THE PULMONARY LESIONS PRODUCED BY THE INHALATION OF DUST IN GUINEA-PIGS.

A REPORT TO THE MEDICAL RESEARCH COUNCIL.

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(With Plates VI–X.)

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1. INTRODUCTORY.

THESE experiments on the lesions produced by the inhalation of certain dusts in guinea-pigs were undertaken on behalf of the Medical Research Council, which very generously provided the necessary funds and assistance.

Over 100 animals, including the controls, have been used in the course of these observations, extending over a period of two years, observations which form, in part, a sequel to the experiments of Mavrogordato (1918) also carried out on behalf of the Medical Research Council.

The object of this investigation was partly to complete certain of Mavrogordato's experiments, partly to examine the effects of various other dusts upon the lungs.

In addition, the controversial question of the origin of the dust-cells, and the modes of elimination of the dust particles from the lungs, have been carefully studied.

And, finally, evidence has been brought to bear upon the alleged relationship between the degree of harmfulness of a given dust and the degree of sharpness and of angularity of its constituent particles.

My acknowledgments are many. I am indebted to Dr J. S. Haldane for suggestions on many points. It is with pleasure that I take this opportunity of expressing to him my gratitude for many kindnesses. I have to thank Professor Sir Charles Sherrington for his continual interest in my work and for according me every facility in his laboratory. I am also deeply indebted to Dr A. G. Gibson for valuable comment on the lung lesions, while to the Managing Director, and to Dr White, of the Worcester Royal Porcelain Co., I must express my gratitude for information on the dusts used in connection with the manufacture of china. I am also indebted to this establishment for the benefit of a personal visit. And, finally, I have to thank Prof. Mellor for supplying certain of the dusts used in these experiments, and the Mining Research Laboratories of the University of Birmingham for igniting the samples of earth and shale.

I have to thank Mr F. Haynes for taking over the technical portions of this research and for doing a number of the autopsies, and Mr W. Chesterman of the Anatomical Department for his skilful microphotographic rendering of difficult material.

2. LITERATURE.

The bibliography of Dust-Inhalation is so vast that no useful purpose would be served by mentioning other than the more important memoirs bearing directly on the subject-matter of this paper. The memoirs are summarised in chronological order, further comment being reserved for the discussion on pp. 455 to 463.

Arnold, in 1885, published the most important of the early contributions. In this lengthy monograph, which contains no little repetition, Arnold brings out the following points:

The inhalation of soot, emery and sandstone particles by rabbits and dogs caused

definite lung changes. These comprised thickening and infiltration of the alveolar walls, processes so marked as sometimes to result in the formation of nearly solid areas of lung. Dust particles—both intra- and extra-cellular—were observed in the lung substance, in the bronchi, and in the bronchial glands.

From the inspection of the figures illustrating Arnold's sections, made, of course, at a time when staining methods were not in current use, one suspects that the chief lesions were those of an interstitial pneumonia with well-marked fibrosis. Arnold noted, furthermore, that some of the dust left the lungs and was deposited in the lymphoid tissue both beneath the bronchial epithelium and in the bronchial glands. Without committing himself definitely, he was of opinion that soot particles, at any rate, found their way from the alveoli into the lymphoid tissue *via* the lymphatics. In this way he accounted for the tendency for dust to disappear from the pulmonary alveoli in course of time. The large cells, often containing dust particles, which Arnold observed in the alveolar cavities, he regarded as being derived by proliferation from the alveolar epithelium.

Tchistovitch (1889) studied the phagocytosis of foreign bodies in the lungs with special reference to the origin of the cells which ingest them. He injected carmine particles into the trachea of frogs and guinea-pigs. He also caused the latter to inhale lamp-black in a special chamber.

Tchistovitch observed that phagocytosis occurred, in all these experiments, after a latent period, which, in the case of guinea-pigs exposed to fine soot particles, was approximately 14 hours. He claimed that the phagocytic cells were always macrophages originally derived from the leucocytes of the blood, and that the alveolar epithelium, though often covered with soot and other particles, neither ingested them nor ever showed signs of proliferation. In all these respects the views of Tchistovitch are diametrically opposed to those of Arnold.

Claisse and Josue (1897) repeated the lamp-black experiments just described on dogs, rabbits and guinea-pigs. They confirmed Arnold's view that dust particles tend to be eliminated from the lungs *via* the lymphatics to the bronchial glands and, ultimately, to the spleen. With regard to the effects produced by the dust they say: "Il se produit donc, à la longue, chez nos animaux, une infiltration charbonneuse considérable des poumons et des ganglions bronchiques....Les poumons sont en pleine activité épithéliale,...mais nous n'avons pas constaté chez nos animaux purement anthacosiques de véritable réaction inflammatoire."

These authors further deny that siderosis produces lung lesions, which, they assert, are only caused by dusts composed of large and angular particles. The trauma caused by these to the bronchial epithelium results in infection by micro-organisms and consequent lesion to the adjacent lung substance.

Metchnikoff (1901) in accordance with his phagocytic theory of defence, strongly upholds the contention of his pupil Tchistovitch (*vide sup.*) that all the phagocytic cells found in the lung under inflammatory conditions are derived from the leucocytes of the blood.

Chantemesse and Podwyssotsky (1901) claim that the removal of dust from the lung is largely due to leucocytes apparently derived from the blood.

Washbourne and Eyre (1902) note the presence of areas of collapse in the lungs of animals exposed to dust.

Dust particles within the alveoli are eliminated thus: "some pass through into the lymphatics and either reach the bronchial glands, where they are retained, or are retained in the adenoid tissues (peri-bronchial and peri-vascular) and thence may be excreted to the bronchi by means of cells, whilst some are taken up by cells and thus passed into the bronchi are ultimately extruded through the upper air passages."

Oliver (1903) concludes from the study of anthracosis among coal-miners, that a mild degree of pulmonary anthracosis is not inconsistent with health. He also considers that tubercle, when present in such cases "is an accidental infection."

Wainwright and Nichols (1905) made some remarkable observations on the protective effects of coal dust against infection by the tubercle bacillus. One group of guinea-pigs was exposed to coal dust for two months; the animals then received an intra-tracheal injection of a culture of tubercle bacilli. The animals of the other (control) group were not dusted, but received a similar injection. The animals exposed to the coal dust showed "extensive

T.B. of the abdominal viscera and of the glands around the tracheal region, but the lungs were free." The controls, on the other hand, were found to have "extensive T.B. of the lungs and abdominal viscera."

Briscoe (1908) exhaustively studied the effects of foreign bodies of various kinds—micro-organisms, red blood corpuscles, etc., but not dust particles—on the alveolar epithelium of guinea-pigs. He concludes that the alveolar epithelial cells undertake most of the phagocytosis in slight infections, while the polymorphonuclear leucocytes are the most actively phagocytic elements in acute infections. Regarding the origin of the dust, and other large phagocytic, cells of the lung, Briscoe is of opinion that they are derived from the alveolar epithelium by proliferation.

Beattie (1912) made experiments on the effects produced in guinea-pigs by the inhalation of the following dusts:

(i) Coal.

(ii) Shale (as used for stone dusting in collieries against explosions).

(iii) Stone dust containing much silica.

From these experiments Beattie distinguishes between the dusts which are harmful —*i.e.* produce fibrosis—and those which are not. The dusts which cause fibrosis increase the susceptibility to infection. Beattie concludes that "the more irritating the dust the more intense the fibrosis."

Haythorn's (1913) observations on the reaction of the lungs of guinea-pigs to the inhalation of lamp-black leads him to the conclusion that the dust-cells are probably derived from Endothelial Leucocytes—*i.e.* Large Mononuclears and Transitionals. Haythorn is further of opinion that "...carbon pigment once taken up by the cells remains intra-cellular indefinitely unless freed by some process producing general necrosis of the tissues."

Haldane (1916) has furnished the following figures as a striking testimony of the relative rarity of phthisis among coal-miners:

Death-rates from phthisis per 1000 *living at each age-period for England and Wales*, 1901–1902.

Age period	15–25	25–35	35–45	45–55	55–65
Occupied and retired coal-miners	0·7	1·0	1·1	1·5	2·0
Occupied and retired farm labourers	0·6	1·15	1·3	1·4	2·6

From this it is evident that the incidence of phthisis among coal-miners is definitely less than among farm labourers. On the other hand, Haldane points out that the death-rate from bronchitis in coal-miners is high when compared to other occupations. This, however he does not think bears any definite relation to the inhalation of coal dust, since the death-rate from bronchitis among coal-miners has markedly *decreased* in recent years although the amount of dust in the air of the mines has *increased* (largely owing to more powerful ventilation) within the same period.

Haldane is of opinion that the most probable cause of the diminution in the death-rate from bronchitis among coal-miners is to be found in the better ventilation of the mines and the consequent lessening of the respiratory movements. This latter would entail a smaller tendency to emphysema, and, following this, bronchitis.

Mavrogordato (1918) has made the most important contribution to the experimental study of dust-inhalation of recent years.

This author exposed guinea-pigs to different kinds of dust in a special chamber, the dusty atmosphere being maintained by a fan. His main conclusions are as follows:

Coal is rapidly eliminated from the lungs provided that the amount inhaled is moderate.

Flue dust causes plaque formation and patches of broncho-pneumonia.

Shale gives results intermediate between coal and flue dust: no permanent lesions occur, but the rate of elimination is slow as compared to coal.

Transvaal dust causes marked lung changes and remains (largely) in the lungs.

Pure precipitated silica is rapidly eliminated.

A *mixed dust—i.e.* flint and coal—was finally tested. The results of this were not conclusive, but Mavrogordato inclines to the view that the "lungs would practically free them-

selves from flue or crystalline silica dusts if these dusts enter in small quantitites only with coal." The greatest length of time which elapsed in any of these experiments between the last exposure to dust and the autopsy was 10 months.

Haldane (1918) has dealt with the importance of dust-inhalation in various occupational diseases. He points out that the lesions produced by quartz dust, which are usually attributed to the sharpness of the particles, are probably not due at all to this, since microscopically such particles are neither sharper nor more angular than those of other and more innocuous dusts. Haldane also stresses the fact that the harmful dusts are those which are not eliminated with sufficient rapidity from the lungs, while the harmless ones are taken up by cells and quickly eliminated.

