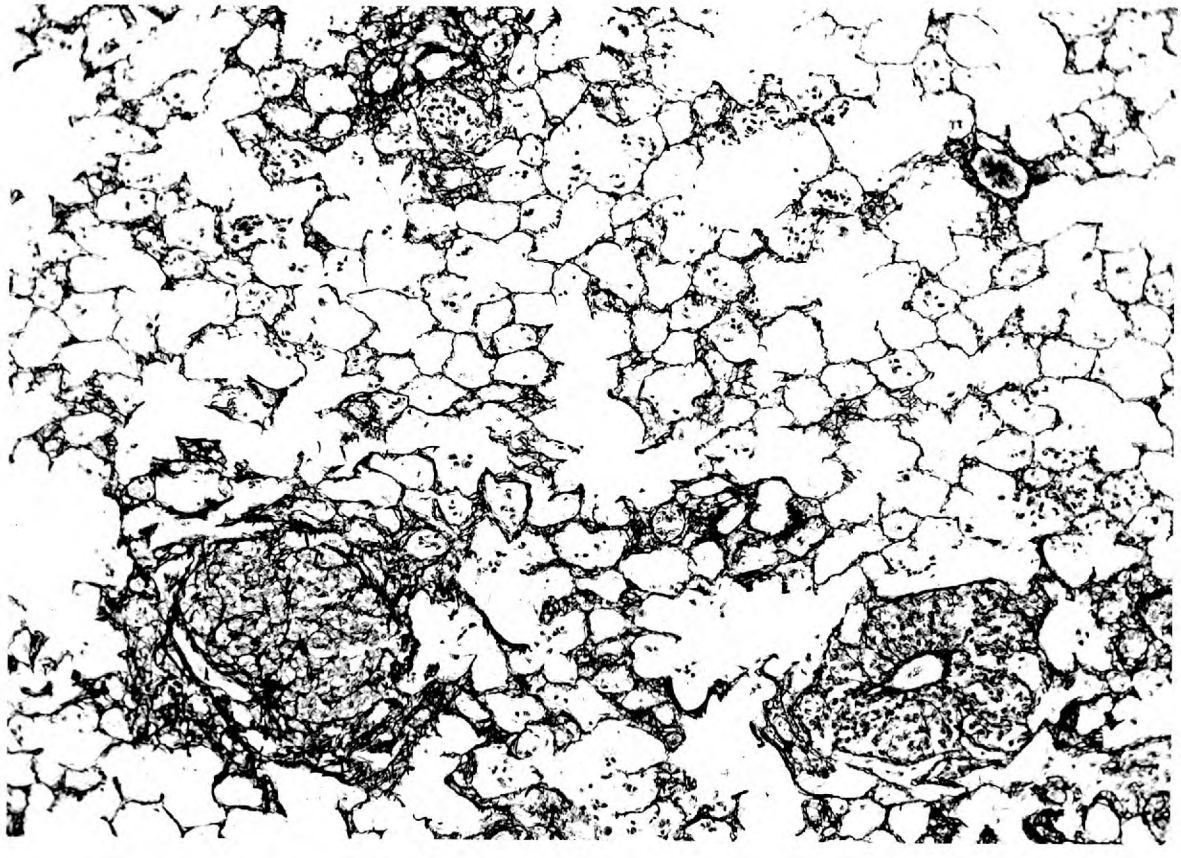


Fig. 24. Guinea-pig lung, 30 days after the injection of 3.0 mg. dead B.C.G. Nodular lesions with a few fine reticulin fibres (grade 1 fibrosis, minimal). Silver impregnation. (X. 100).

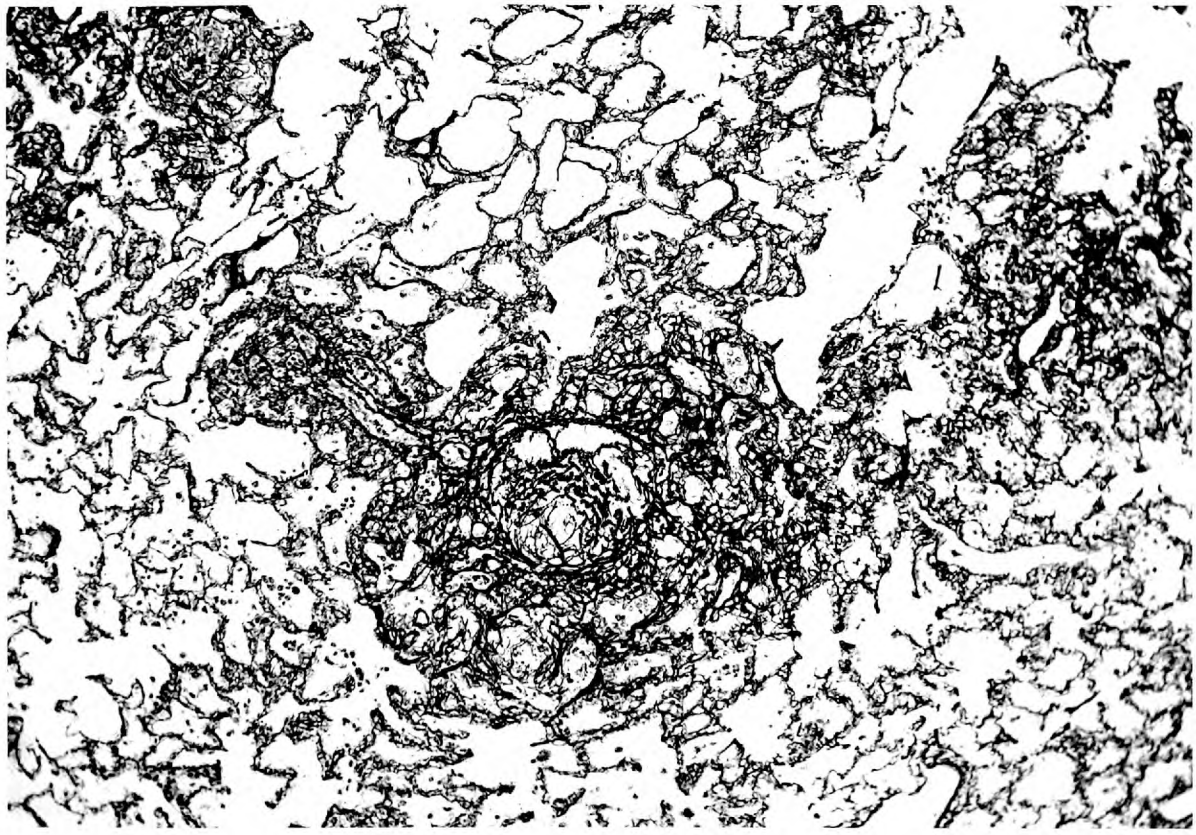


Fig. 25. Guinea-pig lung, 180 days after the injection of 2.5 mg. dead B.C.G. Nodular lesions with a loose reticulin network (grade 1 fibrosis). Silver impregnation. (X. 100).

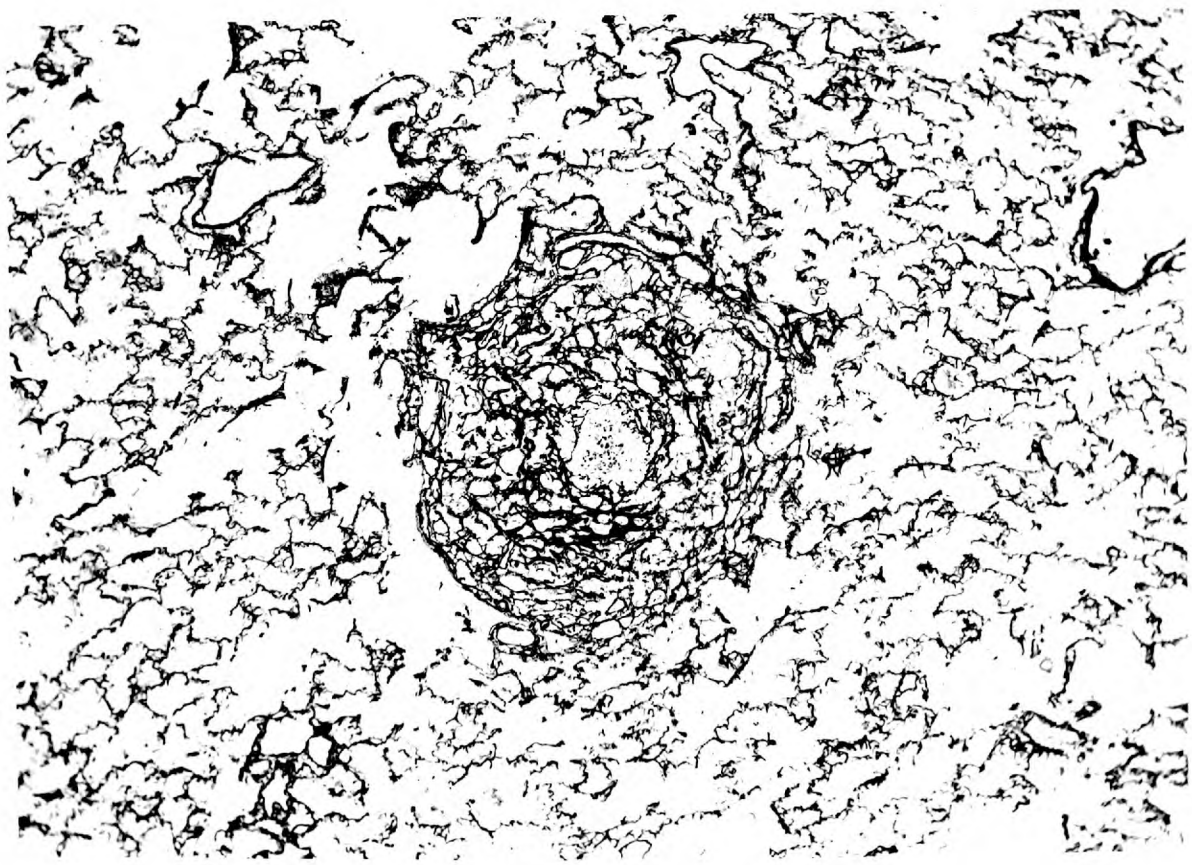


Fig. 26. Guinea-pig lung, 365 days after the injection of 2.5 mg. dead B.C.G. Cellular lesions, smaller in size than at 180 days and with a few fine reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 100).

