THE NATURE OF LESIONS CAUSED BY ASBESTOS AND MICA IN EXPERIMENTAL PNEUMOKONIOSIS IN GUINEA-PIGS

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Summary

1. The pathological changes taking place in the various tissues with special reference to lungs, in response to the inhalation of mica and asbestos dusts, have been studied in guinea-pigs using Hilton and Kettle technique.

2. It has been shown that in the case of lung, mica dust predisposes to nodular formation whereas asbestos dust causes only diffuse fibrosis.

3. The outstanding pathology may be in the lung and the disturbance of lung lesions may dominate the picture, but with long continued exposure to dusts lesions developed in other organs.

4. The role of morphological chemistry of the dust in causing definite pathological changes, with special reference to silicosis, is emphasised and has been shown that in addition to atomic structure of the mineral and nature of the cleavage of the mineral is also intimately related to the production of silicosis.

Introduction

It has been estimated that silica 12 per cent. of which is in the form of quartz constitutes 59 per cent. of the earth's crust (Geological Survey, 1918). As a consequence of such ubiquity, minerals are often associated with quartz and in the extraction, manipulation and use of such natural products quartz may be present in the dust in varying proportions, thus many igneous rocks, such as granite, contain crystalline quartz, it is present in slate, in and between the laminæ and some minerals such as graphite contain only a small proportion as an impurity. Free silica is used in technology and the arts by artificial admixture, like flint and earthenware. When dust is produced from any such mixtures, natural or artificial and inhaled, the essential action is that of silica. It is in fact silicosis, but it is modified by the other constituents which may act as accelerators or may inhibit or neutralise the intimate surface contact which is the necessary condition for the fibrogenic reaction of free silica. The degree of resemblance to typical

lsiicosis is related to the percentage of free silica in the inhaled dust and to the influence of other constituents. Gardner and Redlin (1942) have shown that the amount of free silica found in the lungs does not necessarily correspond with the severity of the reaction or with the duration of the employment. This disease is caused by inhalation of dust particles which can reach the alveoli and remain in the lung in sufficient amount and over a certain period of time (Landahl and Hermann, 1948). The onset is insidious and normally its full development requires frequent if not continuous exposure to dust for long periods within the lung, the dust acting as an irritant which destroys the highly specialised cells of the lung. These are replaced by scar or fibrous tissue which is quite useless for the purpose of respiration. In the early stage only small localised nodules of fibrosis are formed, but as the disease progresses large masses appear until at the most advanced stage practically the whole of the tissue may be destroyed (Meiklejohn, 1946; Boyd, 1938). The pathogenesis of the silicotic nodule is in some way related to the presence of silicious dust in the lung, but how the lesion develops is still largely a matter of conjecture (Kettle, 1930; Wright, 1950 and Holt, 1951). By histological analysis of say, the various stages of the contracted granular kidney, we can describe pretty well how the condition is produced. But in the case of silicosis, surprising it may seem, great difficulty has been encountered in tracing the various stages of the development of the silicotic nodule in human beings (Kettle, 1930). This is due to the reason that early cases do not come for examination, but only the advanced stages being known at the period of autopsy examination. Under industrial conditions men are exposed to mixed dusts and it is often impossible to be certain of the nature of a given lesion when infection such as tuberculosis is also associated with it. Most silicotics die of tuberculosis because the presence of silica in the tissues favours the growth of tubercle bacilli to an astonishing degree. This was shown by Gye and Kettle (1922) who injected a mixture of silica and tubercle bacilli into mice and observed very rapid development of the tuberculous lesion. Briscoe (1939) who has made a detailed study of the chemical activity of the dusts collected from variety of industrial atmospheres is of opinion that there is a strong reason for a fresh approach to this problem of silicosis by carrying out injection and inhalation experiments on animals with dusts whose properties are precisely known. The present study has a two-fold purpose, (i) to learn whether a well-defined crystalline structure of a mineral can evoke a definite tissue reaction when the material was inhaled over a period of time, (ii) and to compare with another mineral of a different chemical architecture with the tissue reaction produced by it in an animal,

A critical study of the tissue reaction to asbestos—a fibrous silicate mineral—and mica—a non-fibrous silicate—was undertaken as detailed structural studies made on these minerals are available (Bragg, 1937; Evans, 1939; and Wells, 1945). In the opinion of Stewart (1947) asbestosis is a more serious disease than silicosis. The disease is acquired either during the crushing of asbestos rock or in the processing of carding the asbestos in the factories. The characteristic microscopic picture in addition to the large amount of silica dust is the presence of large angular particles, which are probably fragments of asbestos fibres and curious golden-yellow bodies with a globular end and segmented body. Asbestos bodies are formed presumably as a part of a natural reparative process and when present in the sputum in clumps are of diagnostic significance (Cooke, 1927; McDonald, 1927; Donelly, 1933; Sayers, 1939; and Stewart, 1947).

In the absence of quartz and of fibrous structure, silicate minerals appear to produce pathological radiographic changes in the lung accompanied by symptoms which are less severe than might be expected from the radiographic signs. The amount of change depends on the concentration of dust and the period of exposure. In a study among workers in mica mines, Dreesen *et al.* (1940) found pneumokoniosis in 8 men exposed to dust concentrations of the 18 to 50 million particles per cubic foot (640 to 1760 per cm.^{3}) for periods lasting from 18 to 46 years. Smith and Wickoff (1933) studying clinical cases of silicosis among mica miners is of opinion that exposure to inhalation of this dust for even a comparatively short period is a definite and serious industrial hazard although the number of workers in this industry is much less than in other industries. Policord in France (1944) on the basis of his inhalation experiments with mica dust on animals lent his support to the view that sericite is the cause of experimental silicosis.

Materials and Methods

Guinea-pigs of average weight of 250 grams were employed as experimental animals and they were housed in individual cages. Food and water were made available in sufficient amount to keep the animals in good health. Groups of 6 were used for each variable in all experiments. Control groups consisted of 6 animals for each mineral group.

Inhalation procedure.—The mineral dust-asbestos or mica- of mesh — 325 (particle size below 40 μ) was administered to the experimental group of animals. Usually 1·5 to 2 grams of the dust were suspended in normal saline and this amount of the dust was administered intra-tracheally, spreading over several days so that each day the animal received only a fraction of the total dosage. The method of administration was a slight modification of

the technique adopted by Kettle (1932). The animals received a single dose on each day till the required dose was over. The animals experienced violent coughing and choking sensation, but very soon they recovered from the effect.

Histology.—The animals were killed at varying intervals and the tissues like lungs, liver, spleen, kidney, and the supra-renal were dissected out after macroscopic examination for any abnormality, and fixed in Bouin's fluid as a routine and some were fixed in Zenker-Formol for comparison. Paraffin sections were stained with Delafield's Hæmatoxylin and Eosin.

EXPERIMENTAL

The experimental animals recovered from the aspiration of the dust solution except for a brief moment of respiratory embarassment, and continued to live for varying periods. The maximum survival period in the case of mica and asbestos were 40 and 60 days respectively after the experiments were started. The maximum number of deaths occurred between the 30th and the 40th day. So, for all practical purposes the histological picture does not give any idea of the succession of pathological findings as the crisis was abrupt and unexpected. The animals dying were systematically examined for gross anatomical changes before tissues were taken out for histological studies. Post-mortem macroscopic appearance of the lungs, liver, spleen, and kidney and supra-renal glands in the case of asbestos treated animals showed no gross macroscopic changes in the organs. But in the cases of animals exposed to mica dust, punctate hæmorrhagic spots were seen on the surface of the kidney and the supra-renal glands only. The other organs showed no gross abnormalities. The various pathological findings in the damaged tissue so far as the present investigations are concerned are illustrated in the following microphotographs (Plates IX-XII).

MICROSCOPIC APPEARANCES

A. Pathological findings in the case of mica treated animals

Lungs.—Histopathological changes are essentially those of bronchiolitis. The bronchiole is surrounded by a ring of alveolii filled with an inflammatory exudate consisting mainly of poly-morphonuclear leucocytes with a moderate amount of fibrin. A fairly constant broncho-dilatation was found affecting some parts of the bronchial tree. In several cases this was accompanied by plugs of debris in bronchi and bronchioles. Attempts at nodular fibrosis was evident. Areas of atalectasis and emphysema were also commonly seen. Dust particles were absent from the bronchioles and alveoli. There were no signs of hæmorrhage (Plate IX, Figs. 1, 2 and 3).

Liver and Spleen.—Liver shows a series of changes varying from patchy hydropic degeneration of liver cells and fatty changes to centrilobular necrosis. The cells around the central veins of the lobules or in the mid-zonal areas are at first affected. The extent of the lesion is intense and sometimes confluent with neighbouring lobules. There was no evidence of hæmorrhage or pigment deposition (Plate IX, Fig. 4). Spleen and tracheo-bronchial lymph glands showed no marked changes.

Kidneys.—The kidneys showed occasional contracted glomeruli than the normal and showed partial replacement of their capillaries by hyalinised tissue (Plate X, Fig. 5). Sometimes adhesions between the parietal and visceral layers of Bowman's capsule led to varying degree of obliteration of the glomerular space. Degenerative cloudy swelling of tubular epithelium was well marked in all cases. These cells appearing crowded with granules are often swollen and contain vacuoles. Occlusion of the tubular lumen was also common. The severity of the renal changes is on the whole roughly proportional to the liver damage. Hæmatogenous pigmentation was marked and chiefly consisted of amorphous granules (Plate X, Figs. 5, 6 and 7).

Suprarenal gland.—The adrenal cortex showed hypertrophy and the cortical cells showed from extensive foamy degeneration to varying degrees of necrosis. The pathological findings were confined more towards the zona glomerulosa although the zona fasciculata and reticulata were also affected to a varying degree. There was no evidence of cortical hæmorrhage (Plate X, Fig. 8).

Muscle.—The skeletal musculature showed hæmatogenous pigmentation but the musculature was not damaged.

B. Pathological findings among asbestos treated animals

Lungs.—Histopathological findings were essentially those found in the case of mica treated animals, but with some exceptions. Histological examination showed generalised fibrosis, thickened alveolar walls and slight œdema in the surrounding alveoli. No typical asbestos bodies were noticed. The particles of dust present in the alveoli ranged from 4 μ to 15 μ in diameter (Plate XI, Figs. 1, 2 and 3).