Sewell (1918) has made a careful study of the phagocytic powers of alveolar epithelium. His technique consisted in administering to rabbits preliminary intravenous injections of carmine; 24 hours after the last injection another—and this time intratracheal—injection was made. The substances introduced into the trachea included Indian ink in normal saline, pigeon's blood, *Staphylococcus pyogenes aureus* in normal saline and spores of *Oidium albicans* in normal saline. The animal was killed 3 to 24 hours after the intratracheal injection. These experiments showed that whereas the phagocytic cells within the alveoli, which Sewell seems largely to consider as being derived from the alveolar epithelium, took up the substance injected into the trachea, they failed to ingest the carmine particles. The latter, on the other hand, were engulfed by the macrophages of the blood. The great merit of Sewell's experiments is that they appear conclusively to demonstrate that the intra-alveolar macrophages or dust-cells are derived from alveolar epithelial cells and do not represent the macrophages of the blood stream.

Permar (1920, 1, 2, and 3), after repeating Sewell's experiments with a slightly modified technique, has come to the conclusion that the "Dust-cells" are endothelial elements derived from the blood capillaries of the alveoli. These cells, after irruption into the alveolar cavities and phagocytosis of pigment particles, slowly make their way back through the alveolar walls into the lymphatics. Stages in the proliferation of the endothelial elements—destined to become dust-cells—are figured by Permar. The fact that the dust-cells, though of vascular origin, are not to be found in the general circulation, is regarded by this author as evidence of their local origin and distribution.

Westhues (1922), after making experiments with a technique very similar to that of Permar, has come to conclusions diametrically opposed, in that Westhues regards the dust-cells as being derived from the alveolar epithelium and not from the endothelial cells of the pulmonary capillaries.

Gye and Kettle (1922, 1 and 2) have investigated the relation between silicosis and tuberculosis along novel lines. These authors found that the subcutaneous injection of mice with finely powdered silica (either insoluble or colloidal) in saline produces characteristic lesions at the site of inoculation. These changes comprise a focus of coagulative necrosis surrounded by leucocytes; this focus is subsequently absorbed and replaced by an inflammatory fibrosis. It was also noted that the injection—simultaneous or previous—with Tubercle Bacilli of such an area was followed by marked proliferation of the bacilli. On the other hand, subcutaneous injection of Tubercle Bacilli, without injection of silica, caused but slight tissue response with relatively slight multiplication of the Tubercle Bacilli, the mouse possessing a high degree of natural immunity towards these organisms. Clearly, then, the presence of silica in subcutaneous connective tissue favours the survival and multiplication of the Tubercle Bacilli. This the authors suggest is due to silica being a cell-poison, with the result that the bacilli thrive in the silica-laden areas of damaged and necrotic tissue. In the case of phthisis subsequent to pulmonary silicosis an additional factor is involved—the disorganisation of the lymphatics of the lung by the silica[1].

[1] On going to press (May, 1924), Fenn's admirable studies on the rate of phagocytosis of coal and quartz particles came to my notice. This observer found that carbon particles were ingested by leucocytes about four times as readily as quartz; quartz, in acid solutions, was ingested more rapidly than carbon, but carbon more readily than quartz in alkaline solutions. The applications of these quantitative studies of Fenn's to other dusts would be of interest. (Fenn, W. O., "Phagocytosis of Solid Particles," Studies Nos. I and III, *Jour. Gen. Physiol.* III. 1921, pp. 439 and 575.)

3. MATERIAL AND METHODS.

The animal used throughout these experiments was the guinea-pig. The dust cloud was raised in the apparatus already employed by Mavrogordato (1918) consisting of a large wooden box bearing a piece of plate-glass as a cover and a two-bladed fan working in a circular channel at its bottom. The animals were placed on a wire grid midway between the top and bottom of the box.

Various methods of killing the guinea-pigs were tried. Killing by chloroform or ether anaesthesia was unsatisfactory on account of the great congestion produced in all the viscera—and in the lungs in particular—just prior to death. The following method gave the most satisfactory results: One of the carotids was rapidly dissected out under chloroform anaesthesia; the artery was severed and the animal bled to death. To reduce collapse of the lungs to a minimum the trachea was ligatured before opening the thorax and removing the lungs. The latter were fixed in corrosive sublimate—formol, a mixture which was found to have the great advantage over Bouin's fluid—so commonly employed for fixing lungs—that red and white blood corpuscles are accurately fixed. The method of distension fixation was tried and not found to be as satisfactory as the technique of immersion fixation of lungs and heart after occlusion of the trachea. It is comparatively easy in one and the same specimen to burst the alveoli in some parts of the lung and not sufficiently to distend them in others.

The lungs were embedded in paraffin wax under reduced atmospheric pressure (450 mm. Hg). This method of embedding is almost essential for the successful penetration of the paraffin into the air-laden lungs. The only other alternative is to keep the pieces of lung for weeks in 70 per cent. alcohol until the air has disappeared, and then imbed at atmospheric pressure in the ordinary manner. One precaution, however, has to be observed when employing the technique of vacuum embedding. The reduction of the pressure has to be slowly made, for unless this is done, the alveoli are distended and may even burst, thereby producing an artefact microscopically indistinguishable from true pulmonary emphysema. The production of this artefact was guarded against by ascertaining the rate and degree of reduction of pressure which could safely be applied to normal guinea-pig lungs, the rate of removal of air from the pathological material being kept well within these limits.

The standard staining method was Ehrlich's haematoxylin and eosin. In cases of suspected fibrosis Heidenhain's iron haematoxylin counterstained with van Gieson's or Mallory's connective tissue stain were employed.

For the bacteriological examination of the sections the Claudius-Neutral Red and Ziehl-Neelsen stains were used as routine methods.

Many other stains were also employed from time to time, but no useful purpose would be served by describing them here.

4. THE HISTOLOGY OF THE NORMAL GUINEA-PIG LUNG.

To establish as far as possible the limits of normality, the lungs of animals which had never been placed in the dusting machine were examined at intervals. This precaution is essential in view of the fact that pigment particles can be found in the lungs of most—if not all—domesticated animals.

As the result of the microscopical examination of the lungs of seven control guinea-pigs, the following points were confirmed or established:

1. The lungs of guinea-pigs kept under the usual conditions of confinement contain fine black intra-cellular dust particles. Although there is no difficulty in finding these with the microscope, they are always relatively scanty when compared to the pigment particles seen in the lungs of the dusted animals. This dust is probably of the nature of fine carbon particles derived from the air, though the possibility of dust from the straw bedding, etc., cannot be excluded.

2. Fibrous (*i.e.* collagen) connective tissue is scanty in the guinea-pig lung as compared to its development in the larger mammals and especially man. In the normal guinea-pig lung fibrous tissue is only found in the following situations:

(*a*) Beneath the pleura as a thin sub-serous layer.

(*b*) Around the large and medium-sized bronchi.

(*c*) Around the larger branches of the pulmonary veins. As a consequence of the paucity of fibrous tissue, the guinea-pig lung is of very delicate texture (see Plate VI, figs. 1 and 2).

3. Elastic tissue is highly developed around the alveoli of normal guinea-pig lung.

4. The presence of eosinophils, often in large numbers, in and around both epithelium and connective tissue of the bronchi and trachea is normal. This observation was first made by Opie (1904).

5. The media of the smaller branches of the pulmonary arteries, as noted by Jordan (1920), forms curious oval thickenings in the wall of these vessels.

6. The lungs of control animals often show small areas of congestion, as already pointed out by Beattie (1912). Examination of such areas reveals congestion of the smaller blood-vessels, capillary engorgement, and (usually) an increase in the number of alveolar epithelial cells. The alveolar walls are consequently somewhat thickened. Such an area of congestion is shown in Plate VI, fig. 3.

7. Small areas of broncho-pneumonia were noted in the lungs of three out of the seven control animals. No symptoms were noticeable in these guinea-pigs. Examination of sections stained by the Ziehl-Neelsen and Claudius-Neutral Red methods failed to show any organisms, although the presence of broncho-pneumonic foci is strongly suggestive of an infection.

8. The bronchial glands of normal guinea-pigs sometimes show scanty black intra-cellular particles. Eosinophil infiltration of both gland substance and capsule is not uncommon, as noted by Opie (1904). Occasionally eosinphils may be found in the afferent lymphatics of the gland.

This infiltration of lymphoid, peri-bronchial and peri-vascular connective tissue is to be regarded as perfectly normal in guinea-pigs.

Lymphoid tissue is widely distributed throughout the normal guinea-pig lung. Not only are small nodules present in the peri-bronchial connective tissue, but they also occur in the lung parenchyma itself.

To ascertain whether the lesions observed in the dusted animals were due solely to the dust and not to the draught created within the machine by the revolving fan, the following control experiment was devised:

Four animals were exposed to the draught within the machine for the same period as the dusted animals—*i.e.* two hours daily for fourteen days (Sundays excluded).

Three of the guinea-pigs were perfectly healthy at the end of the experiment, one coughed, panted, and was very weak from the tenth day of exposure onwards.

Microscopic examination of the lungs of these animals was made 24 hours after the last exposure.

The lungs of the three healthy animals show large areas of normal lung. Interspersed among these, however, are patches of slightly thickened alveoli, and, in each of the specimens, a small area of broncho-pneumonia.

The lungs of the diseased guinea-pig show a massive broncho-pneumonia, with marked "cuffing" of the pulmonary veins by lymphocytes. The examination of sections stained for bacteria by the Ziehl-Neelsen and Claudius-Neutral Red methods failed to reveal organisms.

Lung changes such as those found in the three healthy animals exposed to the draught are so frequent in guinea-pigs kept in confinement that it would be unwarranted to ascribe them to the draught in the machine, which, moreover, is very slight. The massive broncho-pneumonia of the fourth animal was probably due to infection, since guinea-pigs from the normal stock have died from time to time and presented similar lesions.

5. THE LESIONS CAUSED BY THE DUSTS USED IN THESE EXPERIMENTS.

The mode of dusting was as follows: The animals were exposed to the dust for two hours daily over a period of 14 days, Sundays excluded. Hence the total number of days on which the guinea-pigs were exposed was 12. The dusts were measured by volume, and the total amount of dust placed in the machine for each period of two hours was 135 c.c.

(1) INHALATION OF A MIXTURE OF FLINT AND COAL.

Series 1.

Two parts of flint to one of coal by measure, the total volume of dust administered at each dusting period being 135 c.c. The lungs of most of the animals of this series showed, at autopsy, some degree of pigmentation.