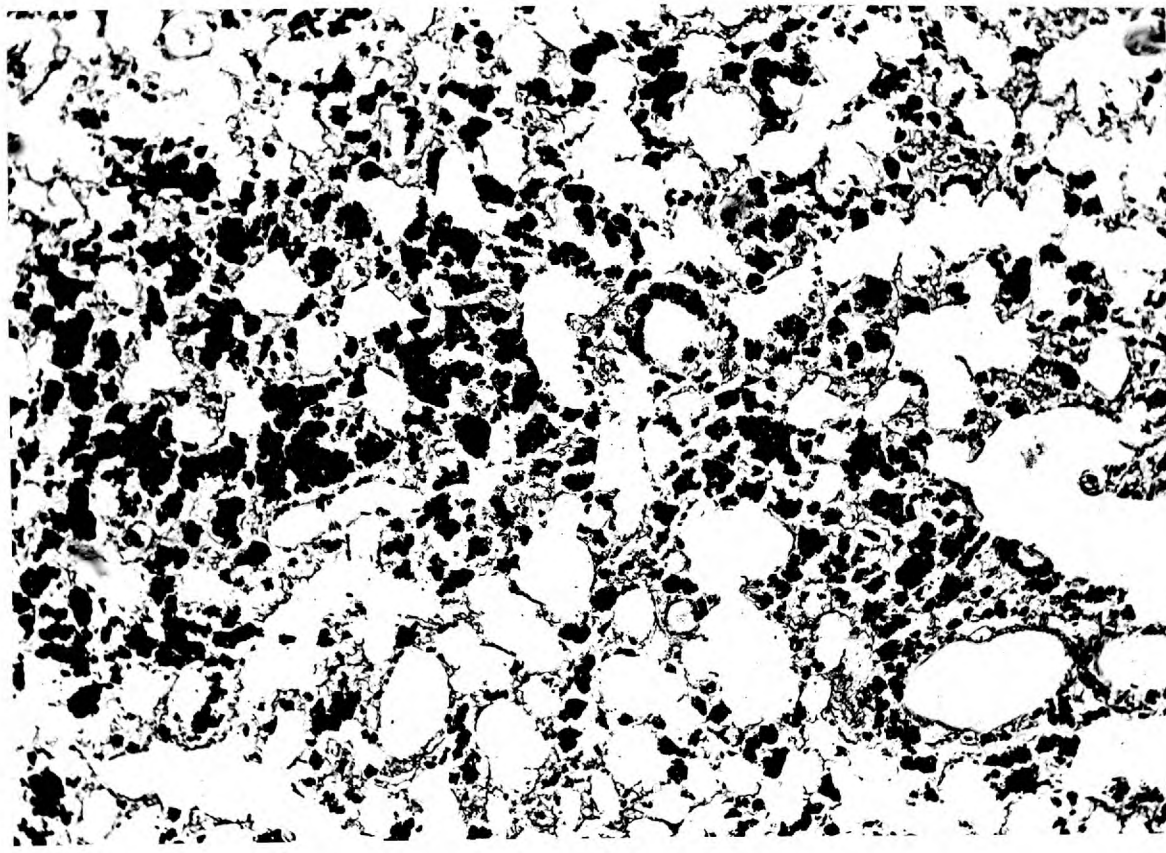


Fig. 27. Guinea-pig lung, 30 days after the injection of 150 mg. of coal-mine dust. Dust diffusely distributed throughout the lungs. No evidence of increased reticulin. Silver impregnation. (X. 100).

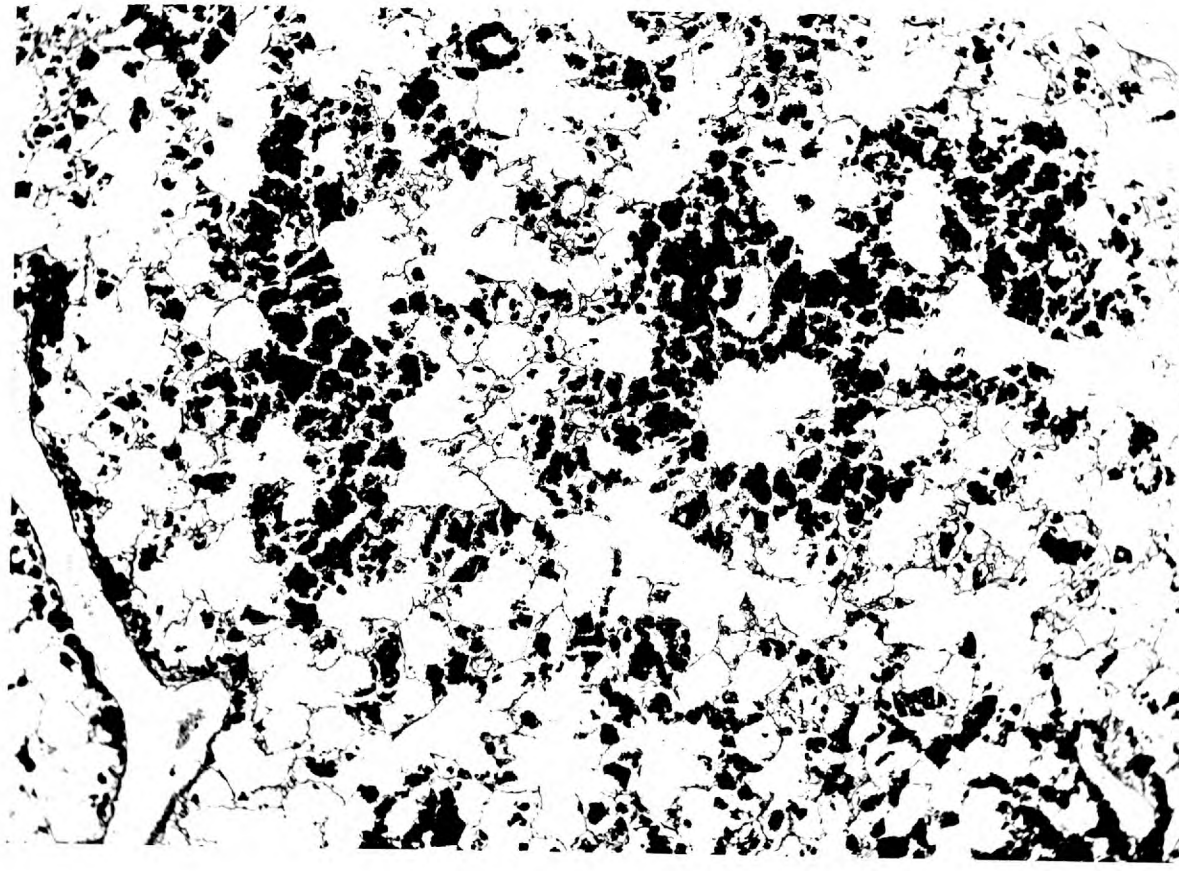


Fig. 28. Guinea-pig lung, 240 days after the injection of 150 mg. of coal-mine dust. Small collections of dust seen in the form of irregular nodules, without any fibrosis. Silver impregnation. (X. 90).

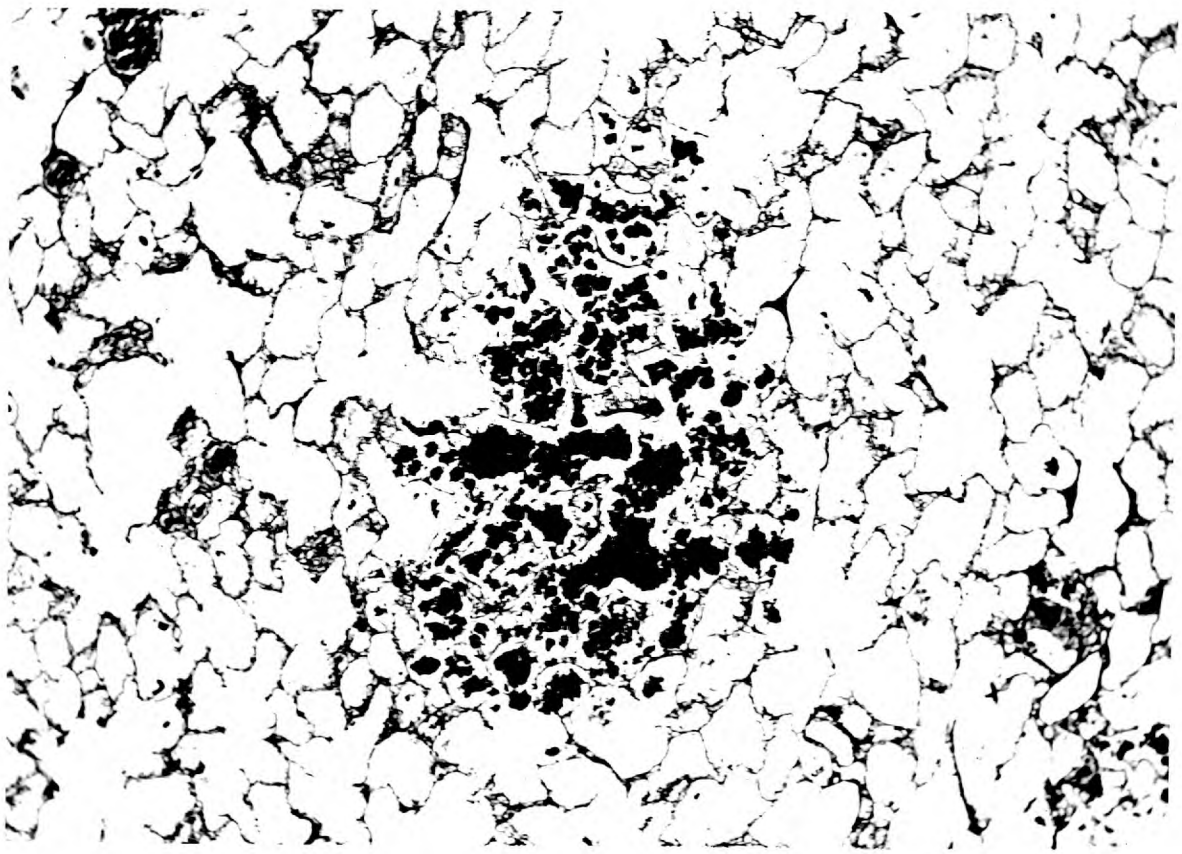


Fig. 29. Guinea-pig lung, 365 days after the injection of 150 mg. of coal-mine dust. Dust collected into compact nodules with scanty reticulin fibres, but no definite fibrosis. Silver impregnation. (X. 100).