Liver and Spleen.—Histopathological findings met with in the liver in the specimens were as follows: All phases of degenerative changes may be observed, although some regeneration of liver cells are observed only at the periphery of the lobules. The majority of the hepatic cells of the central lobule are destroyed (Plate XII, Figs. 4 and 5).

Kidneys.—The kidneys showed marked signs confined to the tubular epithelium. The pathological changes were the same as that observed in the case of mica without pigmentation (Plate XII, Fig. 6).

Suprarenal gland.—The adrenal cortex showed slight histopathological changes in the cortex. Small areas of necrosis were occasionally found in the zona fasciculata and zona reticulata. These appeared as rather diffused areas where the cells showed no nuclear staining. There were no signs of cortical hæmorrhage (Plate XII, Fig. 7).

DISCUSSION

Silicosis is a chronic disease of the lungs due to inhalation of microscopic particles of silica. Not only must the dust contain silica or silicates, but the dust must be finely divided in order that silica may be fixed in the lungs (Smith and Wickoff, 1933). McCrae (1913) reports that in the mine-workers of Transvaal Gold Mines, South Africa, 70 per cent. of the particles in the silicotic lung were less than 1 μ in diameter, and that the largest being 10·5 μ in diameter. The dust particle which reach alveoli of the lung become engulfed by the phagocytic cells which may carry them to lymph glands. When silica particles are inhaled a number of phagocytic silica-laden cells clump together forming patches in which the dust concentration is high. Whether they are in the lung or the lymph gland the cells are stimulated to form collagen, with the result that in these patches fibrous tissue replaces areolar tissue. As the condition advances towards silicosis the fibrous patches take up a characteristic nodular form. Collagen is a protein with a much higher content of glycine, proline, hydroxy-proline, and hydroxy-lysine than do other proteins (Holt, 1951; Everett, 1948).

The theory that silica causing tissue damage is due to traumatic action of silica dust was challenged by Kettle (1930) some 20 years ago. Gye and Kettle (1922) not only pointed out that silica is readily soluble in alkali and in the presence of living matter but also suggested that the deleterious action of silica depended upon its solubility "Just as in a physiological sense no material is of use for energy purposes unless it is soluble, so pathologically with certain obvious exceptions, it is only soluble substances which can exert a substantial or a continuous effect on the tissues". Again in a paper published in 1934, Kettle showed that lesions can be rapidly produced in the lungs of guinea-pigs when a dust is combined with an infective process, whereas typical lesion can only be produced with great difficulty if at all by the dust alone. While silicates by themselves may produce fibrosis in the lungs of animals, Kettle was of opinion that in man silicosis was nearly always associated with tuberculosis. Cases of simple silicosis were in Kettle's

experience rare, and even in those in which tuberculosis appeared to have been grafted on silicotic lung, there often seemed to be strong evidence that the apparently pneumokoniotic lesions were really infective from the beginning. A most serious objection to infection theory is due to (1) the absence of the usual signs and symptoms of pulmonary tuberculosis in well-known cases of fully developed silicosis and (ii) frequently the inability to demonstrate tubercle bacillus in silicotic nodules (Simson and Strachon, 1935; King and Belt, 1938). The toxicity of the injected silicic acid was demonstrated by Gye and Purdy (1922) who studied tissue changes in the liver, spleen and kidney of mice and rabbits after the injection of silicic acid solutions. The 1 per cent. solution used by Gye was of a concentration which would gel at pH 7·0 (Cannan, 1940), and would be highly polymerised. The conditions of his experiment are not at all similar to those found in the lung and the possibility of effects such as gel formation cannot be exculded. Banting (1936) has shown that sodium silicate or silica gel does not produce fibrosis, yet injected quartz particles always give typical silicotic nodule (Kettle, 1932; Fallon and Banting, 1935).

The essential agent in the causation of a true silicosis is the freshly cloven silica particle. The pathological process basically consist of hydration of the silica at the expense of the cell cytoplasm. Because of its atomic lattice structure quartzite, when powdered, yields a more pathogenic dust than other silica formations. Fully hydrated silica is non-toxic and enters freely in the metabolism of plants and animals. The minerals studied in these experiments are of different crystalline structure and of different cleavage, the one forming sheets of plate-like structure and the other on fracture yielding fibrous bundles. The detailed structural peculiarities become clear on examination of their crystal structure.

The asbestos minerals are amphiboles and chrysotile. Chrysotile is also a fibrous mineral and is closely related to amphiboles, but contains more of magnesium and hydroxyl. Most of the material known commercially as " asbestos " is chrysotile, although the fibres are not so long and silky as those of other types of asbestos which are forms of amphibole. Its formula is $(OH)_6Mg_6Si_4O_{11}\cdot H_2O$ and its structure is probably of the type shown in Fig. 1. Though the structure has not been confirmed in every detail (Warren and Bragg, 1930; Wells, 1945), the Si_4O_{11} chains have the same appearance as in an amphibole. In chrysotile, the magnesium atoms lie between the vertices of the tetrahedra on one side and a complete sheet of hydroxyl groups on the other side. This sheet is opposite to a similar sheet attached to Mg atoms to the vertices of another Si_4O_{11} chain. Fig. 1 ·

will make this arrangement clear. The consequence is that the lateral binding between Si_4O_{11} chains is very weak consisting largely of the secondary forces between the opposed (OH) sheets. Its X-ray diffraction pattern has again certain very curious features. If an X-ray diffraction pattern of asbestos

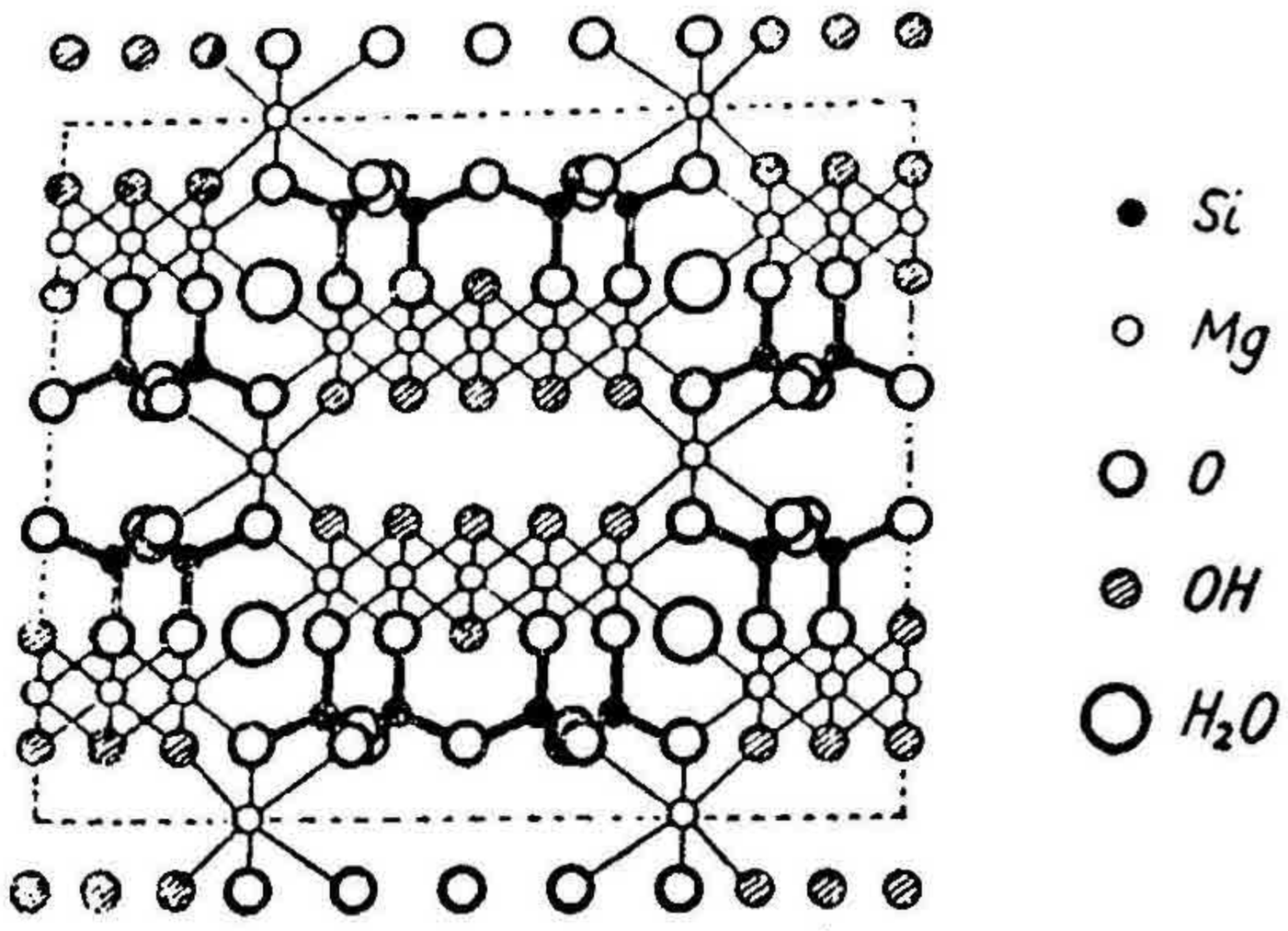

FIG. 1. The Probable Structure of Chrysotile (Asbestos). The Si_4O_{11} group forms double chains, with vertices of the tetrahedra opposed to each other. The Mg atoms lie between the vertices on one side and a complete sheet of hydroxyl groups on the other side. This sheet is opposite to a similar sheet attached to Mg atoms to the vertices of another Si_4O_{11} chain. A structure of this type would account for the ease with which the crystals may be split into bundles of parallel fibres after Wells).

is examined, it shows what is known as fibre structure. Such a phenomenon is rare in minerals though characteristic or organic substances such as rubber, wool and cellulose. A structure of this kind would account for the ease with which the crystals may be split into bundles of parallel fibres (Bragg, 1939).