No. 1. *Animal killed three hours after the last exposure.*

Microscopically there is thickening and cell proliferation of the alveolar epithelium. The lungs contain much intra-cellular dust, some of the dust-cells being free within the alveolar cavities while others are still attached to the alveolar walls. The smaller bronchi contain plugs of dust-cells. The bronchial glands show no more dust than is usually found in control animals (see p. 444).

No. 2. 24 *hours after exposure.*

Microscopically the alveoli show the same changes as in the 3-hour specimen; in addition, there are areas of collapse and, rarely, of oedema. Dust particles numerous and evenly distributed as shown in Plate VI, fig. 5. Bronchi often plugged. Bronchial glands normal.

No. 3. *One month after exposure.*

Large areas of lung are normal except for the presence of a little dust. Elsewhere there is capillary congestion, proliferation and thickening of the alveolar epithelium. Dust-cells are abundant and there is marked eosinophil infiltration of the lung substance. Smaller bronchi sometimes plugged. Some dust in the peri-bronchial lymphatics. Bronchial glands contain more dust than is normal for control guinea-pigs.

No. 4. *Six months after exposure.*

Areas of normal lung alternate with areas of cell-proliferation and capillary congestion. More normal tissue in the lobes than in the apices. No dust in the lymphoid tissue of the lung. There is less dust than in No. 3. There are small areas of incipient *plaque-formation.*

By *plaque-formation* is meant the appearance of conglomerations of thin squame-like cells, the cytoplasm of which is packed with dust particles. The nuclei of such cells are often multiple and degenerate. Vascular connections

are lacking within these areas of plaques, which I interpret as an index of incomplete phagocytic response on the part of the dust cells: the dust is ingested, but the cells degenerate and die before they have removed it from the lung substance. Areas of plaque-formation, if of any size, represent permanently damaged areas of lung substance. I have noticed, however, that while some of the lungs of animals exposed to a given dust contain plaques, the lungs of animals from the same series, but killed several months later, may not show them. Often this can only be ascribed to differences in the susceptibility of the lungs in different animals. But not always, and in such cases I think that the disappearance of the plaques from the lung is due to plaque-formation having occurred *not* in the lung parenchyma but inside the lymphatics of the lung—notably the sub-pleural lymphatics. Under such conditions, the necrotic dust-containing cells are somewhat isolated from the lung parenchyma and fibrosis does not supervene. On the other hand, disintegration of the plaques within the lymphatics may well lead to the removal of the dust to adjacent lymphoid tissue, and notably the bronchial glands. It is significant that some of the smaller plaques appear to be encircled not by proliferated alveolar epithelium but by flattened cells strongly suggestive of the endothelium of a lymphatic. Large plaques, however, I regard as permanent lesions in contact with the smaller—intra-lymphatic—plaques. Plaques are figured in Plate VII, figs. 13 and 14.

No. 5. $9\frac{1}{2}$ *months after exposure.*

The alveolar epithelium shows extensive thickening and cell-proliferation. There are areas of broncho-pneumonia as shown in Plate VI, fig. 6. Eosinophil infiltration is very slight. Dust-cells—often multi-nucleate—are present in fair numbers. The bronchial glands contain a little intra-cellular dust. The changes are more pronounced in the apices than in the lobes.

No. 6. $12\frac{1}{2}$ *months after exposure.*

Large areas of normal or slightly emphysematous lung alternate with small patches of broncho-pneumonia, which latter contain dust-laden plaques. Plaque-formation is especially pronounced beneath the pleura. Changes less marked in lobes than in apices. Plaque-formation in these lungs is shown in Plate VII, figs. 13 and 14.

No. 7. 16 *months after exposure.*

Large areas of lung approximate towards the normal except for the presence of dust-cells in the alveolar walls. In some parts of the lung broncho-pneumonia is present. In these regions (see Plate VII, figs. 8 and 9) the alveolar walls are thickened and the alveolar cavities largely obliterated. There are massses of dust-containing cells aggregated into small plaques. "Cuffing" by lymphocytes of some of the smaller branches of the pulmonary veins has occurred. No fibrosis; no eosinophil infiltration. The lymphoid tissue of the lung contains a little dust, the bronchial glands a good deal. The most damaged areas of the lung are those containing the most dust, but there is considerably less dust in the specimen than in No. 6 of this series.

Series 2.

A few animals were exposed to the same amount of flint and coal for the same dusting periods and for the same number of days as in Series 1, but the

relative proportions of the two dusts were reversed—*i.e.* 2 parts of coal were mixed with 1 part of flint by measure (instead of 1 of coal to 2 of flint).

The records of this series may be summarised as follows:

Two animals, *killed* 11 *days after exposure*, show general thickening and proliferation of the alveolar epithelium, patchy areas of broncho-pneumonia and much dust.

A specimen of lung 3½ *months after exposure* shows marked broncho-pneumonia with massive eosinophil infiltration. The lesions are most pronounced in the lobes—an exceptional occurrence. There is a fair amount of intra-cellular dust.

The lungs of two animals killed 9 *months after exposure* reveal a fair amount of normal tissue, areas of thickened alveolar epithelium and, specially in one of the specimens, patches of broncho-pneumonia without eosinophil infiltration. The bronchiolitis has considerably subsided. Giant cells containing dust particles are present. No fibrosis; no plaque-formation.

(2) INHALATION OF CHINA CLAY.

(AS SUPPLIED TO THE POTTERIES.)

Series 1.

The standard amount of dust (135 c.c.) was administered at each dusting period. At autopsy the lungs showed greyish streaks on their surfaces.

No. 1. *Animal killed* 24 *hours after the last exposure.*

The alveolar walls are somewhat thickened—especially in the apices. Large areas of lung are normal except for the presence of inspissated particles—dark grey to brown—plastered here and there on to the alveolar walls. Only a little of the dust is intra-cellular. The bronchi contain a few dust-cells and a little free dust. The bronchial glands are normal.

No. 2. *One month after the last exposure.*

The condition of the lungs and bronchial glands is as for No. 1 of the series with the following differences: There is slight collapse beneath the pleura in some parts of the lung (see Plate VII, fig. 10), while the dust particles are now nearly black.

No. 3. *Two months after the last exposure.*

In some parts the alveolar walls are thickened and there is capillary congestion. Large areas of lung are normal except for the presence of a moderate amount of intra-cellular dust. The dust-cells are nearly always attached to the alveolar walls.

No. 4. *Three months after exposure.*

The lesions have progressed. There are numerous areas showing great proliferation and thickening of the alveolar epithelium. Capillary engorgement is marked. Dust-cells, both free and attached, are abundant. There is capillary bronchitis in many areas of the lung, but dust particles are scanty among the cell-débris in the lumina of the bronchi.

No. 5. *Four months after exposure.*

The lung changes are as for No. 4 of the series while, in additon, incipient plaque-formation has set in. The lungs still contain a moderate amount of dust, a little of which can be seen within the macrophages in the lymph sinuses of the bronchial glands.

No. 6. *Six months after exposure.*

Thickening of the alveolar walls is general, except where areas of emphysema occur. There is capillary congestion and eosinophil infiltration. There are areas of broncho-pneumonia. Such an area is depicted in Plate VII, fig. 11; in it can be seen plaques and a massive eosinophil infiltration. Dust is present in moderate amount. The lesions are more severe in the apices than in the lobes.

Series 2.

The experiments were repeated, using the same dust, but at half the concentration previously employed—*i.e.* 65 c.c. of china clay being administered at each dusting period.

The lesions caused show no appreciable difference when compared with Series 1. The last animal of Series 2, killed nine months after exposure, contained many plaques in its lungs. The bronchial glands showed some intra-cellular dust.

Series 3.

This series was dusted as follows:

1 exposure per week for 14 days,

2 exposures per week for 14 days,

1 exposure every 2 days for 16 days.

The amount of dust administered at each dusting period was as for Series 2 (*i.e.* 65 c.c.).

It will be seen that 14 exposures (two more than in the other series of experiments) were given over a period of about 6 weeks (44 days) instead of 14 days. Further, the intervals between the exposures were progressively shortened as the experiment proceeded.

Animals examined $2\frac{3}{4}$ and 7 months after the last exposure showed not only lesions less severe than in Series 2, but also evidence of a far more active phagocytosis of the dust particles, which were found in both bronchial and abdominal lymph glands. The last two animals examined, 7 months after exposure, showed neither plaque-formation nor fibrosis.

(3) Inhalation of Felspar.

Standard exposures and concentration of the dust. At autopsy the lungs of the animals of this series were normal in appearance up to three months after exposure. Later specimens showed black spots scattered over the surface of the lungs, and, sometimes, areas of congestion.

No. 1. *Animal killed six hours after the last exposure.*

Microscopically there are large areas of normal lung except for the presence of dark brown dust particles plastered on to the alveolar walls. In some places, and especially beneath the pleura (see Plate VII, fig. 12), the alveolar epithelium is proliferating and thickened. The amount of dust is moderate, its distribution patchy. The bronchial glands are normal.

No. 2. *One month after exposure.*

The lung changes are as in No. 1 of this series. No free dust cells were noted. There is some bronchitis. The bronchial glands are normal.

No. 3. *Two months after exposure.*

There are large areas of proliferating and congested alveolar epithelium. Dust-cells mostly attached to the alveolar walls, though a few are present in the smaller bronchi. Small plaques are present (see Plate VII, fig. 14) and the general lesion is that of a bronchopneumonia with eosinophil infiltration. The bronchial glands contain a little intra-cellular dust.

No. 4. *Three months after exposure.*

Lesions as for No. 3, but the plaque-formation and the bronchitis have increased. There is less dust within the lungs than in No. 2 of the series (killed one month after exposure) and the particles are darker.

No. 5. *Five months after exposure.*

General thickening of the alveolar epithelium. There are areas of emphysema and also abundant plaques. Some diapedesis of the red blood corpuscles. The bronchial glands are free from dust.

No. 6. *Nine months after exposure.*

The extensive changes still persist, but are more intense in the lobes than in the apices. No dust—either free of intra-cellular—within the bronchi. The bronchial glands contain a fair amount of intra-cellular dust.

No. 7. *Twelve months after exposure.*

The lesions are less marked than in No. 6, large areas of lung showing only moderate thickening of the alveolar epithelium (see Plate VII, fig. 13). The eosinophil infiltration has subsided. There is still much dust in the lungs. There is a mild fibrosis, the collagen fibres being derived from the peri-bronchial and peri-vascular connective tissue.