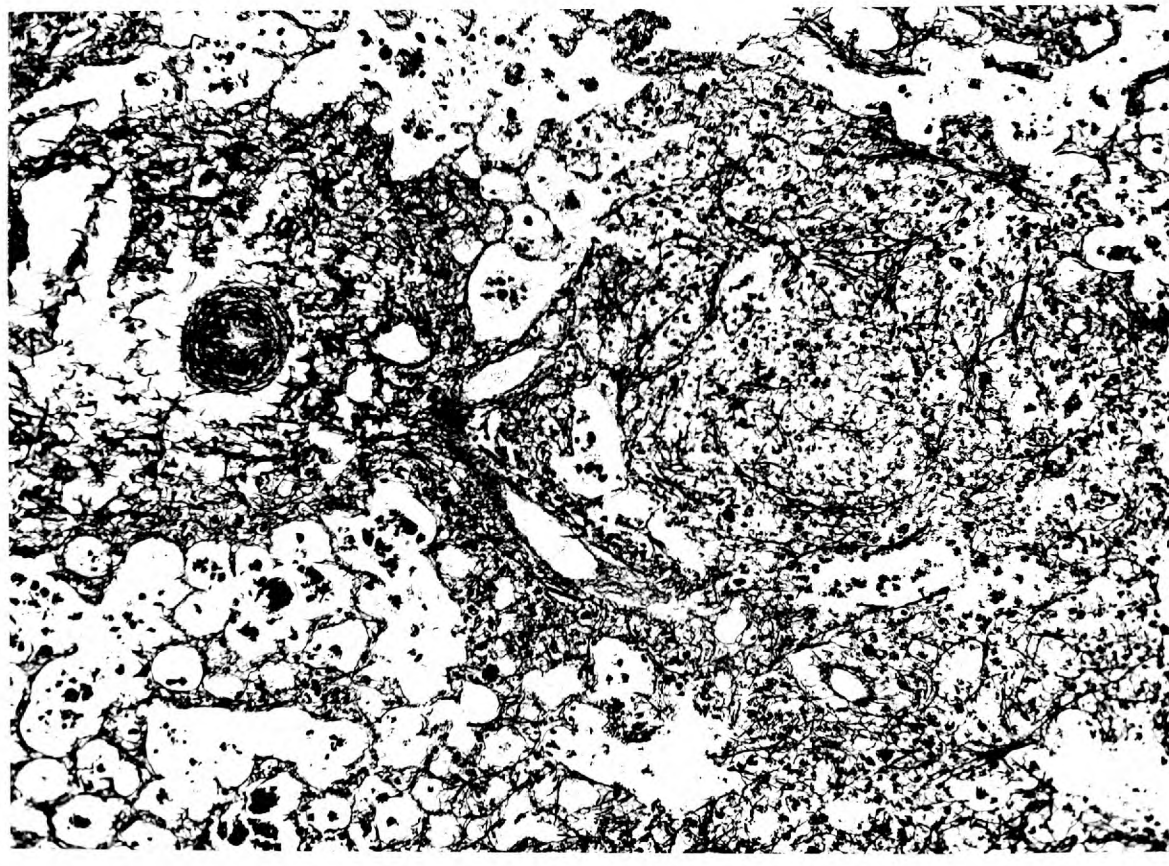


Fig. 30. Guinea-pig lung, 30 days after the injection of 150 mg. of coal-mine dust and 3 mg. dead B.C.G. Shows nodular lesions with fine reticulin fibres (grade 1 fibrosis). Silver impregnation. (X. 100).



Fig. 31. Guinea-pig lung, 240 days after the injection of 150 mg. of coal-mine dust and 3 mg. dead B.C.G. Combined dust and infective lesions with thick and compact reticulin fibres and some collagen. (grade 2 fibrosis). Silver impregnation. (X. 100).

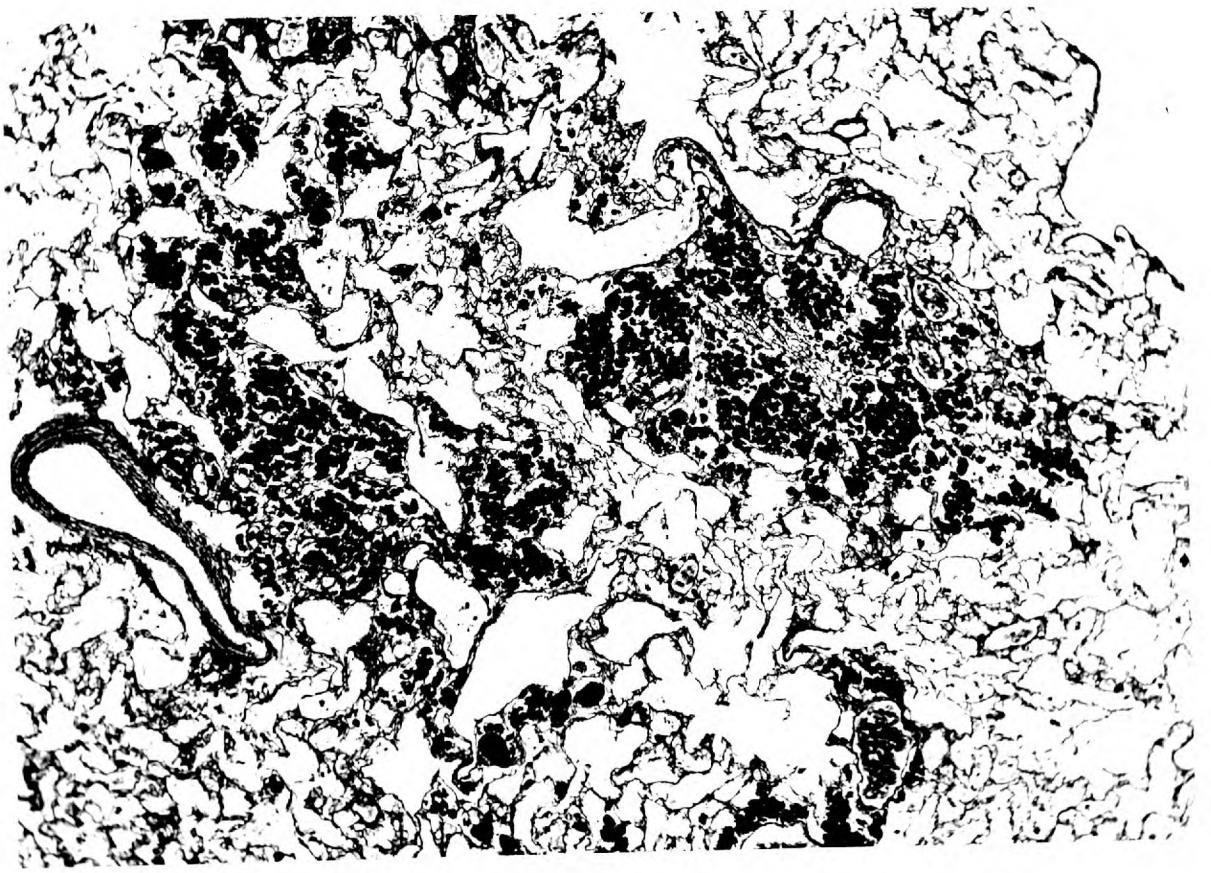


Fig. 32. Guinea-pig lung, 365 days after the injection of 150 mg. of coal-mine dust and 3 mg. dead B.C.G. Nodular lesions due to dust and infection with only a few reticulin fibres arranged loosely within them (grade 1 fibrosis, minimal). Silver impregnation. (X. 100).