The micas exhibit a bewildering variety of composition. The crystalline structure is based on the hexagonal network of linked silicon-oxygen tetrahedran. Although the detailed study of these minerals are not complete, the main lines on which they are built are now clear and the elucidation of their general structure is due to Pauling (1930). These minerals have a flaky structure, easy cleavage and pseudo-hexagonal symmetry, owing to their being based upon sheets of linked silicon-oxygen tetrahedra of hexagonal type. The structural scheme of mica is as follows. Commencing with the hexagonal network of linked tetrahedra, two of these sheets are placed together with vertices of the tetrahedra pointing inwards. These vertices are cross-linked by aluminium atoms in muscovite or by magnesium and iron atoms in phlogopite and biotite respectively. Hydroxyl groups are incorporated,

linked to aluminium, magnesium or iron alone. Thus we have a firmly bound double sheet, with the bases of the tetrahedra on each outer side. The double sheet is shown in detail in Fig. 2.

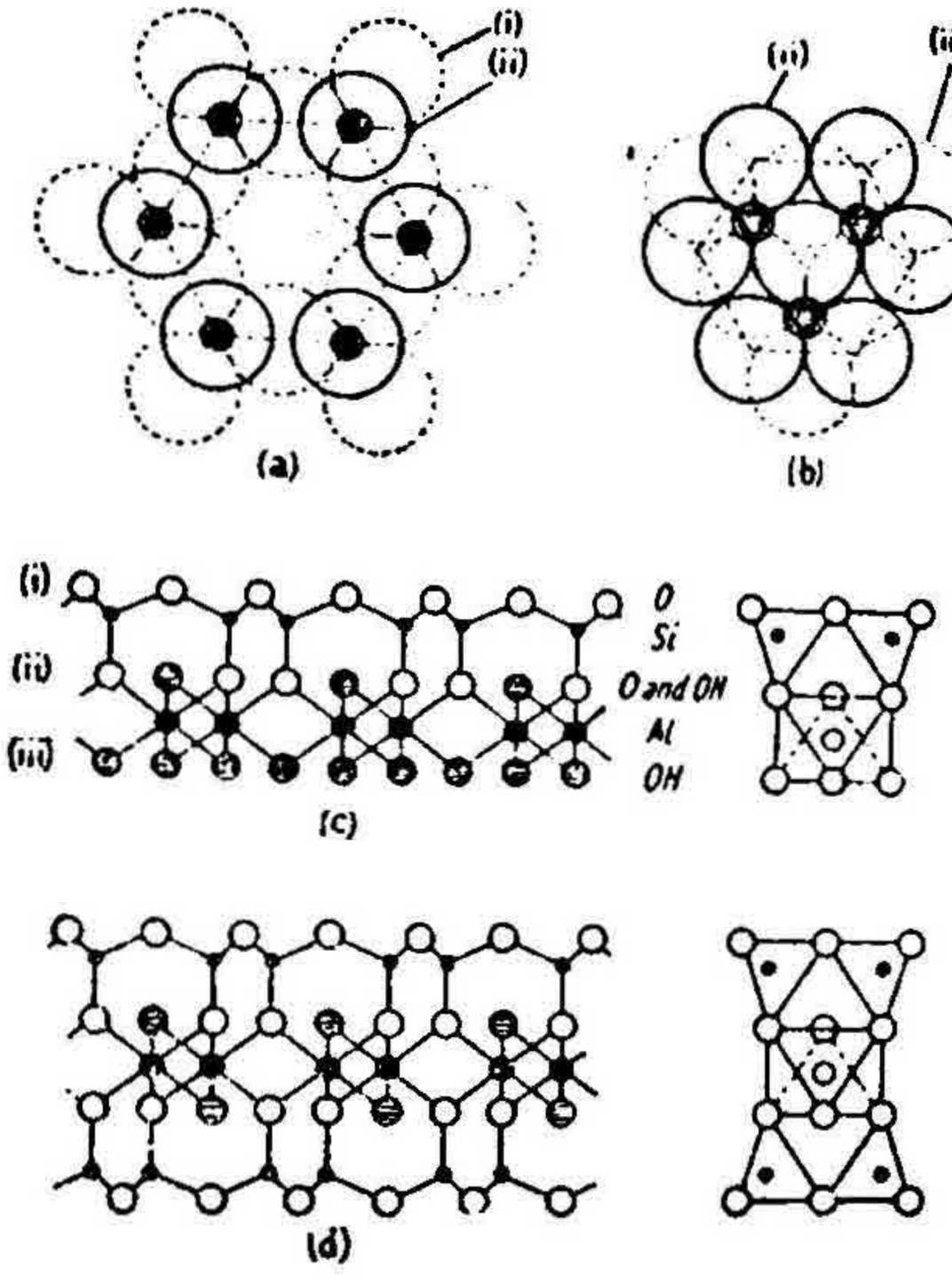

FIG. 2. The Structure of Mica. (*a*) A portion of a sheet of linked SiO_4 tetrahedra, three oxygen atoms of every SiO_4 being shared with adjacent groups. (*b*) Shows the arrangements of the OH groups in a layer of the Al $(OH)_3$ structures. (*c*) and (*d*) The double sheet is shown in detail according to Pauling. The layers of vertices and hydroxyls pack into each other providing situations for Al atoms. The whole forms a firmly bound double sheet (after Wells).

The structure is a succession of such double sheets, with potassium atoms placed between them. The double sheets of mica are very strong, owing to their silicon-oxygen bonds holding the tetrahedra together and the aluminium-oxygen or magnesium-oxygen links between the single sheets of a pair. Pauling (1930) has shown that the gradation in ease of cleavage of the minerals is readily explained by their structure. Cleavage in mica takes place between one double sheet and the next by a breaking of the weak bonds between potassium or sodium and oxygen.

The results recorded in this paper, together with earlier observations (Ramaswamy and Rama Rao, 1952), constitute a considerable body of evidence to show that the pathological lesions in the lungs and other organs are in some way intimately related to crystalline lattice of the mineral. The

presence or absence of bonding between aggregates of mineral units also have a directive action on the pathogenesis of silicosis.

In our series of experiments, in the case of mica, the lung showed evidence of fibrous reaction, although the particles of mica were not seen in the tissue sections. It is evident therefore that the damage to the tissue can be seen long after the tissue is exposed to the harmful dust, although post-mortem and histological studies reveal no evidence of the presence of the primary agent of the reaction. Hæmorrhages into the tissues such as the kidneys must have been the cause for the early death of the exposed animals. In contradistinction to the findings of Policord (1934) mica perse is capable of producing fibrogenic reaction, quite independent of sericite which is chemically quite different from plate line mineral mica. Bhatia (1952) studying the antitubercular activity of (Shankarabhraka) atomised mica, as is used by ayurvedic physicians, has found that subcutaneous injection into guinea-pigs of atomised mica has no effect on tubercular infection and that atomised mica does not evoke any physiological response, such as temperature or other toxic symptoms. In the absence of detailed histological data, it is very difficult to agree with his findings.

Gardner and Cummings (1931) studied production of experimental asbestosis in guinea-pigs and rats for over 2 years with heavy dust concentration in cages. He found that the dust did not reach further than bronchioles, whereas other dusts were in air sacs and alveoli. He concluded that this was due to the fibrous nature of the mineral. The typical asbestos bodies as found in the case of humans was not found in the lungs. In our series of experiments also, we have confirmed his findings. In the lungs the essential lesion is however diffuse and never nodular fibrosis. It is now recognised that the fibrous form of asbestos determines many of the characteristics of the disease. Phagocytosis is also found to be inperfect.

As the adrenal glands present special features in the case of inhalation experiments with mica and asbestos, they deserve special consideration. The clinical findings in the cases of miners exposed to mica and asbestos dust are anorexia, weakness, loss of weight and extreme degree of prostration (Donelly, 1933; Smith and Wickoff, 1933; Sayers, 1939; and Dreesen *et al.*, 1940). These symptoms are partly explainable for the damage that is sustained by the adrenal glands. Apparently slight changes are observed in the case of zona glomerulosa, while greater damage is observed in the zona reticulata, and zona fasciculata presents moderate amount of damage. Contrarily in the case of mica greater amount of damage to adrenal cortex is seen in the case of zona glomerulosa and zona fasciculata. Anatomical

changes, mainly deposition of collagen and connective tissue, are observed in the endocrine glands with advancing age (McGuack, 1951). In the normal animal a delicate balance is kept up between the secretion of mineralocorticoids and the glucocorticoids secreted by the adrenal cortex. Deane and Greep (1946) in a morphological and histochemical study of the adrenal cortex after hypophysectomy, came to the conclusion regarding the independance of the two outer zones of the cortex of the adrenal gland. They showed that the ketosteroids gradually disappear from the zona fasciculata, but persist in the zona glomerulosa after hypophysectomy. The mineralocorticoids favour hyaline deposition in tissues, whereas glucocorticoids oppose it, so that a dynamic interplay of these hormone secretion is a necessity for the proper morphological integrity of these tissues. Under abnormal conditions of stress and strain, as is observed in the case of pneumokoniosis, this balance is upset and consequently the pathological changes dominate the scene. It seems probable that silicates which evoke a fibrogenic reaction in the tissues must behave differently from those silicates that do not evoke such a response. This property is again being determined by the physicochemical properties of the mineral.

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MICA

Pathological Changes Caused by Mica Dust

PLATE IX

FIG. 1. Section of lung of guinea-pig, showing attempts at nodule formation, and fibrosis 40th day. H and E, × 60.

FIG. 2. Lung of guinea-pig, showing patchy character of the exudate. Consolidated areas alternate with areas of congestion, collapse and emphysema. Slight œdema surrounding the alveoli is seen. H and E, × 60.

FIG. 3. Lung of guinea-pig. Alveoli surrounding bronchioles are filled with exudate onsisting mainly of polymorphonuclear leucocytes with moderate amount of fibrin. H and E, × 250.

FIG. 4. Section of liver of guinea-pig. The cell around the central veins of the lobules and in the midzonal regions are affected. The normal structure has been completely altered. H and E, × 250.