(4) INHALATION OF GROUND PITCHER.

(AS USED IN THE POTTERIES. GROUND PITCHER DENOTES THE CRUSHED-UP EARTHENWARE AFTER FIRING.)

Standard exposures and concentration of the dust. At autopsy the lungs were always pigmented.

No. 1. *Animal killed* 24 *hours after the last exposure.*

Microscopically the lungs show a general thickening and proliferation of the alveolar walls. In one apex there is an area of broncho-pneumonia with eosinophil infiltration as depicted in Plate VIII, fig. 15. There is much dust, the particles varying from yellow to black. Some of the dust is intra-cellular; the dust-cells are only rarely free. Bronchitis is present, but the bronchi are dust-free. The bronchial glands are normal.

No. 2. *Three months after exposure.*

Lesions as in No. 1 of the series; in addition, plaque-formation has set in and many of the macrophages of the bronchial glands contain dust particles.

No. 3. *Four months after exposure.*

The broncho-pneumonia has been largely replaced by interstitial changes, which, where pronounced, comprise an invasion of the dust-laden and greatly thickened alveoli by strands of fine fibrous tissue (see Plate VIII, fig. 16). The peri-vascular lymphatics often show accumulations of lymphocytes around and within them. Catarrhal changes are present in the bronchi. The bronchial glands contain intra-cellular dust.

No. 4. *Six months after exposure.*

The lungs show general interstitial changes and also large patches of broncho-pneumonia (see Plate VIII, fig. 17). In some of the areas of plaque-formation many young collagen fibres and fibroblasts are apparent. There are still many black dust particles in the lungs. The bronchial glands contain dust-laden macrophages, apparently blocking the lymph sinuses, in large numbers as shown in Plate VIII, fig. 18.

No. 5. *Nine months after exposure.*

This animal has a large abcess containing cheesy pus on the left side of the head affecting the eye on the same side. The lungs show extensive broncho-pneumonia, some areas of emphysema, and a little normal tissue (see Plate VIII, fig. 19). The bronchial glands contain much dust, the lymph sinuses being blocked by conglomerations of macrophages containing black pigment particles.

(5) Inhalation of Pure Amorphous Silica.

(dehydrated and rendered insoluble by heating.)

Usual concentration and exposures. At autopsy the lungs of the animals of this series showed grey to black pigmentation, and, sometimes, punctiform haemorrhages.

No. 1. *Animal killed a few hours after the last exposure.*

Microscopically there is general thickening of the alveolar walls, the dust-cells (often multi-nucleate) being attached to them. There is general capillary congestion, and, occasionally, haemorrhage into the alveoli. Some eosinophil infiltration. The bronchi are catarrhal. Much dust is present. The greater severity of the lesions in the apex as compared to the lobe is shown in Plate IX, fig. 25.

No. 2. *One month after exposure.*

The changes are as in No. 1 of the series, but plaque-formation has also appeared. Eosinophil infiltration is very slight. The bronchial glands contain intra-cellular dust particles.

No. 3. *Four months after exposure.*

Bronchiolitis and a patchy broncho-pneumonia persist—the latter associated with eosinophil infiltration. Plaques are absent. Bronchial glands normal.

No. 4. $5\frac{1}{2}$ *months after exposure.*

There are large areas of slightly thickened alveoli. In the azygos lobe, however, consolidation is complete. Here the capillaries are engorged and beaded, the alveolar epithelium is absent, and a cellular exudate fills the alveoli. There is much dark brown intra-cellular dust. The peri-vascular connective tissue shows slight hypertrophy. The condition in this azygos lobe is such as to suggest a chronic broncho-pneumonia associated with a slight fibrosis.

No. 5. *Eight months after exposure.*

The bronchiolitis and broncho-pneumonia persist. Elsewhere the alveolar walls are thickened. The most damaged areas show a slight fibrosis. The lungs contain a fair amount of dark brown dust, but there is no plaque-formation.

No. 6. 13 *months after exposure.*

The changes are much as in No. 5 of the series, but there is no fibrous tissue increase. In addition, sub-pleural plaques are in evidence.

No. 7. 23 *months after exposure.*

The lesions in this specimen comprise extensive bronchiolitis, large areas of broncho-pneumonia with eosinophil infiltration, patches of emphysema and sub-pleural plaque-formation. Much dust still remains in the lungs. The bronchial glands contain abundant dust particles.

(6) Inhalation of Pure Flint.

(as used in the potteries.)

Dusting was carried out according to the usual method. At autopsy the lungs showed a streaky pigmentation and, usually, scattered haemorrhagic areas.

No. 1. *Animal killed* 22 *hours after the last exposure.*

The lungs are histologically normal over large areas; elsewhere, and especially in the

apices, there is acute capillary congestion, some haemorrhage into the alveolar cavities, and proliferation of the alveolar epithelium. Attached to the latter are many dust-cells which are only occasionally found free within the alveoli. There is some bronchiolitis. The most damaged areas show slight eosinophil infiltration. (See Pl. IX, fig. 26.)

No. 2. One month after exposure.

The lesions are as in No. 1 only more pronounced. There is also a patchy broncho-pneumonia and incipient plaque-formation. The bronchial glands contain some intra-cellular dust.

No. 3. Four months after exposure.

The condition of the lungs in this specimen is as in No. 2 of the series, except that dust-containing giant cells have appeared, also areas of emphysematous tissue. The bronchial glands are normal.

No. 4. Seven months after exposure.

The patchy condition of the lungs persists. The areas of broncho-pneumonia show no eosinophil infiltration. There are large necrotic dust-containing cells in some parts of the lung; these probably represent very early plaque-formation. Giant cells, containing dark brown dust particles, are abundant.

No. 5. Nine months after exposure.

The lesions are as in No. 4 of the series. There is, however, some eosinophil infiltration of the areas of broncho-pneumonia.

Nos. 6 and 7. 12½ and 13 months after exposure.

The lung changes are very similar in both these specimens, which show large areas of broncho-pneumonia heavily infiltrated with eosinophils. Pigment-containing giant cells are common. There are no plaques, neither is there any evidence of an increase in the fibrous tissue. Much dust still remains in the lungs. The lesions are far more marked in the apices than in the lobes, as can be seen by comparing Figs. 27 and 28 (Pl. IX). The bronchial glands contain a moderate amount of dust.

(7) INHALATION OF COAL DUST.

A. As a preliminary experiment to test the effect of small quantities of coal dust on the lungs, a guinea-pig was exposed to the usual concentration of dust for two days only. It was killed immediately after exposure.

Histologically most of the pulmonary alveoli are normal, the chief changes being a mild catarrhal reaction of the bronchi (see Plate X, fig. 29). The latter contain plugs of cells (chiefly eosinophils) and a good deal of dust (mostly free).

B. Animals were exposed to coal dust under the standard conditions—*i.e.* two hours' exposure daily for 14 days. At autopsy the lungs showed dark streaks over their surfaces, and, occasionally, a few small haemorrhagic areas. The lungs of the last specimen examined (19 months after exposure) were macroscopically normal.

No. 1. Animal killed six hours after the last exposure.

There is a general thickening of the alveolar epithelium, and also some areas of broncho-pneumonia as shown in Plate X, fig. 30. Dust is abundant, both in the alveoli and the bronchi, but it is in process of active phagocytosis by the dust-cells. Bronchitis is fairly general. The bronchial glands contain a moderate amount of dust.

No. 2. *One month after exposure.*

Large areas of lung substance are only slightly thickened; elsewhere there are patches of broncho-pneumonia, heavily laden with dust, and infiltrated with eosinophils. One of the lobes is emphysematous. Bronchial catarrh is marked, the plugs of cells within the bronchi containing a little free coal dust. The bronchial glands contain a moderate amount of dust.

No. 3. *Four months after exposure.*

The lung substance in the lobes is almost normal, but the apices show some areas of broncho-pneumonia. There is less dust present in the lungs of this specimen than in No. 2 of the series. The smaller bronchi are catarrhal and contain a few dust-cells. The lymphoid tissue of the lung contains some dust.

No. 4. *Two animals nine months after exposure.*

These specimens show a general though slight proliferation of the alveolar epithelium associated with occasional sub-pleural patches of broncho-pneumonia. Dust-cells abound; sometimes they are free within the alveolar cavities, though more usually they project into these from their points of attachment to the alveolar walls. The lymphoid nodules in the lung substance contain a little dust. In one of the specimens there are dust particles within the peri-vascular lymphatics, as well as in the endothelial cells of the intima, of branches of the pulmonary vein. The bronchial glands, only examined in one of the animals, contained much dust in the lymph sinuses.

No. 5. 19 *months after exposure.*

There is a good deal of slightly emphysematous but nearly dust-free lung. Some areas of broncho-pneumonia. Many of the bronchi are catarrhal, but do not contain any dust. A portion of one of the most damaged areas in an apex is shown in Plate X, fig. 31. The bronchial glands contain a moderate amount of dust. Neither plaque-formation nor fibrosis were noted in this animal or in any others of the series.

(8) Inhalation of Shale Dust.

(as used for dusting in coal-mines.)

The standard exposures were employed in this series. At autopsy the lungs showed slight pigmentation and an occasional small haemorrhagic area.

No. 1. *Animal killed immediately after the last exposure.*

There are large areas of normal or slightly thickened lung substance. Black intra-cellular dust is present in moderate amount, the dust-cells usually projecting into the alveolar cavities from the point of attachment to the epithelium. Some dust-cells are also present in the bronchi, these latter being catarrhal. There are some small areas of broncho-pneumonia.

No. 2. *One month after exposure.*

General proliferation and thickening of the alveolar epithelium has set in. There are areas of sub-pleural broncho-pneumonia as evidenced by Plate X, fig. 32. Eosinophil infiltration and capillary engorgement are also present. There is some bronchitis. The nodules of lymphoid tissue adjacent to the larger blood-vessels contain a little dust.

No. 3. *Two months after exposure.*

The lesions are of the same type as in No. 2 of the series but more pronounced. The bronchial glands contain dust.

No. 4. *Three months after exposure.*

The changes are less marked than in No. 3 of the series. The amount of dust has also considerably diminished. Emphysema is present in some areas as shown in Plate X, fig. 33.