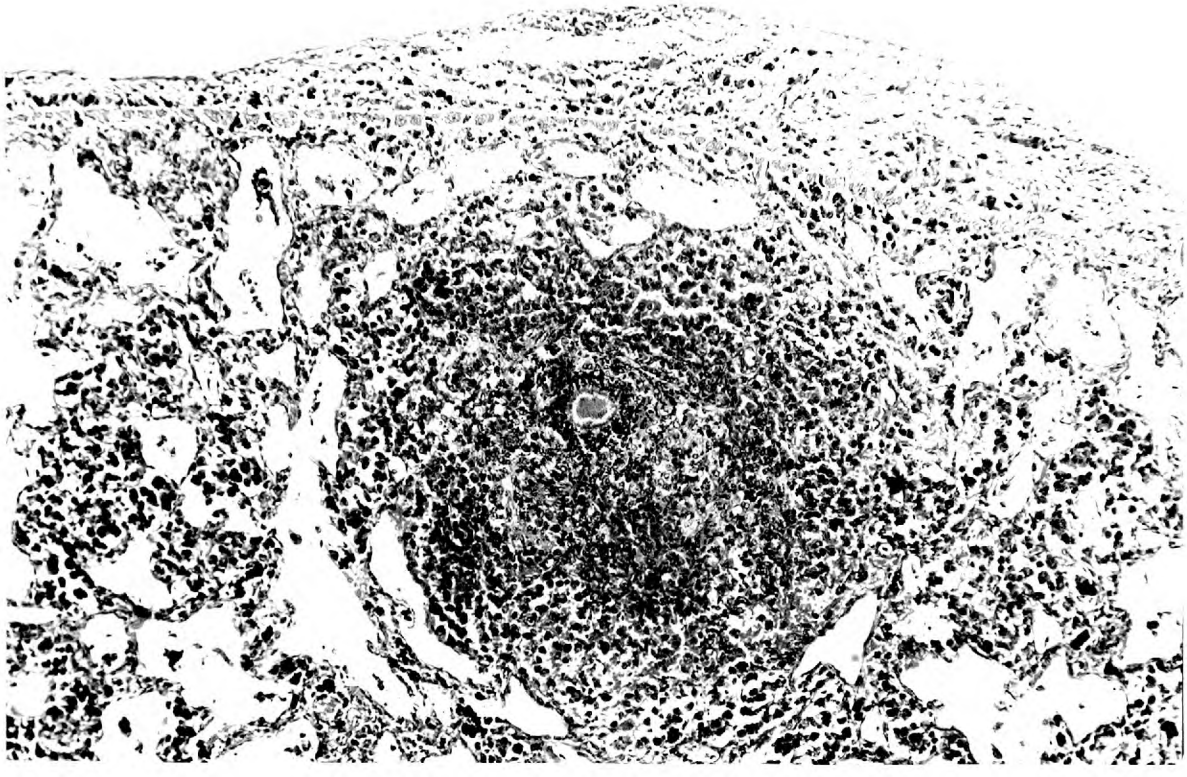


Fig. 33. Guinea-pig lung, 30 days after the injection of 150 mg. of anthracite coal and 3 mg. dead B.C.G. Nodular lesion produced by dust and organisms and composed of dust cells and epitheliod cells. Overlying pleura shows inflammatory thickening. Haematoxylin and eosin. (X. 100).

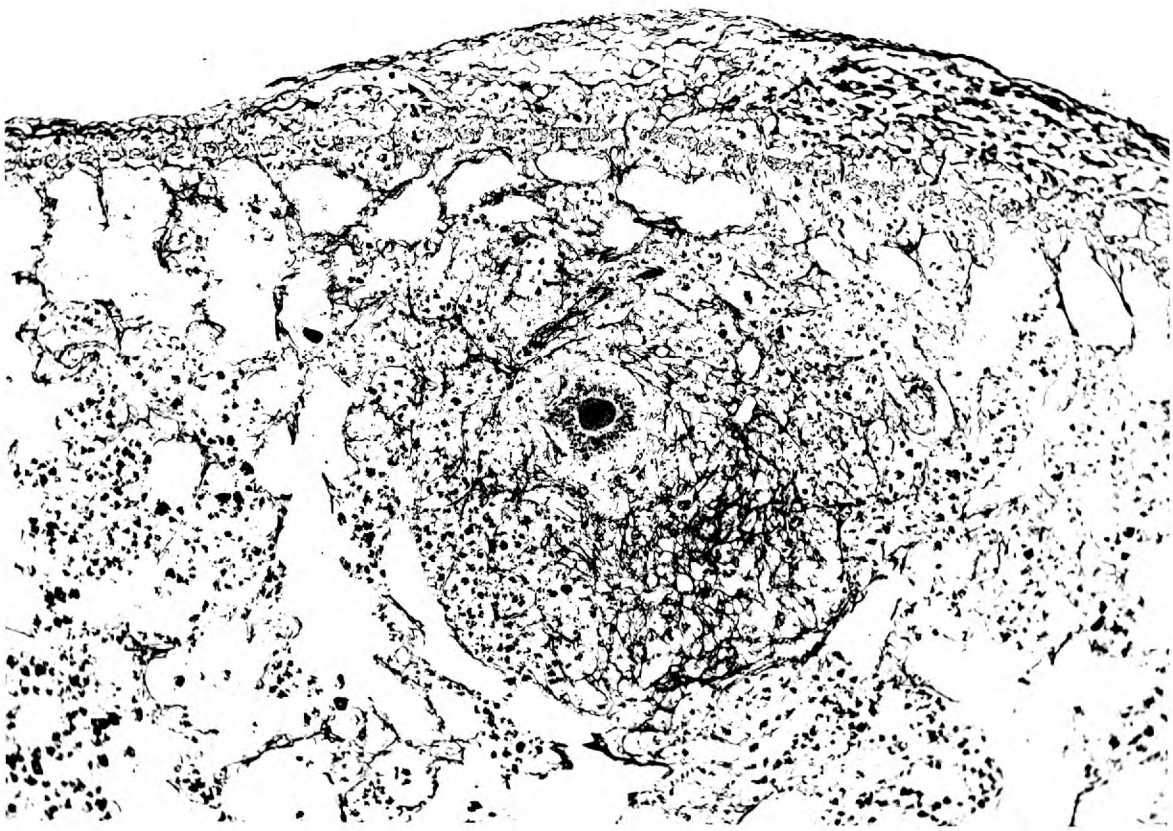


Fig. 34. Guinea-pig lung, 30 days after the injection of 150 mg. of anthracite coal and 3 mg. dead B.C.G. Same area as Fig. 33. Nodular lesion with fine reticulin fibres within it (grade 1 fibrosis). Silver impregnation. (X. 100).

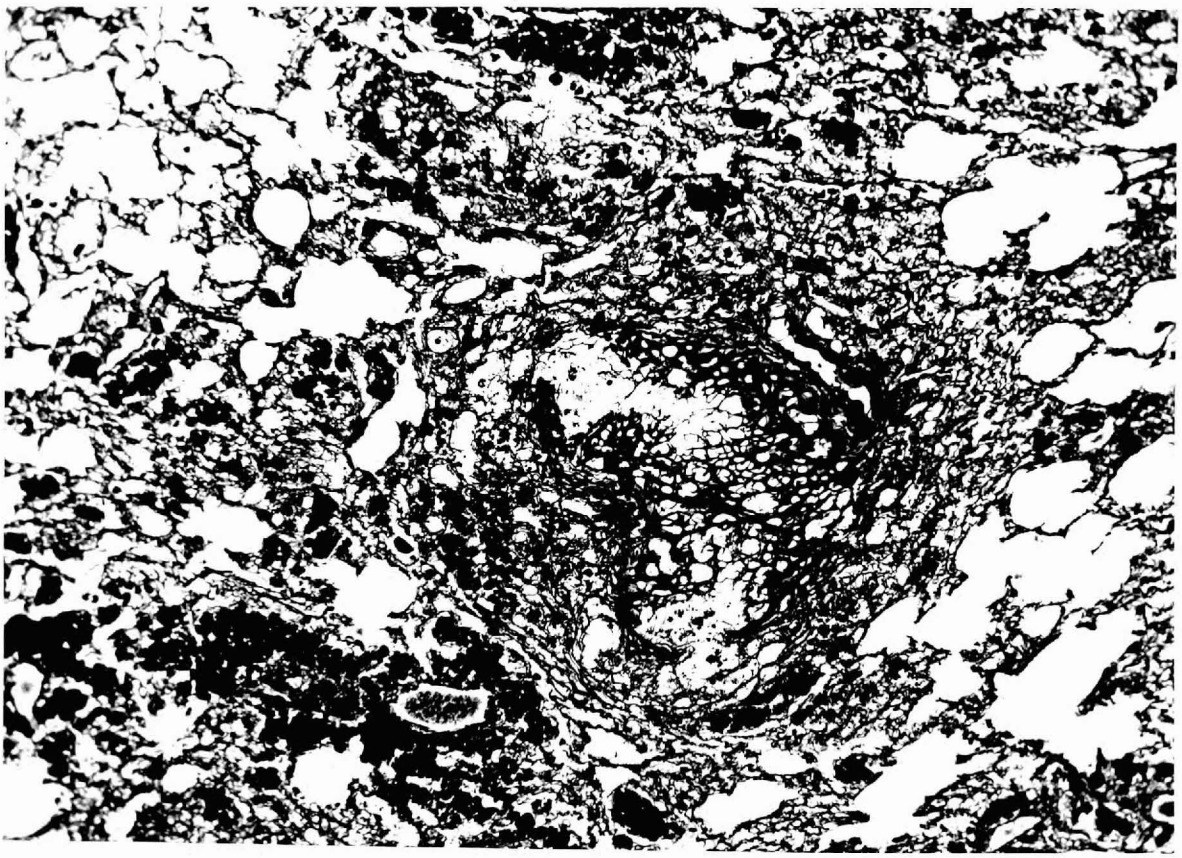


Fig. 35. Guinea-pig lung, 240 days after the injection of 125 mg. of anthracite coal and 2.5 mg. dead B.C.G. Lesions due to the combined action of dust and organisms and composed of thick and compact reticulin fibres together with some collagen (grade 2 fibrosis, maximal). Silver impregnation. (X. 100).