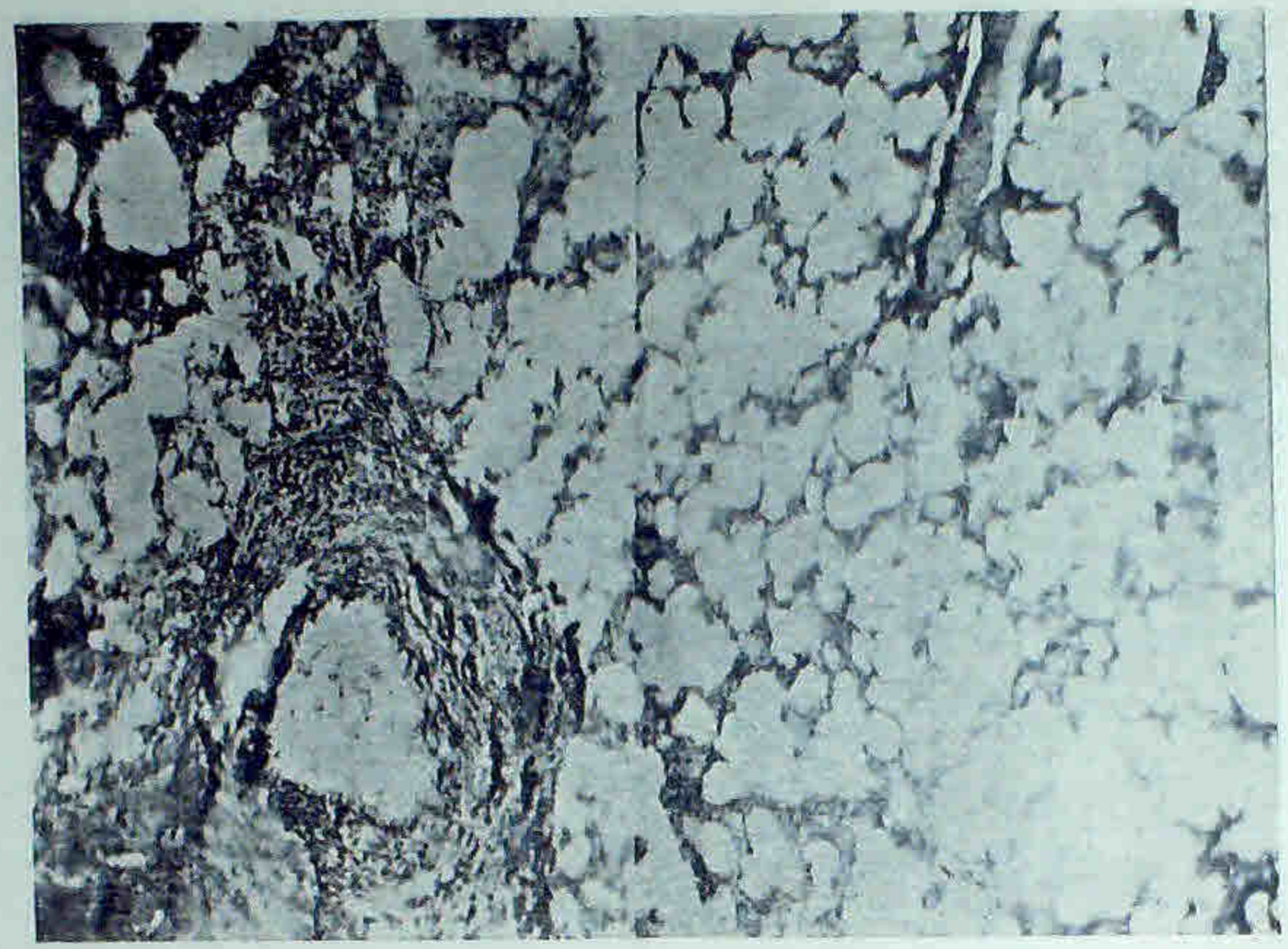

FIG. 1

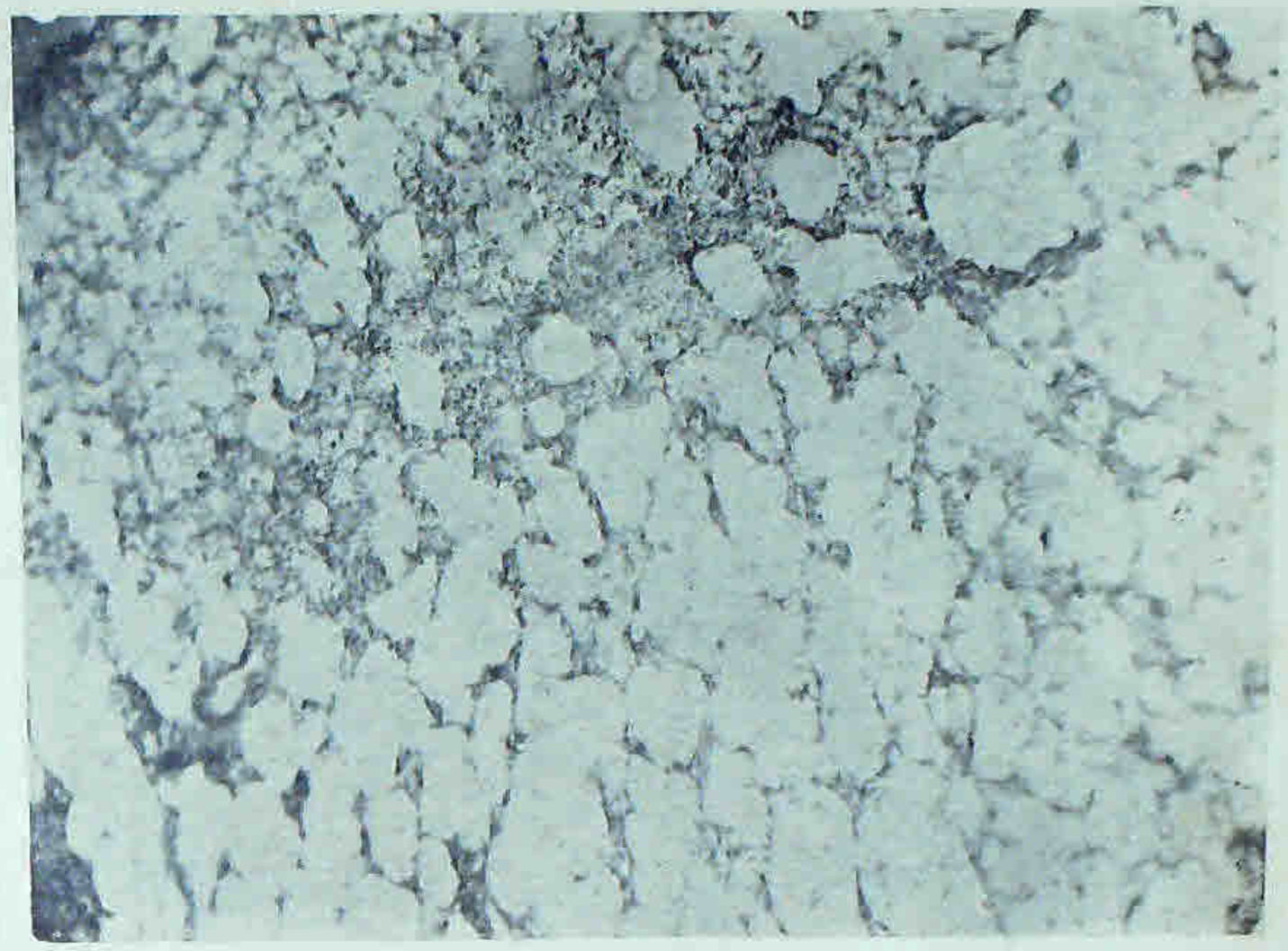

FIG. 2

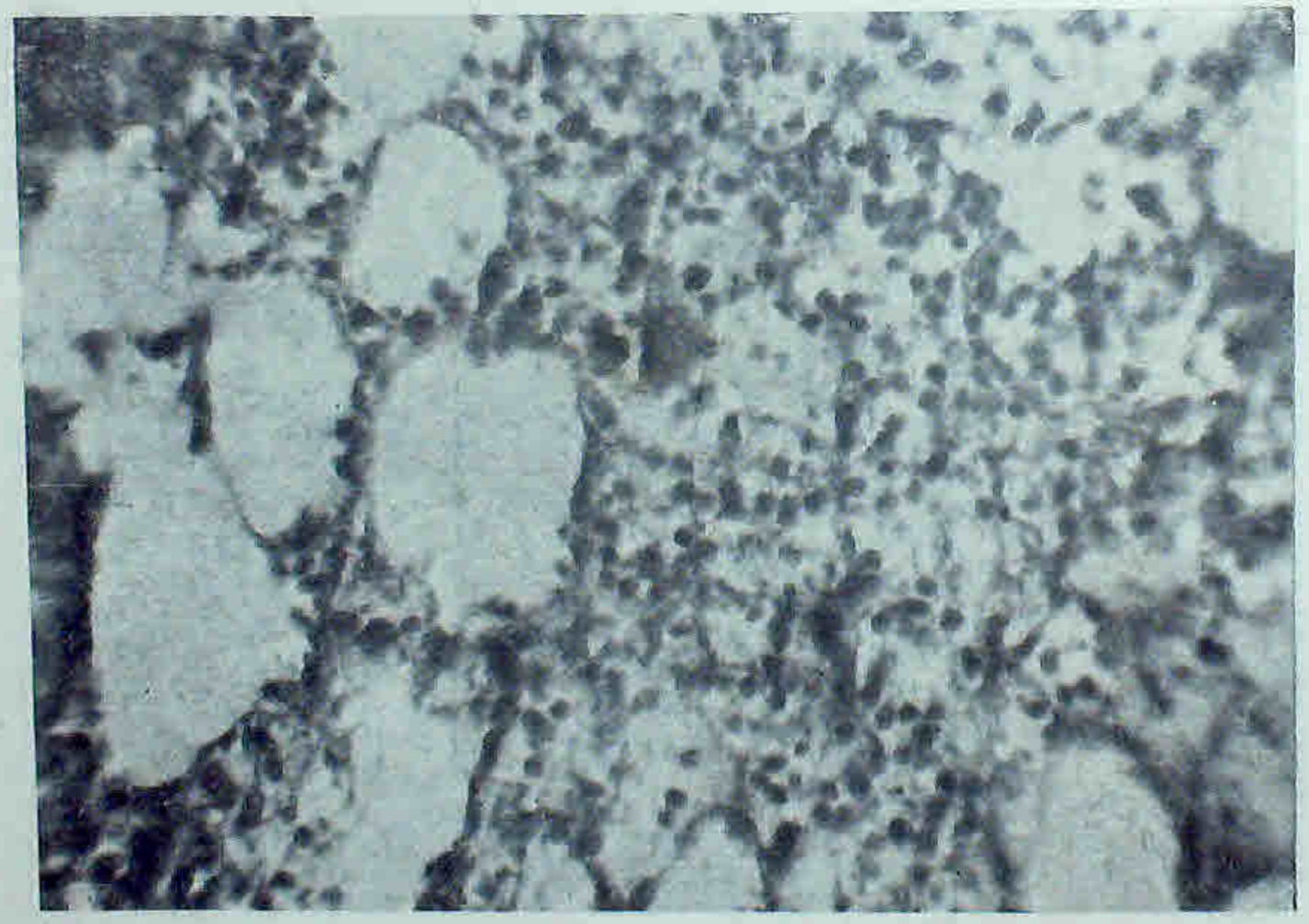

FIG. 3

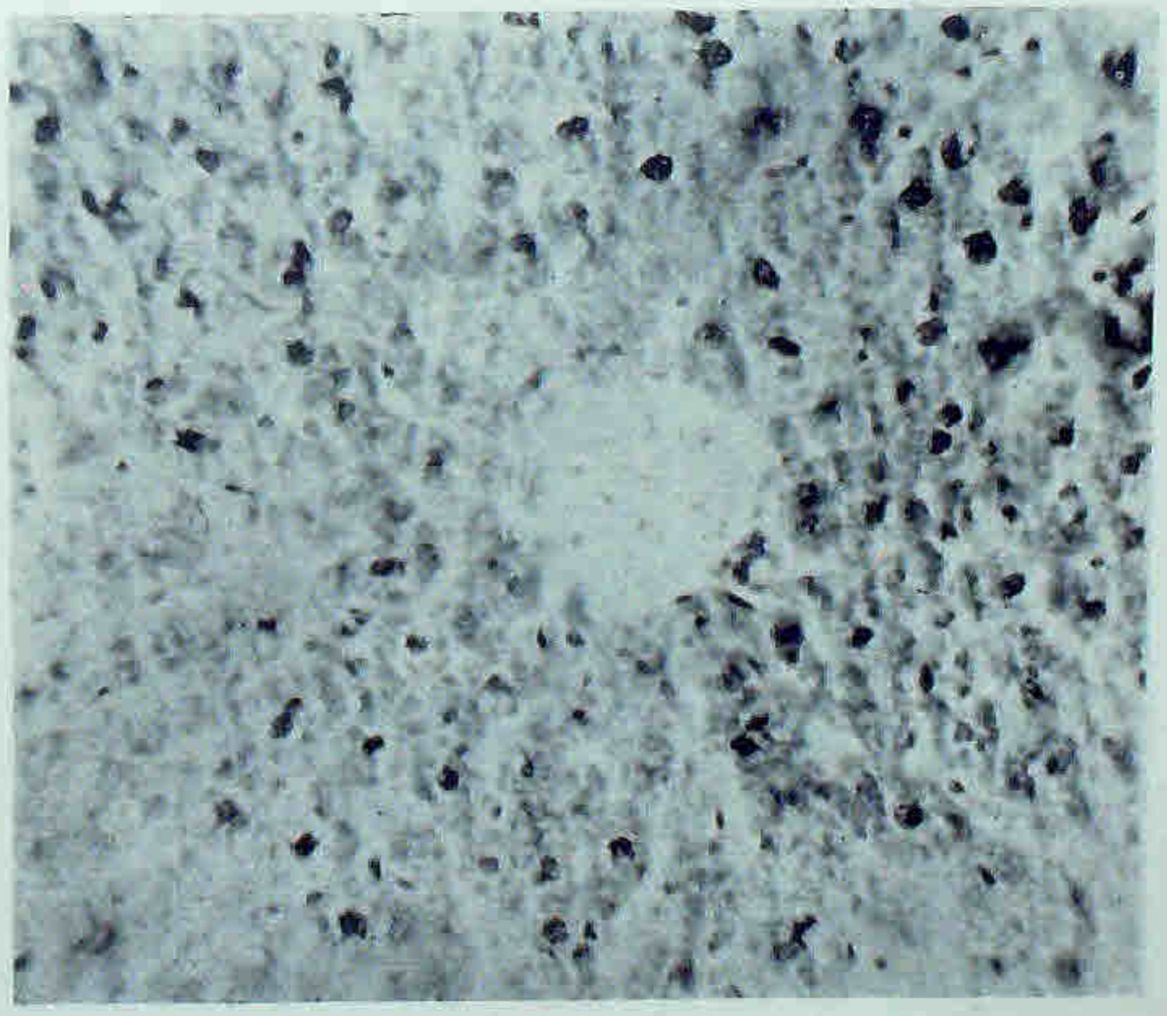