Nos. 5 *and* 6. *Two animals*—8 *and* 8½ *months after exposure.*

There is slight but fairly general thickening of the alveolar epithelium in both these specimens. In one (No. 6) there are a few small areas of broncho-pneumonia (see Plate X, fig. 34) and isolated patches of plaque-formation. There are, however, no gross lesions and the amount of dust in the lungs has undergone considerable reduction. Some of the bronchi are catarrhal. The peri-bronchial lymphoid tissue in the lung contains a little dust in one of the animals (No. 5), while the bronchial glands—only examined in No. 6—contain a fair amount of dust. There is no fibrous tissue increase.

(9) INHALATION OF IGNITED SHALE.

The shale used in these experiments was heated to redness in the electric furnace so as to destroy all organic matter.

Standard concentration and exposures. At autopsy pigmentation of the lungs, chiefly apical, was present.

No. 1. *Two animals killed* 24 *hours after the last exposure.*

In both specimens proliferation and thickening of the alveolar epithelium is general. There are extensive areas of broncho-pneumonia with pseudo-eosinophil infiltration and some "cuffing" of the blood-vessels by lymphocytes. Intra-cellular dust is abundant. The bronchi contain dust and are catarrhal.

No. 2. *Two animals six weeks after exposure.*

Changes slightly less marked than in No. 1. The amount of dust in the lung substance is much the same, but intra-bronchial dust particles are very scanty. The bronchial glands contain much intra-cellular dust.

No. 3. *Two animals four months after exposure.*

There are fair-sized areas of normal lung alternating with patches of broncho-pneumonia. Dust is still present in considerable amount. A few isolated areas of plaque-formation. The bronchial glands contain abundant intra-cellular dust particles.

No. 4. *One animal six months after exposure.*

The condition of the lungs is much as in No. 3. Intra-cellular dust is present in fair amount. Bronchi catarrhal and dust-containing.

No. 5. *One animal eleven months after exposure.*

A few areas of consolidation. Large areas of normal or nearly normal lung substance. A few small areas of plaque-formation. The dust is considerably reduced in amount. There is no fibrosis. One of the areas of consolidated tissue contains giant cells within which are granular oxyphil bodies. These, while certainly not bacterial, are of uncertain origin. Probably they represent the granules of eosinophil cells previously phagocytosed.

(10) INHALATION OF DRIED EARTH.

Ordinary garden earth passed through a sieve in order to eliminate the coarser particles.

Usual concentration and exposures.

At autopsy the lungs showed marked pigmentation in No. 1, very slight pigmentation in the succeeding animals.

No. 1. *Two animals killed three days after last exposure.*

In both specimens there is general thickening of the alveolar epithelium, some areas of

haemorrhage into the alveoli, and numerous intra-cellular particles. There is slight bronchial catarrh, and, in one of the specimens, a few broncho-pneumonic patches.

No. 2. *Two animals killed two months after exposure.*

The lesions have slightly regressed. Much dust is still present both in the lungs and in the bronchial glands.

No. 3. *One animal killed seven months after exposure.*

Areas of proliferated alveolar epithelium and broncho-pneumonia (with eosinophil infiltration) are still to be found. There is slight bronchitis but no intra-bronchial dust. The lung substance contains a fair amount of dust. No plaque-formation.

No. 4. *Two animals nine months after exposure.*

There is widespread thickening of the alveolar epithelium. A few small areas of broncho-pneumonia. The lungs contain a fair amount of intra-cellular dust while the bronchi are dust-free. Neither plaque-formation nor fibrosis. The lungs are microscopically negative for organisms.

(11) Inhalation of Ignited Earth.

Sifted garden earth heated to redness in the electric furnace.
Standard concentration and exposures.

Only three animals have been examined owing to an epidemic of pneumonia which killed all the other animals in this, and also large numbers in the other, groups.

No. 1. *Two animals killed* 24 *hours after the last exposure.*

In one of the animals there is intense pulmonary congestion—undoubtedly agonal. Apart from this the lesions are similar in both specimens, and comprise small areas of thickened alveolar epithelium. Intra-cellular dust is plentiful. There is no bronchitis but much intra-bronchial dust.

No. 2. *One animal killed* $3\frac{1}{2}$ *months after exposure.*

There are areas of proliferated alveolar epithelium and plaque-formation (the latter very scanty). Some bronchitis. Over the lobes there is an extensive, old, organised pleurisy with some fibrotic ingrowth into the underlying lung substance. Both lungs and pleura are microscopically negative for bacteria. The bronchial glands contain a fair amount of intra-cellular dust.

(12) The Bacteriological Examination of the Sections.

The results furnished by staining sections of the lungs of those animals which showed pronounced lesions are summarised and briefly commented on below.

The Ziehl-Neelsen and Claudius stains were used as standard methods for each group of animals. Cresylecht Violet and Carbol-Thionin were also used in some instances. Since these two stains never revealed organisms not demonstrated by the standard methods, no reference is made to them in the following summary.

Summary of bacteriological examination of the lungs of Control and dusted animals.

Controls. Lungs and bronchi (medium and small) negative for micro-organisms in the three animals examined.

1. *Flint and coal* (*Series* 1). A few gram-positive Streptococci and Diplococci in the bronchi. Lung substance negative.
2. *China clay* (*Series* 1 *and* 2). Lungs and bronchi negative.
3. *Felspar.* Negative.
4. *Ground pitcher.* A few gram-positive Streptococci were once noted within a plug in a bronchus.
5. *Precipitated silica.* Negative.
6. *Flint.* Negative.
7. *Coal.* A gram-positive Streptococcus present in small numbers.
8. *Shale.* Negative.

Neither tubercle bacilli, nor, indeed, any lesions even remotely suggestive of pulmonary tuberculosis, were ever noted.

As shown by these findings, the presence of bacteria, even in the areas of broncho-pneumonia, was very rare, and, when present, they were always very scanty. The lungs of the control animals, including one broncho-pneumonic specimen, were negative. So also were the lungs of all the other groups with the exception of three.

The presence of bacteria in cases of human pneumoconiosis is, of course, well known. Thus, the expectoration of coal-miners "is found teeming with Staphylocci and many putrefactive organisms" (Summons (1907)). The fact that the broncho-pneumonic lesions were not found to contain organisms after treatment with bacterial stains should not be regarded as proof that such lesions neither contain organisms nor that they possibly represent, in part, the tissue-response to an infection. The microscopic examination of the consolidated portions suggests a chronic condition, primarily induced by the dust particles. It is hence possible that micro-organisms might have secondarily infected such areas in small numbers. And in such cases, only cultural methods —which are outside the scope of these observations—would be of use in detecting them.

6. DISCUSSION OF THE RESULTS.

Below are given the conclusions derived from the study of the lesions caused by the dusts used in these experiments.

At the outset I would stress the fact that such deductions as can be made from the study of the response of guinea-pig lungs to dust particles do not admit of a wholesale application to human beings.

It is essential clearly to recognise the limitations of observations such as these.

Firstly, the pulmonary response of the guinea-pig is often different in degree from that of man. Thus, fibrosis, the outstanding lesion directly due to the inhalation of flint dust in man, was never produced by intake of pure flint into the lungs of guinea-pigs in these experiments. This, I think, is due to an important histological difference in the two types of lung: that of man contains much fibrous tissue, that of small rodents very little.

Secondly, the nasal filter of the guinea-pig—from such examination as I have made of it after exposure to dust—appears to be greatly superior to that

of man. This means, of course, that the actual dust content of the inspired air would be less in the guinea-pig than in man.

Thirdly, an important difference between experimental and human pneumoconiosis arises from the fact that it is necessary, for practical reasons, when working with animals, to restrict the exposures to as short a period as possible —two hours daily for 12 days in the case of most of these experiments. A man, employed in a dusty profession, inhales dust for many years. Whence the necessity for using very high concentrations of dust in the case of animal experiments in order to obtain definite lesions after so short a series of exposures.

All this indicates the necessity for caution in the interpretation of animal experiments in terms of human pneumoconiosis. A dust which causes pulmonary damage in guinea-pigs but not, so far as is known, in man (*e.g.* china clay) can only be regarded as potentially dangerous—*i.e.* harmful if breathed at high concentrations—for human beings in the absence of other evidence.

(1) Inhalation of a mixture of Coal and Flint.

It is well known that coal dust can be breathed in considerable concentration over prolonged periods without causing any damage to the tissues. It is equally well established that flint dust, even in the infinitesimal concentration in the flint-knapping industry, is extremely harmful. These experiments involving the inhalation of a mixed dust were performed so as to test Mavrogordato's hypothesis that a dust which stimulates phagocytosis—*e.g.* coal—if mixed with an inert dust—*e.g.* flint—will induce phagocytosis, and consequent elimination, of the flint particles as well as the coal.

This group of experiments contained the two following series:

Series 1. Coal and flint mixed in the proportion of one of coal to two of flint by measure (*i.e.* 25 per cent. and 75 per cent. by weight).

Series 2. Two parts of coal to one of flint by measure—*i.e.* the proportions of the dusts were reversed.

Analysis of Series 1 *and* 2. The immediate effects consist in proliferation of the alveolar epithelium, capillary engorgement and bronchitis. A patchy broncho-pneumonia with eosinophil infiltration appears after the first month. The amount of dust in the lungs undergoes diminution from the first to the sixth month. After $9\frac{1}{2}$ months there is a change towards the normal; large areas of lung clear up though some extensive patches of broncho-pneumonia were still present in the latest specimen examined (16 months). Dust particles are usually present in the bronchial glands from one month after exposure onwards. The chief difference between Series 1 and 2 is that the lungs of the animals of the latter series never contained plaques. No fibrosis was noted in any of the specimens examined.

The histological evidence indicates that while coal and flint produce a brisk phagocytic response, the small amount of flint remaining behind in the lungs was sufficient, particularly in Series 1, to leave lasting though not extensive damage. But there is little doubt that the early phagocytic response initiated by the coal is responsible for the rapid elimination of much of the flint.

It is therefore conceivable that the dusting of gold-mines with coal dust might aid in the elimination of the very harmful particles of quartzite. According to the monograph of Silicosis of Watt, Irvine, Steuart and Johnson (1916), in the case of "machine miners who had worked $4\frac{1}{2}$ years underground, over 50 per cent. were found to be affected (including 'borderland' cases) with Silicosis. After $8\frac{1}{2}$ years the incidence of Silicosis is over 75 per cent. while after $15\frac{1}{2}$ years practically none of the men engaged in drilling operations are free from Silicosis." Furthermore, Silicosis is nearly always complicated by pulmonary tuberculosis (certainly in its later stages)[1].