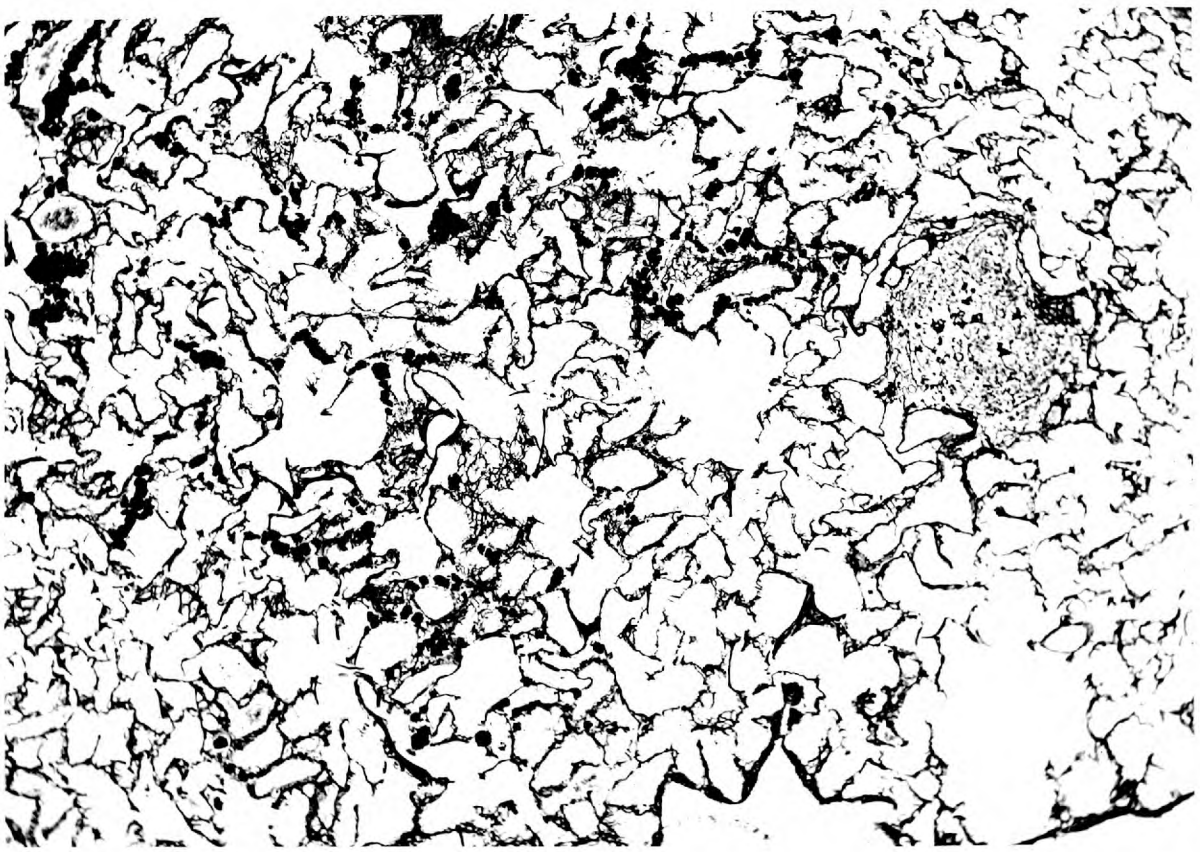


Fig. 36. Guinea-pig lung, 365 days after the injection of 150 mg. of anthracite coal and 3 mg. dead B.C.G. Lesions much smaller and less severe than at 240 days; only occasional reticulin fibres are seen in them. Silver impregnation. (X. 100).

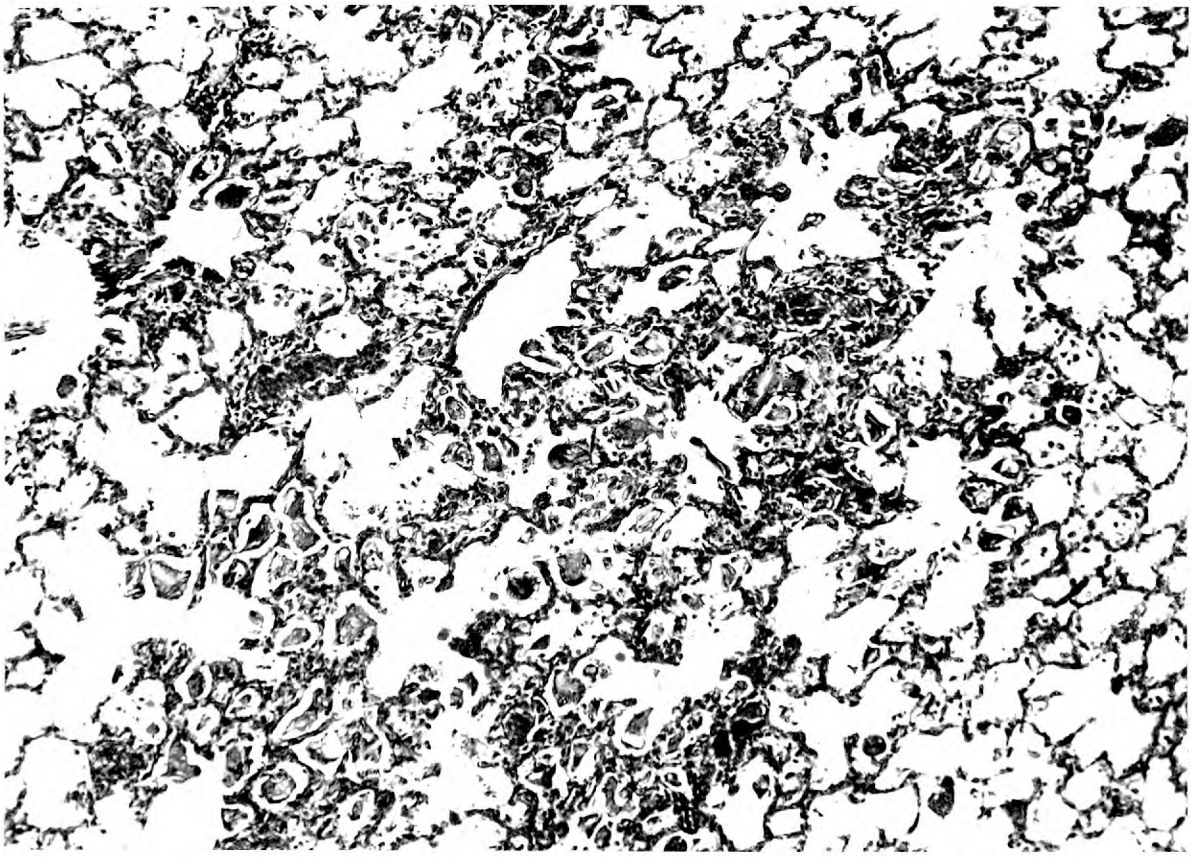


Fig. 37. Guinea-pig lung, 30 days after the injection of 125 mg. of Kaolin. Diffuse exudative reaction with many giant cells, containing dust particles within them, and collected together in certain areas. Haematoxylin and eosin. (X. 100).

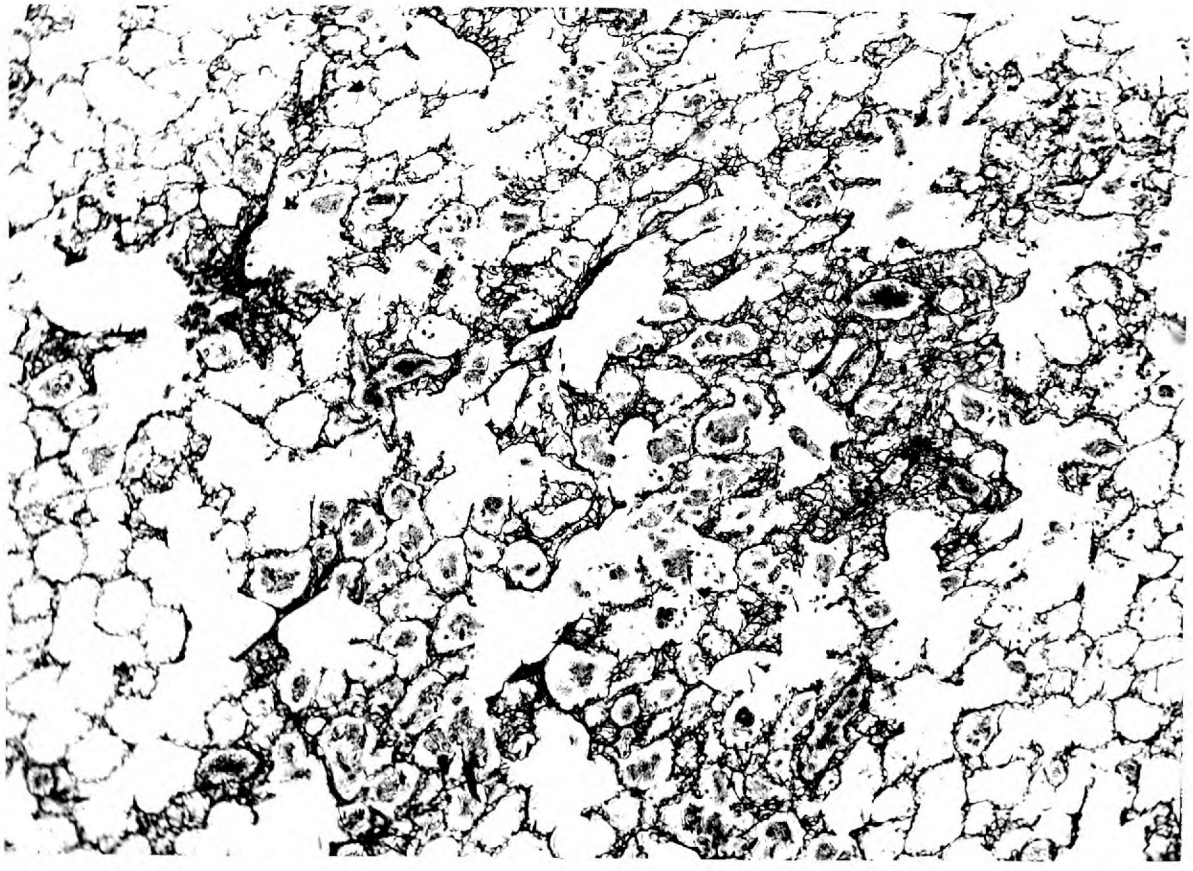


Fig. 38. Guinea-pig lung, 30 days after the injection of 125 mg. of Kaolin. Same area as Fig. 37. Shows a loose network of reticulin fibres among some collections of cells (grade 1 fibrosis, minimal). Silver impregnation. (X. 100).