FIG. 4

Patho'ogical changes caused by Mica dust

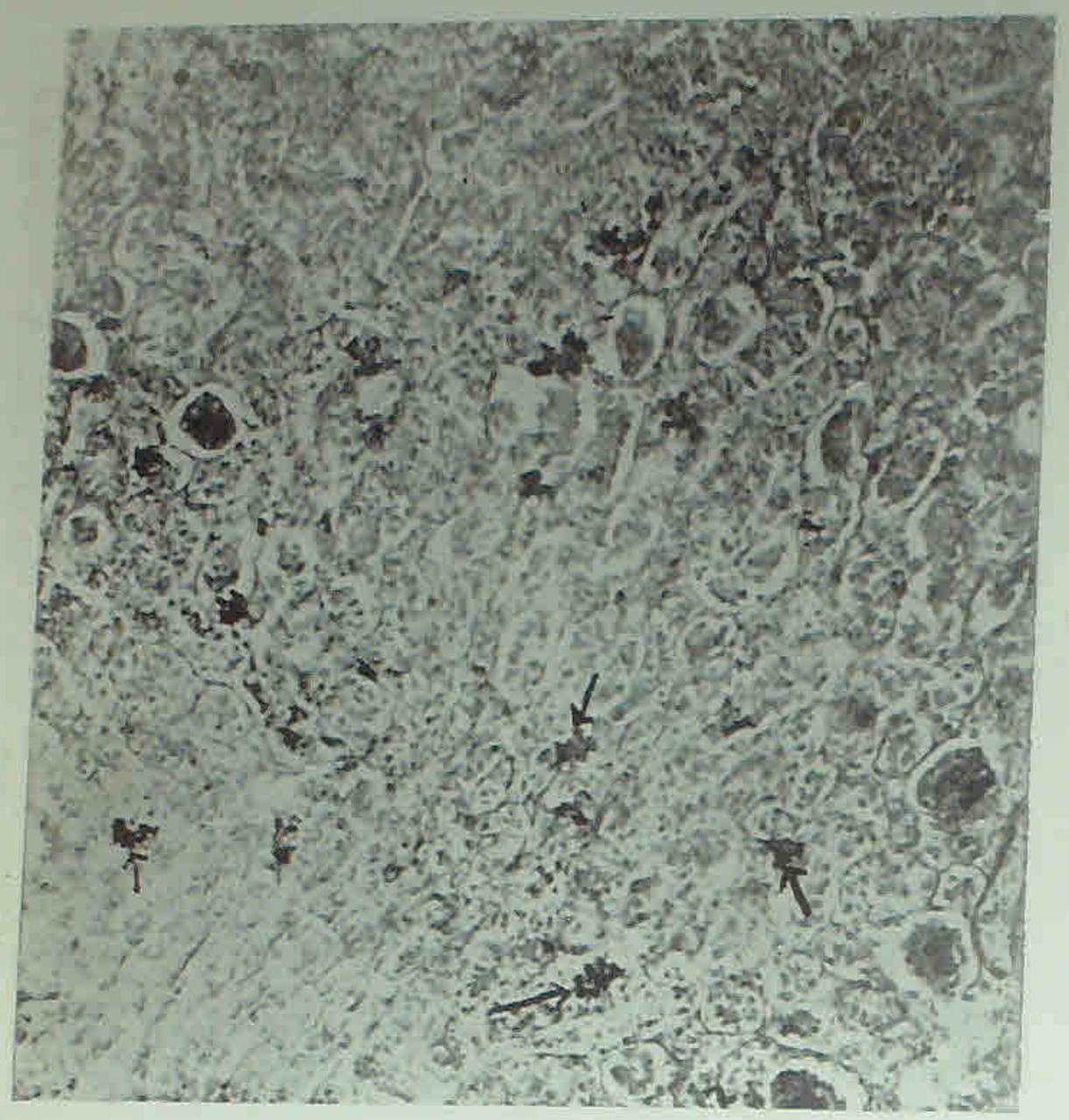

FIG. 6

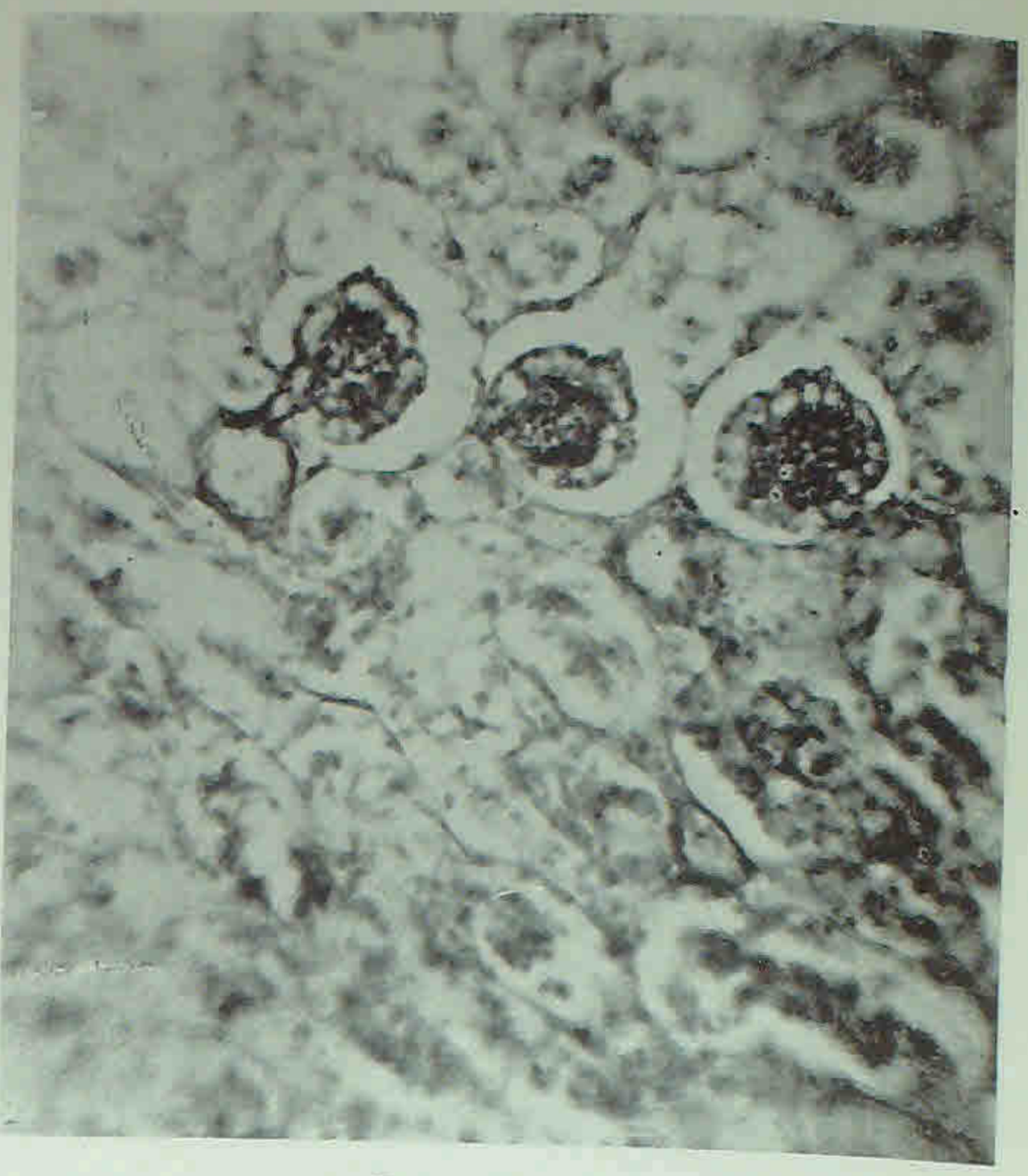

FIG. 5

Z.G.