These observations on guinea-pigs indicate that Haldane's (1918) proposal to dust gold-mines with coal dust might well be beneficial to the health of the miners. Possibly, too, coal dusting might be employed in some of the other trades which involve the inhalation of flint. And, finally, it is conceivable that pulmonary tuberculosis might favourably respond to a carefully graded inhalation of coal or soot particles.

(2) INHALATION OF CHINA CLAY.

China clay—also known as kaolin—is a fine white powder, used in the manufacture of earthenware and china. Chemically it is a silicate of alumina and "has very nearly the empirical composition $Al_2O_3.2SiO_2.2H_2O$" (Mellor, 1914).

Three series of experiments were undertaken with this dust: the standard quantity of dust (135 c.c.) was put into the dusting machine for each period of exposure in Series 1. In Series 2 half the quantity was employed. In Series 3, while both the total amount, and each dose, of dust were the same as in Series 2, the dusting sessions were spaced out over a considerable period (over six weeks, instead of 14 days) as shown on p. 448.

Analysis of Series 1 *and* 2. The behaviour of the guinea-pig lung in the first two series towards this dust is interesting. Until three months after exposure the pulmonary reaction is very slight, being limited to mild proliferation of the alveolar epithelium. From three months onwards, a patchy broncho-pneumonia, with massive eosinophil infiltration, appears. The last specimen from Series 1, killed six months after exposure, showed plaque-formation and a pretty general bronchitis in addition to the above-mentioned changes.

In Series 2 exactly similar lesions were noted—even in the latest animal, examined nine months after exposure.

The bronchial glands showed some dust several months after exposure.

In Series 3 it was thought that the spacing out of the exposures would facilitate the removal of the dust, and that the phagocytic cells of the lung might thus be "trained" to deal with china clay.

The microscopic findings confirmed this supposition. The lesions were less marked than in Series 2, while a far more active phagocytosis was observed. Dust particles were removed to the bronchial, and even the abdominal, lymphatic glands.

[1] In a memoir which has recently come to my notice, Mavrogordato reports on some further experiments with coal and silica. He exposed animals to inhalations (*a*) of silica preceded by coal, (*b*) of silica mixed with coal, (*c*) of silica, followed by coal, and (*d*) of silica alone. His results indicate that "once Silica is fixed in the lung tissues, Coal exerts no eliminative effect,...but that a prior or even simultaneous exposure to coal dust appears to set up a condition in the lung which is inimical to the fixation of silica" [Mavrogordato, "Studies in Experimental Silicosis" (1922)].

From the above it can be seen that there is a long latent period—at least one month—during which phagocytic response of the lungs is almost absent. In fact, the dust particles can be seen lying against the alveolar epithelial cells which only very rarely, at this stage, show any signs of engulfing them. From three months onwards phagocytosis occurs, but the cells seem to die soon after they have taken up the dust. Consequently, much remains in the lungs, thereby initiating plaque-formation and broncho-pneumonia.

The great degree of harmfulness of china clay towards the guinea-pig lung is shown by the fact that even when inhaled at half the standard concentration the lesions produced are quite as severe as when inhaled in the standard quantity. By spacing out the doses, however, it seems possible fairly efficiently to train the lungs to deal with this dust. This is in striking contrast with the effects produced by daily inhalations of even smaller quantities of dust.

Curiously enough, there does not seem to be any evidence that china clay is particularly harmful to the lungs of human beings.

When this substance is used in the manufacture of china and earthenware, Dr White, of the Royal Worcester Porcelain Works, tells me that it is not breathed by the workers; the clay, as received at the works, contains about 10 per cent. of water and therefore does not fly. Further, the mixing is done wet. These facts I was able to note for myself when visiting the above establishment.

Although industrial medicine furnishes no definite evidence that the inhalation of china clay is productive of pulmonary lesions in man, I feel that these experiments indicate that china clay is perhaps a potentially dangerous dust. That is to say, that it might well be harmful to man if breathed in higher concentrations than those which apparently obtain under present-day industrial conditions.

(3) Inhalation of Felspar.

Felspar, like china clay, is an alumino-silicate and is also used in the manufacture of china and earthenware. Its empirical formula is $K_2O.Al_2O_3.6SiO_2$.

The lung changes produced by the inhalation of this dust at the standard concentration were slight up to one month after the last exposure, since they only comprised some proliferation and thickening of the alveolar epithelium. From two months onwards plaque-formation and areas of broncho-pneumonia are in evidence, although the amount of dust in the lungs undergoes some diminution. The last specimen examined (9 months after exposure) shows a mild fibrosis of the lung substance immediately surrounding the peri-bronchial and peri-vascular connective tissue. Dust appears in the bronchial glands one month after exposure, but its presence is not always constant.

The phagocytic response to this dust is slow and was not appreciable until one month after exposure. And, when it does occur, it is feeble.

The evidence, then, is that felspar, when inhaled under the conditions of these experiments, is a very harmful dust. Like china clay, it produces but little initial reaction, though severe and permanent lesions subsequently follow. Even after the short exposure to which the guinea-pigs were submitted felspar eventually produced a slight fibrosis as well as plaque-formation.

Felspar, though used in the china and earthenware industries, does not seem to be responsible for injury to the lungs of the workers, since, as pointed out to me by Dr White, it is used wet.

These experiments indicate that felspar, like china clay, must be regarded as a dust which would probably prove very harmful if inhaled by human beings.

(4) INHALATION OF GROUND PITCHER.

Ground pitcher is the term employed in the Potteries for crushed earthenware after firing. Dr White informs me that ground pitcher is not inhaled by workers in china manufactories. Further, its use is very limited, being restricted to filling up small holes, etc., in porcelain, for which purpose it is made up with water.

The inhalation by guinea-pigs of this dust produces marked initial lesions; the lungs of an animal killed 24 hours after the last exposure, show great proliferation and thickening of the alveolar walls, and also bronchitis. Broncho-pneumonia and plaque-formation appear three months after exposure, fibrosis from the fourth month onwards. The latest specimen, examined nine months after exposure, showed no improvement, normal tissue being very scanty. Phagocytosis is more active than in the lungs of animals exposed to china clay or felspar. Dust is found in the bronchial glands from three months onwards, from which time it seems steadily to accumulate therein.

The histological evidence furnished by these experiments is that while ground pitcher produces marked initial damage to the lungs, phagocytosis of the dust is too slight, and its onset too slow, for the onset of permanent pulmonary lesions to be avoided. The fibrosis caused by this dust was the most definite in any of these experiments.

Ground pitcher is not, to my knowledge, inhaled by any type of worker, though these experiments indicate that its inhalation by human beings would probably provoke very serious lesions.

(5) INHALATION OF PURE AMORPHOUS SILICA.

This dust was administered so as to test the effect on the lungs of silica in the amorphous as opposed to the crystalline state. The dust was dehydrated and rendered insoluble by heating.

The first changes (a few hours after exposure) comprise proliferation of the alveolar epithelium, also capillary engorgement and some haemorrhage into the alveoli. The onset of plaque-formation is early. Bronchiolitis and a patchy broncho-pneumonia are in evidence from the fourth month onwards. The animals killed 4 and $5\frac{1}{2}$ months after exposure show a slight fibrosis. A specimen examined 8 months after exposure presented similar lesions, except that there are no plaques; another, after 13 months, shows sub-pleural plaques but no fibrous tissue increase. The last guinea-pig of this series, examined 23 months after exposure, shows marked bronchiolitis, plaque-formation, and several large areas of broncho-pneumonia, but no fibrosis.

Fibrosis was not constant in all the animals, presumably for the reason already suggested—viz. that the concentration of the precipitated silica was a "borderland" concentration.

The presence of plaques in the two latest specimens (13 and 23 months) shows that permanent damage was caused by this dust.

The above results, together with those of Mavrogordato (1918) on the inhalation of soluble silica, may be discussed here. Gye and Kettle (1922, 1 and 2) have shown that the subcutaneous injection of either soluble or insoluble silica promotes a typical lesion. The only difference observed after injection of the soluble, as opposed to the insoluble, form of silica was the more rapid onset of the lesion with the former. And this, according to Gye and Kettle, is due to the colloidal (*i.e.* soluble) silica acting directly as a tissue-poison, whereas the insoluble SiO_2 can only inflict damage after undergoing hydration —and thereby becoming soluble—in the tissues. Certainly, that would seem the logical conclusion to be derived from the experiments of these authors.

The effects, however, of the inhalation of silica and of siliceous compounds are difficult to reconcile with this view for the following reasons:

(i) Shale contains from 55 per cent. to 60 per cent. of silica (Geikie, *Text-book of Geology*, 4th ed.) of which 35·2 per cent. consists of quartz (Miller). Yet both human and experimental evidence is unanimously in agreement that shale can be inhaled in large quantities without harm.

(ii) The effect of soluble silica on the guinea-pig lung has been investigated by Mavrogordato. This author noted that the lungs of such animals, after 2 hours' exposure for 12 days, were "practically dust-free." In other respects they were normal.

(iii) On the other hand, the inhalation of pure precipitated (amorphous) silica, rendered insoluble by heating to *circa* 800° C., caused pronounced and early lesions frequently accompanied by fibrosis.

One can only assume from (ii) and (iii) that the lungs are far more sensitive to insoluble than to soluble silica, which latter, according to Mavrogordato, causes no lesions whatsoever, presumably because it does not remain in the lungs.

I think that the above remarks show the necessity for extending the experiments of Gye and Kettle to the lungs. The injection of silica into subcutaneous connective tissue is one thing, the inhalation, or insufflation, of the same substance into the lungs, another.

In view of the fact that the lung is habitually dealing with dust throughout life, and that connective tissue is not, it seems premature to apply to lung the deductions from the response of connective tissue towards silica until that of the lung has been studied along similar lines.

There is another interesting point in connection with this dust. It is often maintained (see Parkes and Kenwood, 1920) that the degree of harmfulness of a dust is proportionate to the degree of angularity and of sharpness of its constituent particles. Haldane (1918) has already thrown doubt on this view. Now, precipitated silica and flint are chemically identical, but the former is amorphous, the latter crystalline. Yet the inhalation of the amorphous silica produced lesions even more severe than did the crystalline silica. Again, china clay is amorphous, yet very harmful to the lungs. The evidence of these experiments is totally opposed to the text-book view that the more finely crystalline a dust, the more damage will it inflict upon the lungs.