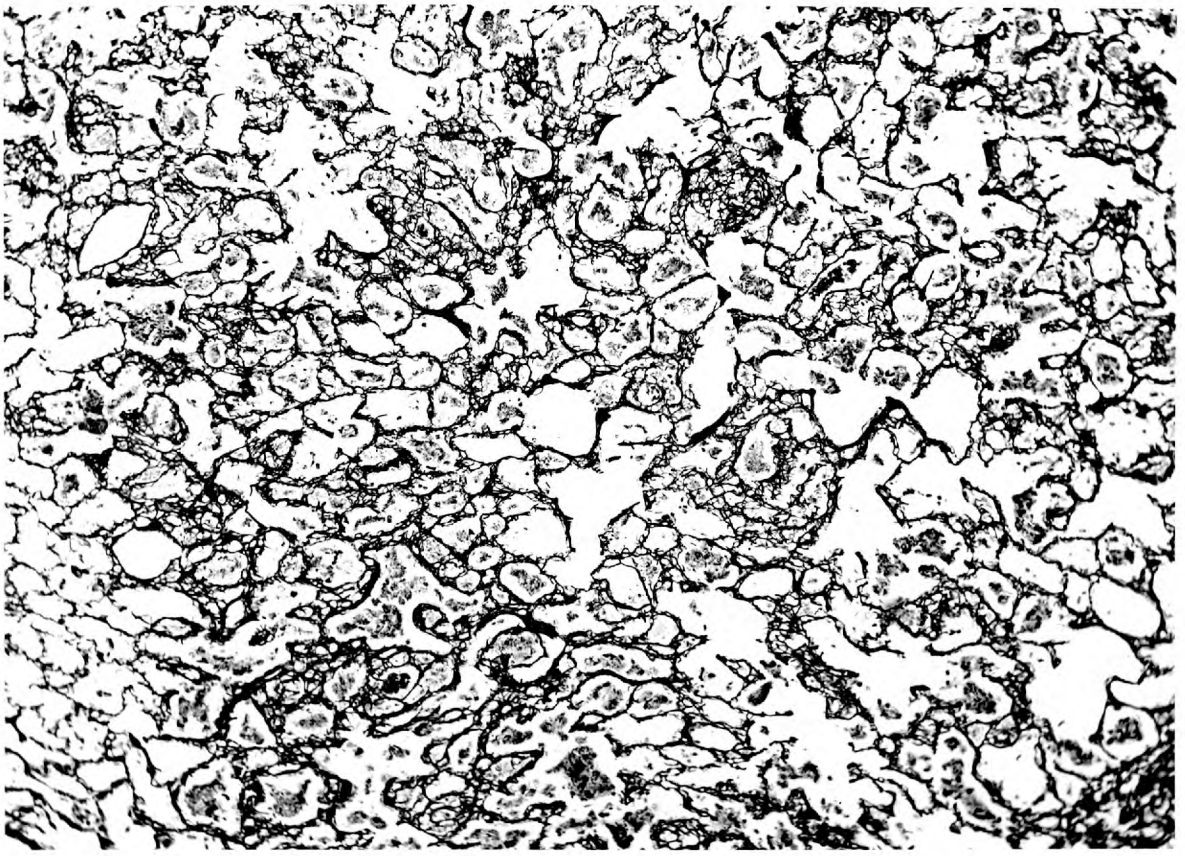


Fig. 39. Guinea-pig lung, 240 days after the injection of 125 mg. of Kaolin. Fine reticulin fibres seen in the giant cell collections (grade 1 fibrosis). Silver impregnation. (X. 100).

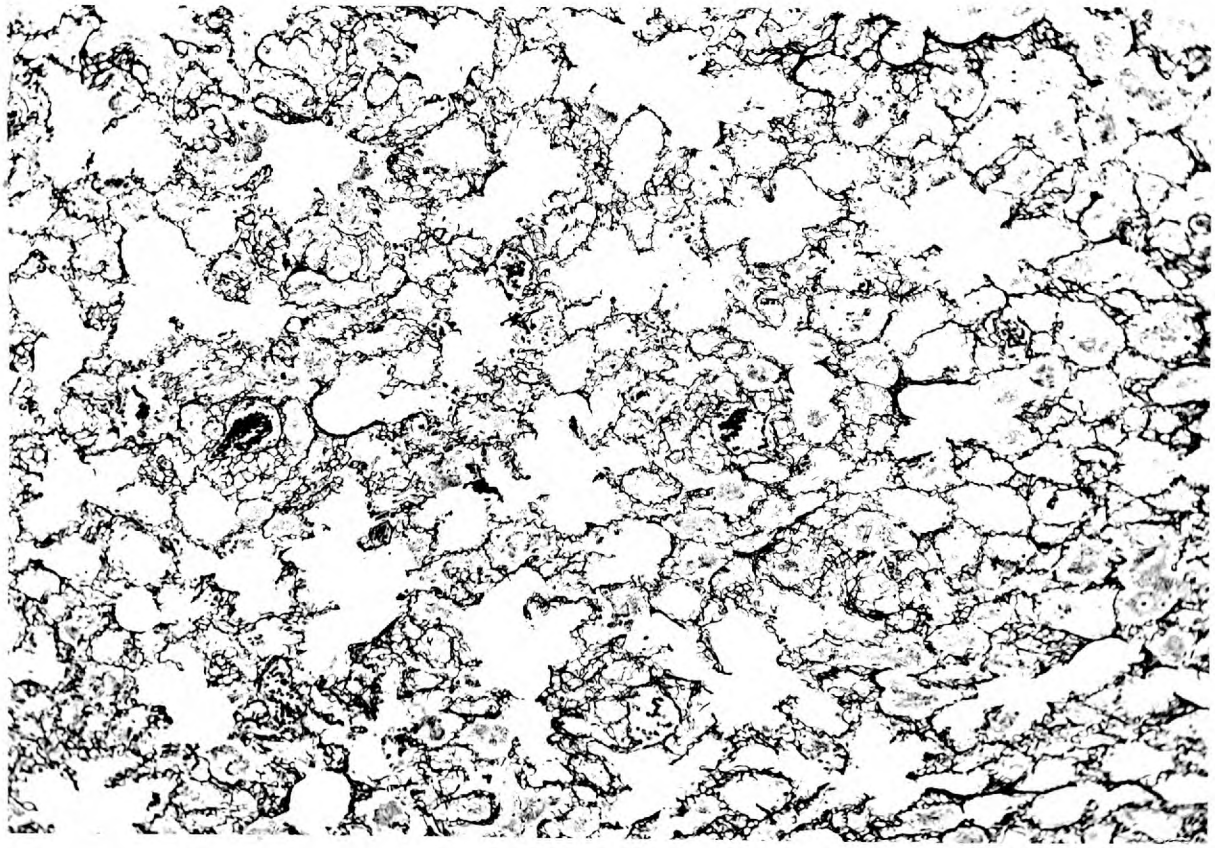


Fig. 40. Guinea-pig lung, 365 days after the injection of 125 mg. of Kaolin. No increase of reticulin in the lesions beyond that seen at 240 days. (grade 1 fibrosis). Silver impregnation. (X. 100).

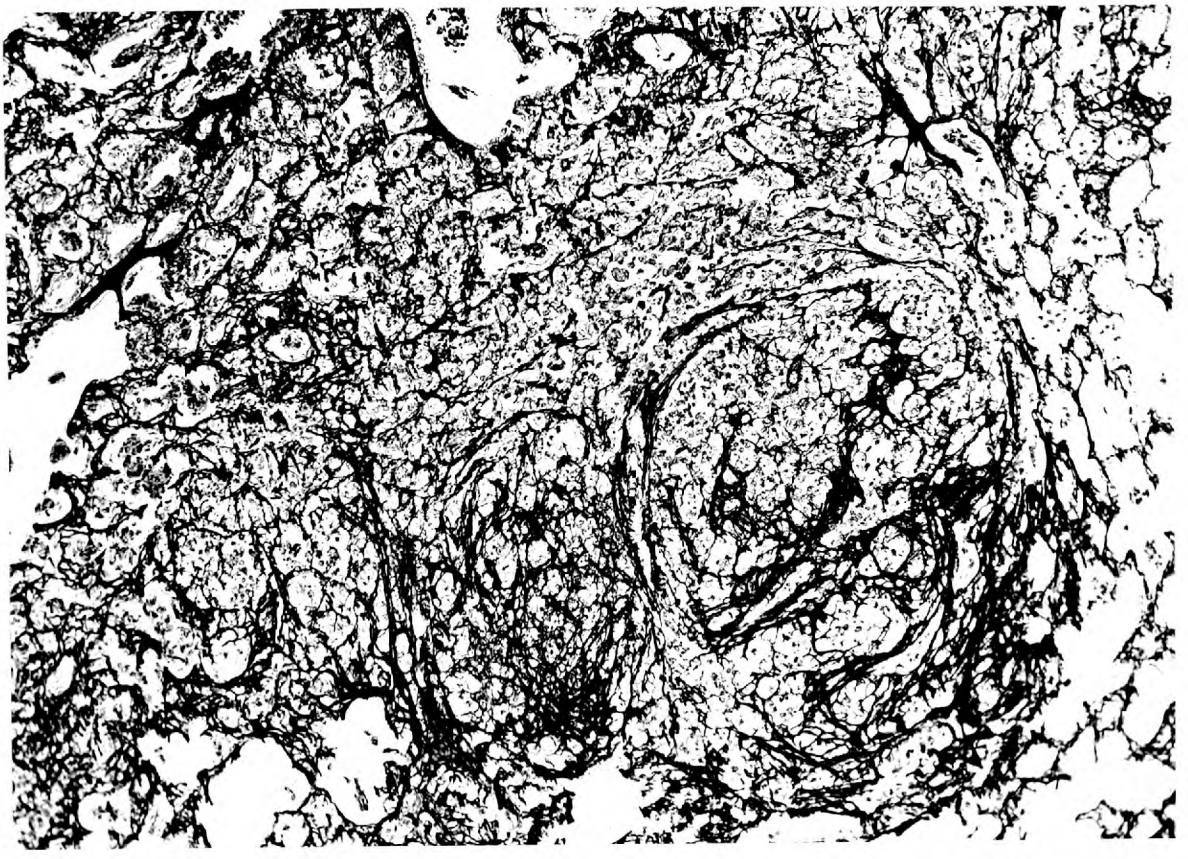


Fig. 41. Guinea-pig lung, 30 days after the injection of 125 mg. of Kaolin and 2.5 mg. dead B.C.G. Large areas of the lung show combined dust and infective lesions in which are loosely arranged strands of reticulin (grade 1 fibrosis). Silver impregnation. (X. 100).