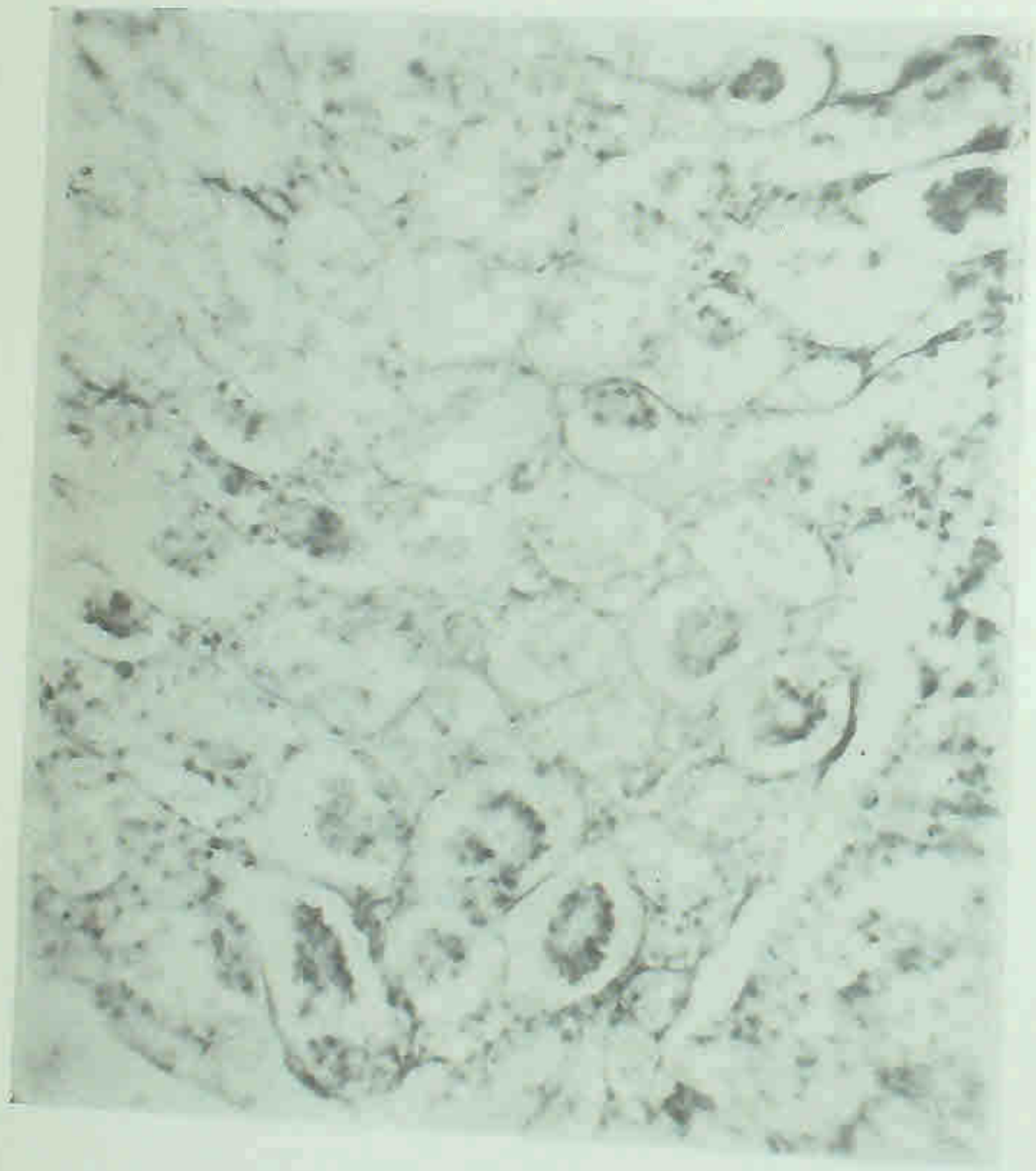

FIG. 7

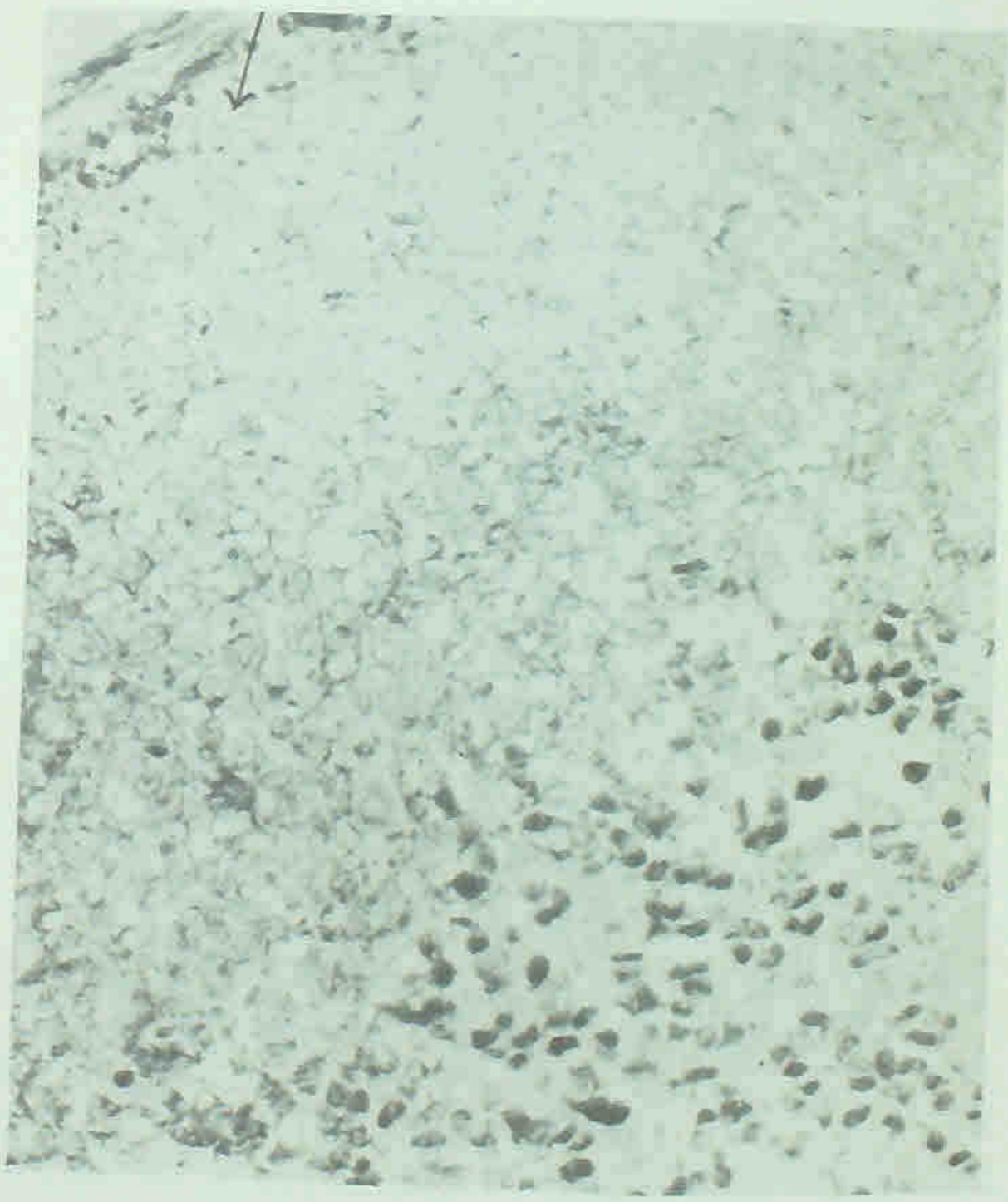

FIG. 8

Pathological changes caused by Mica dust

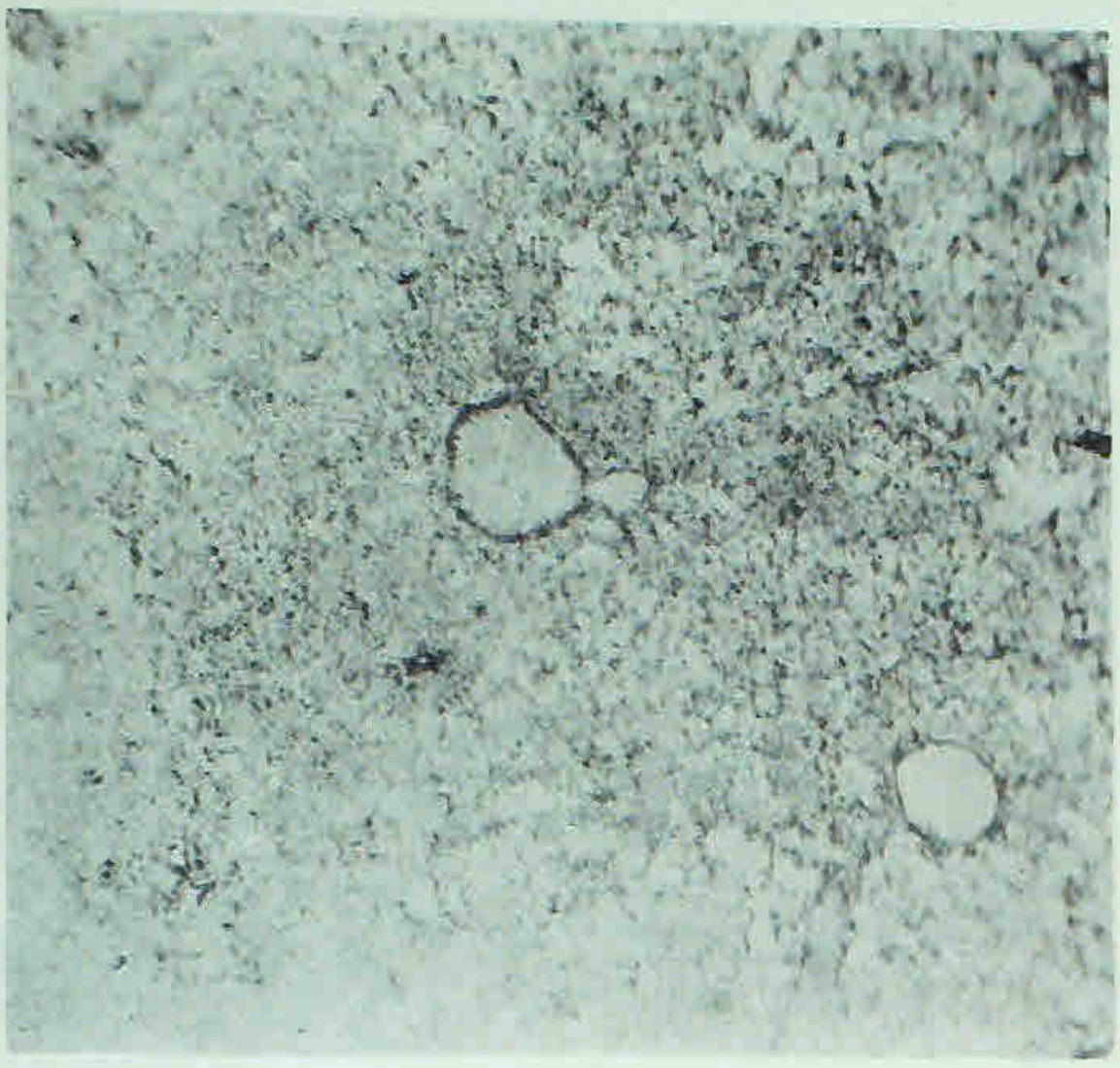

FIG. 1

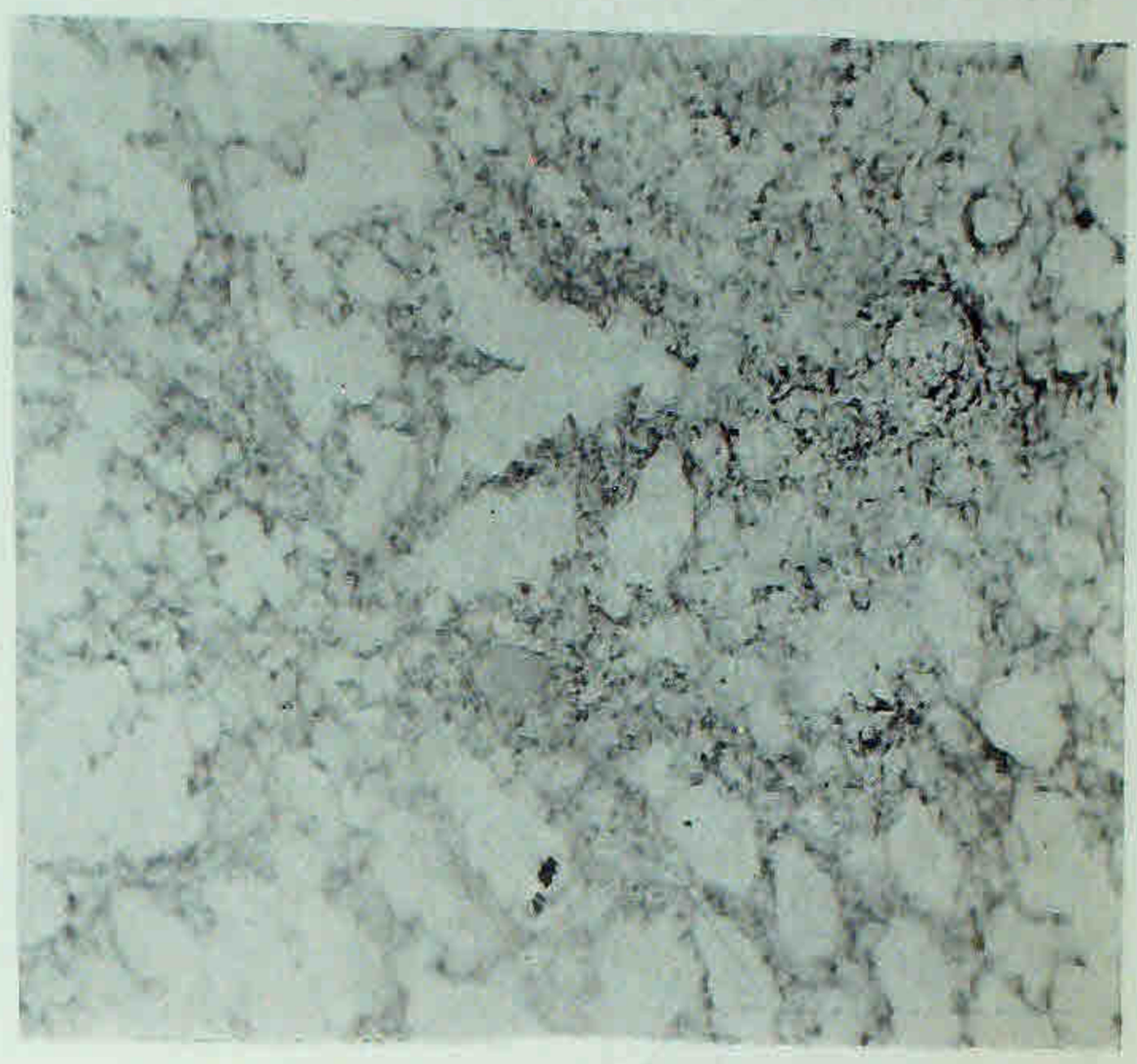

FIG. 2

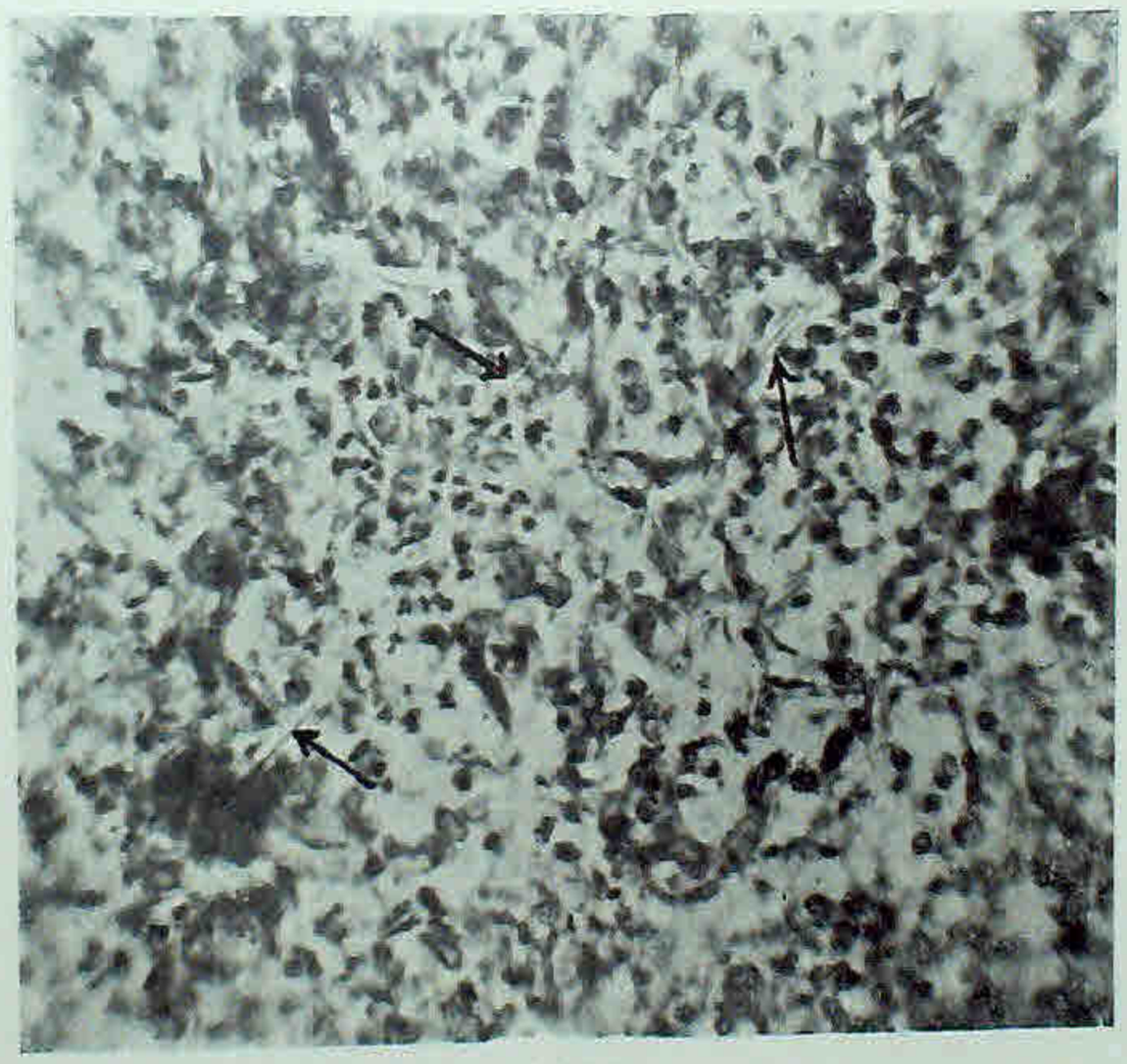

FIG. 3

Pathological Changes caused by Asbestos dust

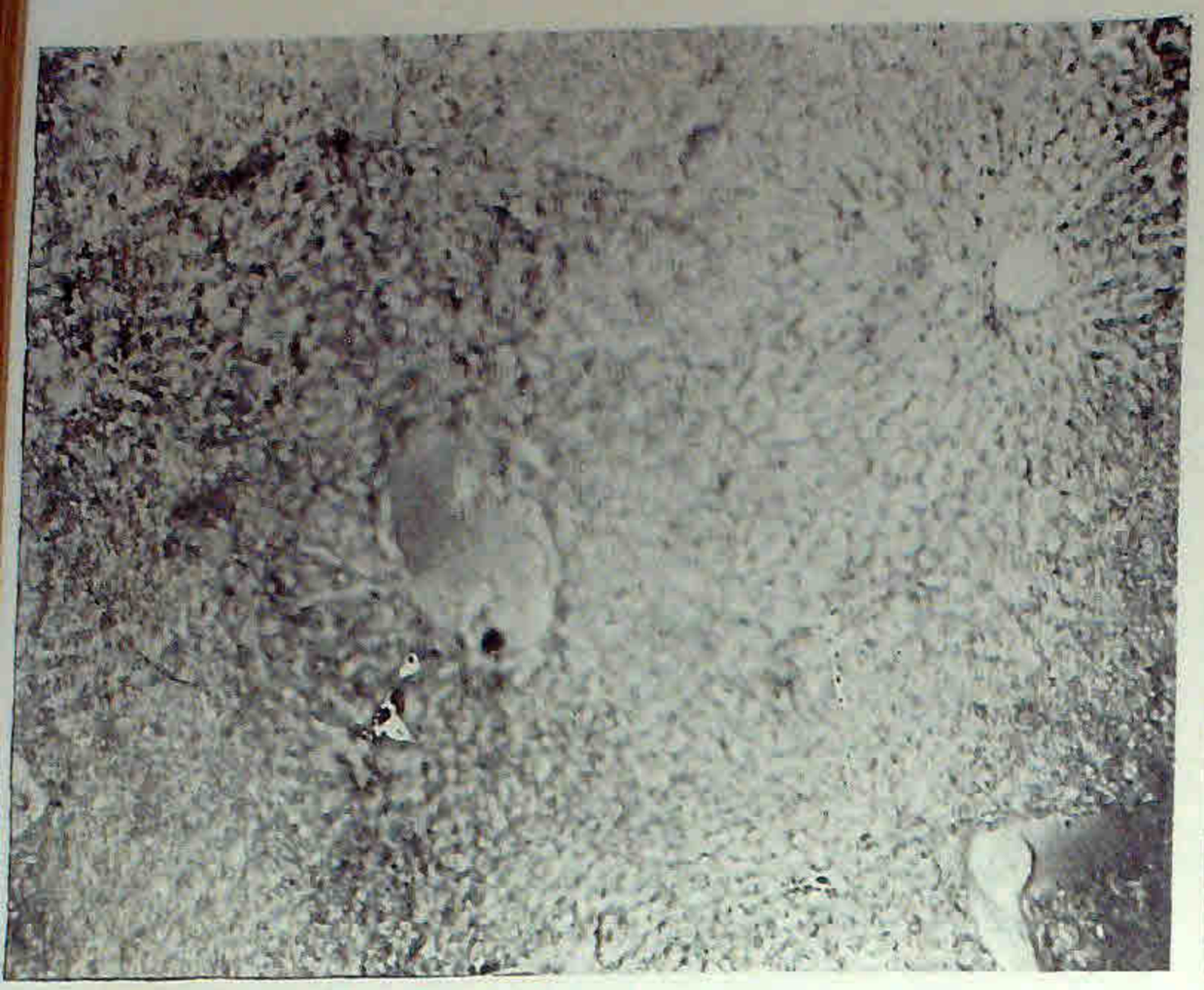

FIG. 4

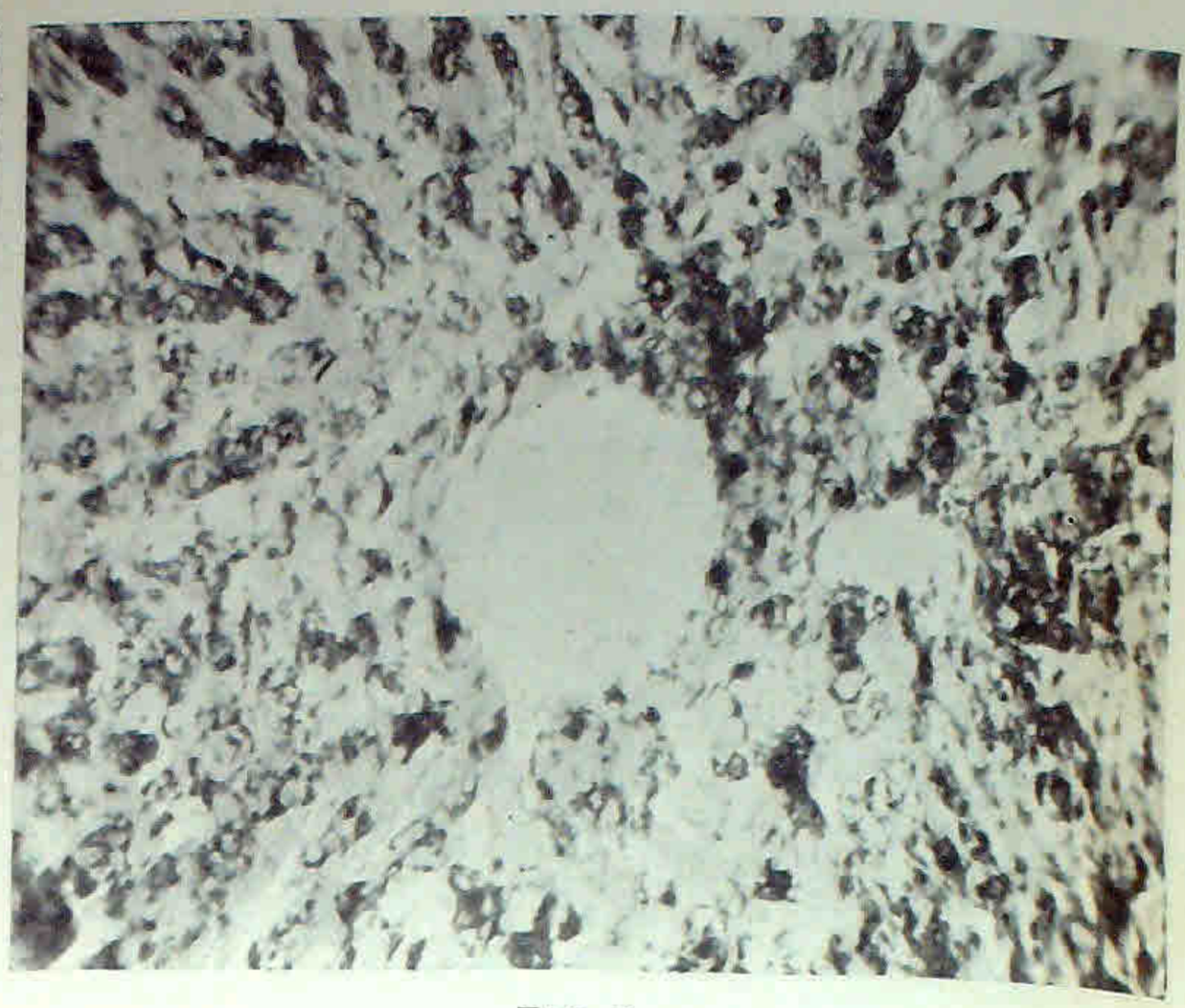

FIG. 5

Z.G.

FIG. 6

FIG. 7

Pathological changes caused by Asbestos dust

PLATE X

FIG. 5. Section of kidney of guinea-pig, showing glomerulonephritis. Capillary tufts show slight separation. H and E, × 250.