(6) INHALATION OF FLINT.

The inhalation of flint dust is notoriously deadly as is testified by the mortality in certain trades—especially flint-knapping—from pulmonary disease.

Flint dust is inhaled by certain types of workers in the china-making industry. China articles, before being baked are "placed"—*i.e.* carefully packed—in finely powdered flint in large fireclay pans called "saggars." Some dust, in spite of all precautions, is generated during the placing of the china and its unplacing after it has been baked.

Flint used to be inhaled during the process of "china scouring" (*vide* Parkes and Kenwood, 1920). This comprised the removal of the flint dust from the fired china by brushing. The Managing Director of the Royal Worcester Porcelain Works informs me that mechanical scouring is nowadays the standard practice.

This series of experiments was carried out not so much to confirm what has long been known concerning the lesions caused by breathing flint, as to have a standard of comparison between this dust and the mixed flint and coal, and the precipitated silica series.

The early stages (27 hours after exposure) comprise small areas of haemorrhage and some proliferation of the alveolar epithelium. A patchy broncho-pneumonia and incipient plaque-formation was established one month later. Specimens examined 4, 7 and 9 months after exposure merely show an extension of these lesions. The last two animals of this series, killed 12½ and 13 months after dusting, show similar changes, much dust, but no plaques. Fibrosis was never present. The absence of plaques from the lungs of the last two specimens is curious; possibly the lungs originally contained small plaques which disappeared later in the manner suggested on p. 446.

The absence of fibrosis is in contrast with human experience of flint dust. It should be remembered that exposure over a long period is apparently necessary in man before fibroid changes are induced in the lungs, whereas the total number of hours of exposure to which the guinea-pigs were submitted was 24. Furthermore, as already mentioned, the scanty development of fibrous tissue in the guinea-pig lung is undoubtedly responsible for the difficulty in eliciting a fibrous tissue increase in response to dust inhalation.

(7) INHALATION OF PURE COAL.

Coal dust is generally admitted to be the least harmful of the many dusts inhaled by human beings; indeed, as already pointed out, there is both statistical and experimental evidence that in some ways it is actually beneficial. It is even possible that carefully graded inhalations of coal dust might be of use in the treatment of pulmonary tuberculosis.

Animals were exposed to coal dust so as to have a standard of comparison with the other dusts used in these experiments—notably the mixed flint and coal.

Pure coal, as is well known, elicits a rapid and marked phagocytic reaction. The lungs of a specimen killed six hours after exposure show general proliferation of the alveolar epi-

thelium and some broncho-pneumonia. Much dust is present. These lesions persist until the fourth month after exposure. Both dust and lesions have undergone diminution in two specimens examined nine months after exposure, while the lungs of the last animal, examined after 19 months, show a marked decrease in the amount of dust, much nearly normal lung substance and some patches of broncho-pneumonia.

Phagocytosis was active in all the specimens. Neither plaque-formation nor fibrosis were ever noted. Coal dust undergoes a rapid elimination from the alveoli, and the brisk phagocytic response of the lungs towards coal is the explanation for their tolerance towards repeated doses of this dust.

(8) Inhalation of Shale.

Shale is extensively used for dusting in coal-mines as a preventive measure against explosions. It is inhaled in considerable quantity by the miner. These experiments have furnished results confirming the previous observations of Beattie (1912) and Mavrogordato (1918).

The reaction of the lungs towards shale was found to be rather like their reaction towards coal, but less intense. The onset of phagocytosis is slower, though in the later specimens the dust has undergone considerable diminution. The lesions undergo regression from three months onwards after exposure. The last two specimens, killed 8 and 8½ months after dusting, show only slight thickening of the alveolar epithelium, and, in one of the animals, slight plaque-formation and a few small areas of broncho-pneumonia. Fibrosis was never noted.

Shale, then, is a dust which tends to be eliminated from the lungs, though not with the rapidity of coal. Its inhalation by guinea-pigs produced no permanent lesions except a very mild degree of plaque-formation in one animal. The coal-miner inhales shale dust mixed with coal and the latter is a notable stimulator of phagocytosis within the lungs. There consequently is no reason to deny the claim that shale dust is comparatively harmless—in spite of the fact that it contains over 50 per cent. of silica by weight.

(9) Inhalation of Ignited Shale.

The shale used in these experiments, after heating to redness in the electric furnace, was administered exactly as for the preceding group.

It was thought that the reason why coal and shale are comparatively harmless when inhaled might be due to the presence of organic matter which stimulated phagocytosis in the case of these dusts.

The response elicited by this dust is similar to that provoked by ordinary shale. Phagocytosis is slow but steady. In the last specimen examined (11 months after exposure) the dust was considerably reduced in amount; there were a few small areas of plaque-formation, but no fibrosis.

Thus, the supposition which led to this experiment being made was not confirmed in practice. The organic matter in shale does not seem to be the factor inducing phagocytosis of this dust.

(10) Inhalation of Dried Earth.

The aim of testing the effect of earth on the lungs was twofold: firstly, to ascertain if any lesions were produced by the inhalation of such a ubiquitous

substance, and, secondly, to compare the effects of dried with ignited earth as for shale.

The initial lesions are a thickening and proliferation of the alveolar epithelium accompanied by a patchy broncho-pneumonia. This persisted for as long as 9 months (when the last two animals were examined). Phagocytosis of the dust is but slight. Yet neither plaque-formation nor fibrosis have occurred. The lungs are microscopically negative for organisms.

While the phagocytic reaction towards this dust is slight, permanent and serious lesions have not been produced. The lungs show a peculiar tolerance towards it, and ordinary earth appears to consitute an exception to the generally correct view that the harmful dusts are those which are not eliminated.

(11) INHALATION OF IGNITED EARTH.

This was the same earth, administered in the same way, as the preceding group, but heated to redness in the electric furnace before administration.

The aim of the experiment was to test the supposition that it was the organic matter content of certain dusts which was the stimulant towards phagocytosis. Although dried earth produced but little phagocytic response it was thought that the absence of its organic matter might transform it into an actively harmful substance.

Unfortunately, an epidemic of pneumonia destroyed most of the dusted animals in both this and other groups.

The initial lesion (24 hours afterwards) is a patchy proliferation of the alveolar epithelium. The only animal ($3\frac{1}{2}$ months after exposure) which survived the epidemic does not admit of accurate interpretation in that there was an old, organised pleurisy over the greater part of the lobes. This, presumably, was a relic of a previous pneumonia contracted during the epidemic. Discounting this, and the underlying fibrosis, derived from it, the only lesions which can indubitably be ascribed to the dust are proliferated alveolar epithelium and scanty plaques.

In view of the absence of further material all that can be said regarding ignited earth is that its earlier lesions do not differ from those produced by ordinary earth.

(12) THE BRONCHO-PNEUMONIC LESIONS.

The type of broncho-pneumonia present in the lungs of the guinea-pigs employed for these experiments merits mention. Unlike broncho-pneumonia as ordinarily found in human beings, the cellular increase is primarily due to a proliferation of the cells of the alveolar epithelium. It is not chiefly an exudate of leucocytes except in such areas where eosinophil infiltration of the lung substance is also present. Further, this eosinophil invasion is secondary to the proliferation of the tissue cells. Only very rarely were necrotic foci seen. On the evidence furnished by human pathology many of these lesions could undergo modification (in time) in the sense that such damaged areas of lung could become functional again. This, in fact, has undoubtedly occurred in some of the animals which were kept for a sufficiently long time after exposure.

7. THE ORIGIN OF THE DUST-CELLS.

In addition to the other series of experiments, a special group of animals, exposed to a single but intensive exposure of three hours with coal, was examined. The aim of this was to avoid the inevitable overlap of the different stages of pulmonary reaction which occurs in animals subjected to repeated inhalations of dust.

As may be seen in the bibliographical section (see pp. 439 to 442) two schools of thought are in active controversy regarding the origin of the dust-cells. The one, animated by Metchnikoff (1901) and his pupils (1889), asserts that dust-cells are always leucocytes which have migrated from the blood stream. The other, of which Arnold (1885) may be regarded as the prototype, claims that dust-cells are always derived from the alveolar epithelium. Many recent observers hold the former view.

The evidence furnished by the examination of sections of dust-containing lungs is, on the whole, in favour of the epithelial origin of many of the dust-cells.

I have been led to this conclusion by the following observations:

1. The more actively a dust undergoes phagocytosis within the lungs, the more rapidly does proliferation of the alveolar epithelium set in. The reverse is also true; dust particles which evoke a poor phagocytic response can be seen plastered against the alveolar epithelium, which remains, sometimes for several weeks, relatively unchanged. This proliferation of the alveolar epithelium and the concomitant appearance of dust-cells suggests the derivation of the latter from the former.

2. Swollen cells, attached to the alveolar walls, and definitely spaced between normal alveolar cells, may often be seen projecting into the alveolar cavities. Such cells, which often contain dust particles, have every appearance of modified alveolar epithelial cells.

3. Only rarely have I observed anything which could be interpreted as the migration of leucocytes from the capillaries in the early stages of the ingestion of dust particles. Yet dust-cells, and proliferated alveolar epithelium, are usually much in evidence.

4. Metchnikoff (1901) claims that the dust-cells are invariably derived from leucocytes of the macrophage type, which have migrated very early in life from the blood stream into the alveolar walls. There they lie dormant, until awakened by dust particles or other foreign bodies. I can only say that I have never been able to find such cells in the alveolar walls of normal guinea-pig lung or even in dust-laden lungs soon after exposure.

5. Permar (1920, 1, 2, 3) holds that the dust-cells are derived from the endothelium of the pulmonary capillaries. Although some of the pigment may be taken up by such cells, I doubt whether the rôle played by the endothelial cells in these experiments was other than secondary for the following reasons:

(*a*) Only very rarely have I noted anything which could be interpreted as

endothelial elements undergoing transformation into dust-cells as described and figured by Permar. This remark applies even to lungs in which active phagocytosis was taking place. On the other hand, stages in what I regard as the formation of dust-cells from the alveolar epithelium were clearly visible.

(*b*) I have often observed dust-containing cells forming an integral part of the alveolar epithelium. The position and connections of these elements are such that they can only be explained by regarding them as modified alveolar epithelial cells. If they are not, how have they come to form an integral part of the epithelium, and what has happened to the displaced alveolar cells?