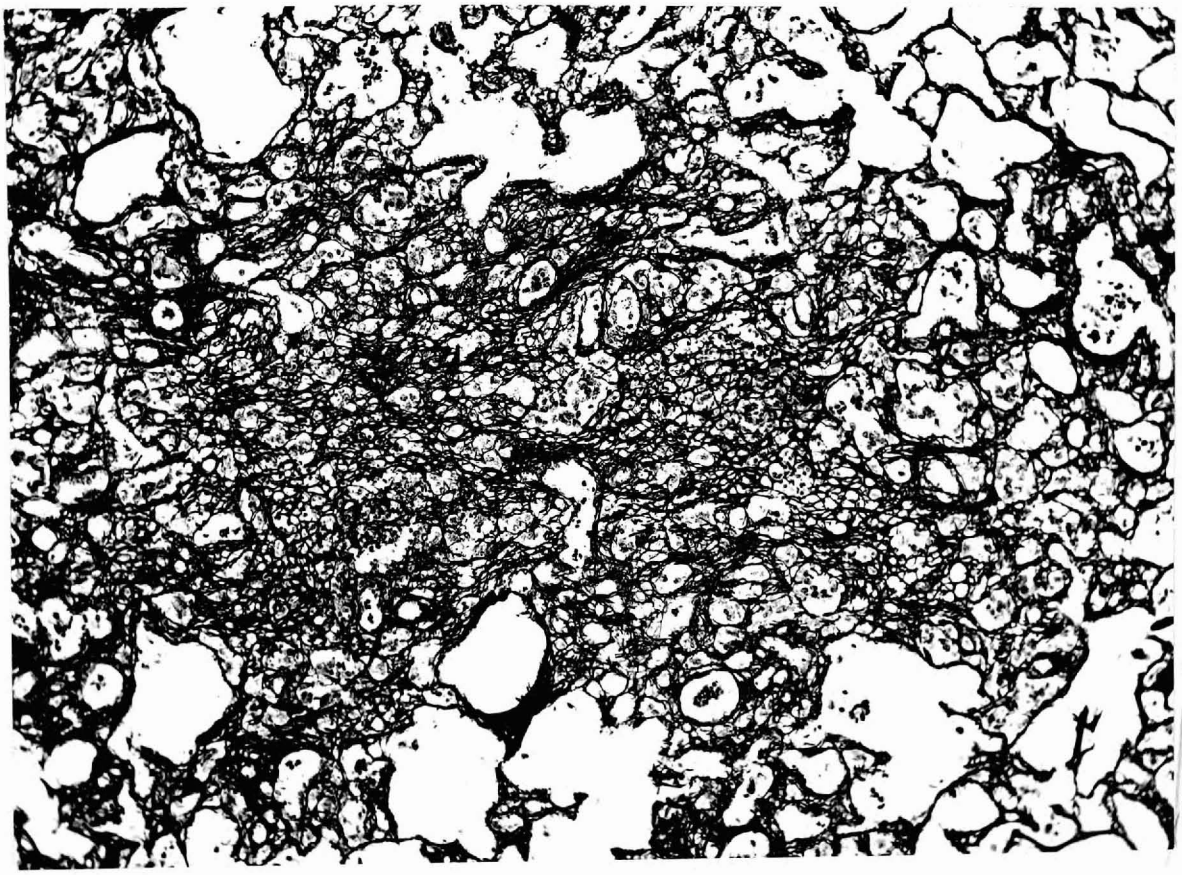


Fig. 42. Guinea-pig lung, 180 days after the injection of 125 mg. of Kaolin and 2.5 mg. dead B.C.G. Lesions show numerous thick strands of reticulin arranged in a compact manner (grade 2 fibrosis, maximal). Coarse reticular fibrosis of the alveolar walls in the neighbourhood of the lesions, is seen. Mild degree of emphysema. Silver impregnation. (X. 100).

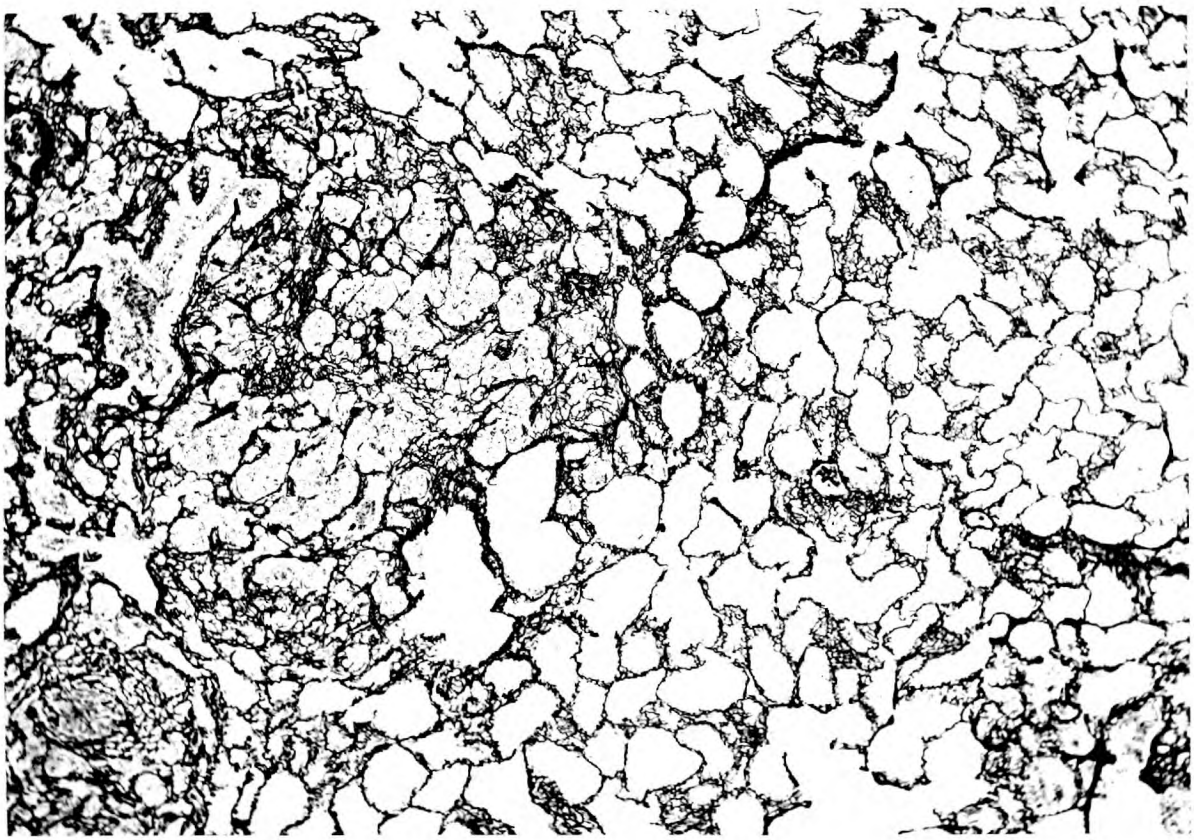


Fig. 43. Guinea-pig lung, 365 days after the injection of 150 mg. of Kaolin and 3 mg. dead B.C.G. Lesions less severe than at 180 days and show a loose reticulin network (grade 1 fibrosis) with mild reticulinosis of alveolar walls. Silver impregnation. (X. 100).

GENERAL SUMMARY

SECTION I

STUDY OF THE RATES OF ELIMINATION OF VARIOUS DUSTS FROM THE LUNGS OF RATS AND THE TISSUE REACTIONS PRODUCED BY THEM

A. QUARTZ

CORUNDUM (ALUMINA)

B. ANTHRACITE COAL

BITUMINOUS COAL

ANTHRACITE + BITUMINOUS COAL MIXTURE

C. RADIO-ACTIVE TANTALUM CARBIDE

The rate of elimination of pathogenic and non-pathogenic dusts from the lungs of rats and the tissue reactions produced by them have been studied experimentally. Quartz and synthetic corundum (alumina) of comparable particle size (nominal size 0.8 - 1 μ) and dosage were injected intra-tracheally and the rate of elimination studied by recovering the dust left behind in the lungs at varying intervals of time, by means of chemical estimations. It was found, in both cases, that the main loss of dust occurred during the first 60 days, when nearly 40% of the injected material was lost. Although the total recoveries from the lungs for quartz and corundum were so similar, the pulmonary lesions produced by them were markedly different, the quartz producing grade 5 fibrosis at 150 days, while the corundum produced no more than grade 1 fibrosis even at 365 days. Fibrogenic dust

(quartz) moved to the tracheo-bronchial lymph nodes in greater amounts than the nearly inert dust corundum, particularly in the later stages.

Anthracite coal from a South Wales mine and bituminous coal from a Cumberland mine, both of similar ash content, were injected in equal doses into the lungs of rats. A mixture of equal amounts of the two dusts was also used. The tissue reactions were studied histologically at regular intervals, and the dust was recovered from the lungs and lymph nodes chemically. The lesions produced by the three samples used were identical, being minimal simple foreign-body type of reaction without any fibrosis, up to 400 days. The amounts of dust recovered showed differences, 30% of the anthracite being lost from the lungs within 120 days, while nearly 50% of the bituminous was lost over the same period. The recoveries for the anthracite-bituminous mixture were intermediate. The tracheo-bronchial lymph nodes of animals injected with bituminous coal contained more dust than those injected with anthracite, from 300 days onwards.