FIG. 6. Kidney, showing hæmatoidin crystals present scattered throughout the tissue. H and E, × 120.

FIG. 7. Kidney, showing marked swelling and degeneration of tubule cells and occlusion of lumina in some places. H and E, × 250.

FIG. 8. Section of suprarenal gland of guinea-pig. Zona-glomerulosa is markedly affected. H and E, × 120.

ASBESTOS

Pathological Changes Caused by Asbestos Dust

PLATE XI

FIG. 1. Section of lung of guinea-pig. Group of bronchioles showing marked dilation. Bronchiolar plug contains fibrinous exudate. The lung shows stage of hepatisation and is solid with dust-laden cells. H and E, × 60.

FIG. 2. Lung of guinea-pig, showing patchy character of the exudate. Typical picture of bronchopneumonia is seen. H and E, × 60.

FIG. 3. Lung of guinea-pig, showing crystal of asbestos in the alveoli. No typical asbestos bodies are seen. H and E, × 250.

PLATE XII

FIGS. 4 and 5. Section of liver of guinea-pig, showing marked hydropic degeneration. Central lobular necrosis is seen. 60th day. H and E, × 60 and × 250 respectively.

FIG. 6. Section of kidney of guinea-pig, showing tubular degeneration and occlusion of lumina. H and E, × 250.

FIG. 7. Section of suprarenal gland of guinea-pig The cortical portion shows no marked changes. H and E, × 120.