An inert dust - tantalum carbide - was injected into the lungs of rats before and after it was irradiated. The radio-active dust was used in two doses of isotope with a half-life of 120 days. In vivo activity measurements were carried out on all animals and the rate of elimination of the dust studied, the degree of activity indicating the quantity of dust in the lungs. It was found that most of the dust

was eliminated rather rapidly, there being only about 12% of the total dose deposited left behind at 100 days. Neither the radio-active nor the inactive tantalum carbide produced fibrotic lesions in the lungs.

SECTION II

THE ACTION OF VARIABLE AMOUNTS OF TRIDYMITE ON THE LUNGS OF RATS

The tissue reactions produced in the lungs of rats by variable amounts of tridymite, a crystalline modification of pure silica containing 99% free silica, were studied by injecting 12.5 mg., 25 mg. and 50 mg. doses intratracheally. An electro-dialysed sample of the dust was also used in a 25 mg. dose.

Tridymite was found to be nearly five times as fibrogenic as quartz which is also a crystalline modification of silica. 12.5 mg. of tridymite produced grade 1 fibrosis at 30 days, grade 3 at 60 days and confluent fibrosis (grade 5) at 300 days. 25 mg. produced grade 5 fibrosis at 60 days and 50 mg. produced grade 5 lesions at 52 days. Further purification of the dust by electro-dialysis did not reduce its pathogenicity, as maximum fibrotic lesions were produced just as rapidly as with the ordinary preparation used in the same dose. The reason for this very toxic behaviour of tridymite is not clear, although it is possible that the crystal structure of the dust influences its biological properties in some manner which is not understood.

SECTION III

THE ACTION OF ANTHRACITE AND BITUMINOUS COAL DUSTS MIXED WITH A SMALL AMOUNT OF TRIDYMITE ON THE LUNGS OF RATS

The effect of small amounts of tridymite - a dust which has been found to be very fibrogenic - when mixed with anthracite and bituminous coal and injected into the lungs of rats, has been studied.

Histological studies revealed that 30 mg. doses of anthracite and bituminous coal did not produce any fibrosis in the lungs or hilar lymph nodes up to a period of 350 days. 1 mg. of tridymite did not produce any lesions in lungs that were free of infection, whereas in those which, unfortunately, were found to be the seat of spontaneous bronchiectasis, fibrous lesions with some collagen were present by 300 days. The hilar lymph node showed fibrous lesions (grade 1) from 300 days onwards. The mixture of anthracite and tridymite, and bituminous and tridymite, on the other hand, produced pulmonary fibrosis (grade 1) at 90 days, early grade 2 fibrosis at 240 days and fully developed grade 2 at 300 days. No differences were observed between the lesions due to these two mixtures.

Small amounts of tridymite are probably eliminated from the lungs without the production of any lesions, and then give rise to fibrosis in the hilar lymph nodes. When this same amount of tridymite is used with an inert dust such as coal, definite fibrous lesions result, probably because the

lymphatics are saturated by the large amount of coal dust, which causes retention of most of the tridymite in the lungs, with the production of lesions.

SECTION IV

THE EFFECT OF CORTISONE ON ESTABLISHED SILICOTIC FIBROSIS IN THE LUNGS OF RATS

The effect of cortisone on established silicotic fibrosis in rats has been studied experimentally. Rats were given intratracheal injections of 50 mg. of quartz, a dose which has been found to produce silicotic fibrosis within a reasonable period. After 100 days, the rats were divided into two groups and one group treated with subcutaneous injections of cortisone acetate (5 mg.) three times a week, the other group remaining untreated. The lung histology of animals from both groups was studied at regular intervals, and the collagen content of the lungs and the silica content of the lungs and lymph nodes estimated chemically. Cortisone treatment was carried on for 200 days.

Cortisone did not bring about the resolution of fibrous tissue in pre-existing silicotic nodules nor alter the pattern of fibrosis. The maturity of the lesions was also unaffected, being grade 5 in both treated and control animals. The actual amount of lung tissue involved was different, it being somewhat less in the cortisone-treated animals. Chemically, there was less collagen and more silica in the lungs of treated animals than in the controls.

The tracheo-bronchial lymph nodes, on the other hand, showed more silica in the controls than in the cortisone-treated animals. It is inferred that cortisone retards the phagocytic transport of dust from the lungs to the lymph nodes. It does not have any appreciable effect on established silicotic fibrosis by way of modifying the maturity of the existing lesions or bringing about resolution; but it may retard the formation and subsequent maturation of fibrous tissue, since there was less total collagen though more quartz in the cortisone-treated rats than in the controls.

SECTION V

THE INFLUENCE OF COAL, MINE DUST AND KAOLIN ON AVIRULENT TUBERCULOUS INFECTION PRODUCED BY DEAD ORGANISMS (B.C.G.) IN THE LUNGS OF RATS AND GUINEA-PIGS

Anthracite coal, artificial coal-mine dust and kaolin were injected intratracheally in rats and guinea-pigs with and without suspensions of killed B.C.G., in order to study the influence of these dusts on avirulent tuberculous infection. Killed B.C.G. was also injected by itself. The lung histology was studied at regular intervals.

Dead B.C.G. produced mild reticulinosis in both rats and guinea-pigs up to 500 days. Mine dust produced no lesions, while anthracite coal has also been found to be non-fibrogenic. Kaolin by itself produced no more than mild reticulinosis in both animal species. Dust and organisms combined produced far more fibrosis than either alone over the same period of time, and the mixed lesions were very much like

those found in human lungs with progressive massive fibrosis. Coal-mine dust and B.C.G., and anthracite and B.C.G. were similar in the reactions produced, the lesions reaching grade 2 fibrosis between 180-240 days in both rats and guinea-pigs, and then regressing. With kaolin and B.C.G. the lesions were more marked in rats than in guinea-pigs, reaching grade 3 and grade 2 fibrosis respectively at 180 days, and then diminishing in severity. No satisfactory explanation is available for the partial resolution of lesions after the peak period between 180 and 240 days. It may be that the stimulus producing the lesions, being dead organisms, would not have exerted a continued action, unlike live organisms which are known to flourish in lesions produced by dust, thus providing the continued stimulus necessary for the progress of fibrous lesions.

CONCLUSIONS

Biological experiments have been conducted using various mineral dusts, with a view to studying their pathogenicity, elimination from the lungs, behaviour when used in combination with infective agents and other aspects of the lesions produced by them.

The rate of elimination of a dust from the lungs of experimental animals does not seem to bear a definite relation to its pathogenicity in the period immediately following its introduction, although in the later stages the movement of a pathogenic dust, such as quartz, from the lungs to the lymph nodes seems rather more rapid than that of an inert dust like corundum. Anthracite coal is more slowly eliminated from the lungs than bituminous coal. Radio-active tantalum carbide is rapidly eliminated from the lungs irrespective of the dose of isotope injected.

Tridymite, one of the crystalline modifications of pure silica, is very pathogenic when introduced into the lungs of rats, producing maximum lesions in a short time, and being almost five times as pathogenic as quartz. The marked pathogenicity may be related to surface properties, or it may be due to the crystal structure of the dust which influences its biological properties in a manner not fully understood.

Very small amounts of tridymite do not produce any lesions in the lungs although they do so in the hilar lymph nodes. The same amount used with coal, which is non-pathogenic, in rats, produces pulmonary fibrosis, due no doubt to a slowing up of the lymphatic transport of tridymite from lungs to lymph nodes caused by the coal, and leading to retention of tridymite in the lung, where it exerts a fibrogenic effect. Under normal conditions this amount is cleared from the lungs to the hilar nodes.

Cortisone has no significant effect on established silicotic nodules in the lungs of rats, as far as the maturity of fibrous tissue is concerned; nor does it bring about resolution of the lesions. Collagen estimations indicate that there is a lessening in the actual amount of fibrous tissue formed during the period of cortisone-administration as compared with controls. It is unlikely that cortisone would be of any permanent benefit in silicosis.

The combined action of dusts such as coal, coal-mine dust and kaolin, with killed B.C.G., produces more fibrosis in the lungs of rats and guinea-pigs, than any of the dusts

or the organism by themselves, in the same period of time. Despite the different degrees of susceptibility to tuberculosis of rats and guinea-pigs, the reactions produced by dust and avirulent tuberculous infection in their lungs are essentially similar - one enhances the effect of the other.

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ABSTRACT OF THESIS

Various mineral dusts of known particle size, solubility and composition have been used intratracheally in biological experiments for the purpose of investigating their pathogenic properties, elimination, antidotal action and relation to infection; the reaction of the resulting lesions to drugs has also been studied.

Comparison of the rate of elimination of a pathogenic dust, which produced marked pulmonary fibrosis and a nearly-inert one which produced only mild fibrosis, did not reveal any significant difference. Different types of coal, however, differed in the rate of elimination while producing identical pulmonary lesions.

The pulmonary reactions produced by tridymite, a crystalline modification of pure silica, were remarkable. Severe lesions developed very rapidly and the dust proved almost five times as pathogenic as quartz. Very small amounts of tridymite disappeared from the lungs (partly into the lymph nodes) and produced no pulmonary lesions, but when used in combination with inert coal dust, fibrosis developed, the coal having no antidotal effect on the tridymite.

Cortisone was not found to have any appreciable effect on established pulmonary silicosis from the point of view of the maturity of fibrous tissue formed during the administration of the drug, as compared with silicotic controls receiving no cortisone.

Finally, the influence of coal, mine dust and kaolin on avirulent tuberculous infection produced by dead organisms has been studied. From the results it is evident that dust and organisms together produce more pulmonary fibrosis than either factor alone during the same period of time. The mixed lesions due to dust and infection were much more cellular and the coal dust much less loosely packed than in the lesions due to coal dust alone; they had the "granular" appearance which is rather characteristic of many of the lesions seen in human lungs with progressive massive fibrosis.