6. The infiltration of the dust-laden lung is a general, but secondary, occurrence. Furthermore, such cells, in the guinea-pig lung, are usually eosinophils, or sometimes polymorphs, and never has it been possible to detect dust in these cells, in these experiments.

7. Sewell's (1918) experimental evidence—already summarised on p. 442—seems strongly to support the contention that dust-cells originate from the alveolar epithelium.

8. And, lastly, there is no *a priori* reason why modified alveolar epithelial cells should not engulf dust particles, since Briscoe's (1908) very careful investigations have shown that the first phagocytic reaction towards bacteria comes from the alveolar epithelium, and that only secondarily do the leucocytes of the blood stream intervene.

The above evidence, then, is in favour of the view that many of the dust-cells are mostly derived from the alveolar epithelium. I say "mostly" derived from this source for the following reasons:

(*a*) An unbiassed observer who looks through a sufficient number of sections of dust-laden lungs not infrequently encounters ambiguous appearances which can easily be interpreted so as to suit the requirements of any of the theories already discussed. I feel sure that this has only too frequently been done in the past.

(*b*) I can see no reason—except the tendency of mankind to pigeon-hole facts in narrow but convenient compartments—to regard dust-cells as *always* derived from leucocytes (or endothelial cells) or *always* from alveolar epithelium.

The plasticity of tissue-response is very great, but only too often plasticity on the part of the interpreters is lacking.

See also the Appendix of this paper (p. 468).

8. THE MODE OF ELIMINATION OF DUST PARTICLES FROM THE LUNGS.

These observations are based on the personal study of sections of guinea-pig lung. No startling differences were noted in the elimination of dust from the lungs of these and other animals—man included.

As is well known, the first stage in this process consists in the phagocytosis of the dust by cells which frequently appear to be derived from the alveolar epithelium.

Once dust particles have been engulfed they tend to undergo removal from the alveoli in one of the following ways:

1. *Bronchial elimination.* Some of the dust-cells become detached from the alveolar walls and lie free within the alveolar cavities. They ultimately pass up the bronchial tree and are eliminated from the lung. Doubtless the rôle played by the dust-cells, once they have absorbed the dust, is passive. The ciliary action of the bronchial epithelium, aided by the forced expirations of coughing, would be sufficient to eliminate both dust-cells and extra-cellular dust particles, provided these have attained a point above the terminal bronchioles —the epithelium of the latter being devoid of cilia.

2. *Lymphatic elimination.* The dust-cells remain attached to the alveolar walls for a considerable time; next, they enter the lymphatic vessels of the lungs.

The intra-cellular dust particles may pursue a slow course through the pulmonary lymphatics, finally coming to rest in the lymphoid tissue adjacent to the lungs. Thus, most of the intra-lymphatic dust comes to rest in the bronchial glands, next, in order of frequency and amount, in the tracheal glands, and, lastly, in certain of the abdominal viscera, notably the mesenteric lymph glands and the spleen. For dust to be deposited in either of these two latter sites it is necesary that it should have been inhaled over long periods, or in very high concentration. Dust within lymphoid tissue is largely, but by no means always, intra-cellular.

In the case of those dusts which do not actively stimulate phagocytosis the dust-cells may die within the pulmonary lymphatics. This gives rise, I think, to the small "intra-lymphatic plaques" already mentioned.

Dust may also be deposited in the peri-vascular lymphatics. Such dust is often extra-cellular. Haythorn, however, claims (1913) "that carbon pigment once taken up by the cells remains intra-cellular indefinitely unless freed by some process producing general necrosis of the tissues." I am inclined to doubt that this is always the case, having observed coal particles lying undoubtedly free in the cleft-like lymphatic channels of peri-vascular and peri-bronchial connective tissue. Yet the dust must (presumably) have been engulfed by dust-cells in order to pass from the alveolar cavities into the lymphatics. One can therefore only suppose that the dust-cells have disintegrated after taking up the pigment.

Haythorn's statement is open to yet another objection. It is now generally recognised that the life of blood leucocytes and other phagocytic cells is not commensurate with the life of the organism. The leucopoietic (*i.e.* white-cell forming) organs are in a state of constant activity during life. This implies that the genesis and death of the leucocytes occurs many times in the life of the organism as a whole. Yet Haythorn claims that a leucocyte—for he regards dust-cells as large mono-nuclear and transitional leucocytes—becomes endowed with a life span many times the normal, provided it has engulfed dust particles. This would seem to be highly unlikely.

3. *The dust remains in the lung substance.* In this case the dust is taken up by dust-cells which then degenerate and form the masses of large squame-like cells known as plaques. Plaques often form large conglomerations lying within the disorganised lung substance. Invasions by fibroblasts are common, and loss of vascular connections, almost invariable features in any fair-sized area of plaque-formation. Such a plaque, in contrast to the small "intra-lymphatic" plaques, is, I think, a permanent pulmonary lesion. There also often appears to be a difference in the distribution of these two types of plaque within the lung; the larger—and permanent—plaques may be found in any parts of the lung which are heavily laden with an inert dust. The "intra-lymphatic" plaques, on the other hand, are usually found in the lymphatics beneath the pleura.

The route taken by a dust which is undergoing elimination from the lung would appear to vary according to the nature of the dust.

Thus, the dusts which freely stimulate phagocytosis are eliminated both *via* the bronchi and *via* the lymphatic system. Such dusts comprise both coal and shale.

Those dusts which stimulate phagocytosis but scantily seem to be chiefly eliminated through the lymphatic system. Such dusts comprise china clay, felspar, ground pitcher, precipitated silica and flint. These are also the dusts which tend to remain in the lungs and cause serious, and sometimes permanent lesions.

In brief, the more harmful a dust is to the lungs the less far is it removed from the alveoli, and the less complete is its removal.

9. SUMMARY.

1. The lesions produced by the different dusts employed in these experiments, and the conclusions to be derived thereform, have already been discussed in Section 6 of this paper (see p. 455, *et seq.*).

2. Plaque formation has been discussed. Evidence has been produced to show that the large plaques which tend to be formed in the parenchyma represent permanent pulmonary lesions. Small plaques, on the other hand, may be formed within the pulmonary lymphatics, in which case they may disintegrate in course of time. Such plaques have been termed "intra-lymphatic plaques."

3. Evidence has been brought forward against the view that the degree of harmfulness of a dust is proportionate to the degree of angularity and sharpness of its consituent particles. Nor has the supposition that the dusts which stimulated phagocytosis were those which contained organic matter been confirmed experimentally. Although silica may be regarded as a cell-poison and that this may account for the inability of the phagocytes to remove it, there are probably other factors than this, since shale—universally admitted to be one of the least harmful dusts—contains from 55 per cent. to 60 per cent. of silica. As to why one dust is harmful, and another not, is a problem as yet unsolved.

4. The origin of the dust-cells has been studied. Reasons have been given to justify the belief that dust-cells are frequently derived from the alveolar epithelium. This, however, does not exclude the possibility of some participation of leucocytes or endothelial cells in the phagocytosis of dust particles.

5. The chief modes of elimination of dust from the lungs have been discussed. It has been shown that the dusts which stimulate phagocytosis tend to be eliminated from the lung by both bronchi and lymphatics, while such elimination as occurs of the dusts which elicit but a feeble phagocytic response is through the lymphatics rather than the bronchi.

October 1923.

10. APPENDIX.

Since writing the section on the phagocytosis of dust particles (p. 464) I have had brought to my notice the following evidence bearing on this question.

Guieyesse-Pellissier (*C. R. Soc. Biol.* LXXXIII. 1920, p. 809, and *Ibid.* LXXXII. 1919, p. 1214) noted that intra-tracheal injections of olive oil in dogs and rabbits are rapidly absorbed and digested in the lungs. The cells responsible for this are derived from the alveolar epithelium. I have been able to verify this statement. All stages in the swelling up and desquamation of alveolar epithelial cells can be observed. Oil droplets can be identified in varying stages of digestion in both attached and free cells. The alveolar walls are largely formed of naked, beaded capillaries. Even after prolonged search I was unable to find more than two or three possible pictures of capillary endothelial cells undergoing detachment. In this case there can be no doubt that the part played by the alveolar epithelium in the phagocytosis of oil droplets is predominant, while the rôle of the endothelial cells is negligible.

Further, the statement of Permar and many others that "epithelia" are incapable of phagocytosis is extremely doubtful, since Guieyesse-Pellissier (*C. R. Soc. Biol.* LXX. 1911, p. 527) has described the phagocytosis of spermatozoa by the epithelial cells of the vas deferens subsequent to its ligature, while Regaud and Tournade (*C. R. Assoc. des Anat.* XIII. 1911 (Paris), p. 245) have noted a similar phenomenon in the epididymis following sterilisation of the testis by X-rays. In both cases a certain degree of de-differentiation of the epithelial elements occurred prior to their becoming phagocytic. I would also point out that the Sertoli cells of the seminiferous tubules normally ingest dead spermatozoa and other cellular débris.

On the other hand, Permar, after careful experimentation, states that the intra-alveolar phagocytes were endothelial in origin. Were Permar's experiments strictly comparable to the inhalation of air-borne particles? Possibly not, for the following reason. Permar first stained the endothelial leucocytes by intravenous injections of isamine blue, etc., then administered an intratracheal injection of carmine suspendid in normal saline. Now, it is possible that the marked endothelial response, noted by Permar, was partly or largely

due to the introduction of a fluid into the lungs, an act which of itself must affect the local vascular system. At any rate, it is certain that the pulmonary response to dust particles in a fluid is not strictly comparable to the inhalation of dust particles in air.

One must admit, *either*, that Permar's observations were incorrect—a view to which I do not subscribe for a moment—*or* that, according to the nature of the dust particles and their medium of dispersion (*i.e.* air or saline) a different type of phagocytic response may be elicited, just as the different types of blood leucocytes respond to different stimuli.

It may also be pointed out that the absence of mitotic figures in adult alveolar epithelium is by no means evidence that cells cannot be produced from it. In addition to the possibility of amitosis, the study of the behaviour of epithelia both in tissue cultures and during regeneration has shown that migration of epithelial cells can occur on a large scale. I suspect that a phenomenon which I have noted *in vitro*, the detachment of alveolar epithelial cells followed by movement or growth of the adjacent elements to cover up the vacated space, occurs also in the dust-stimulated lung.

Finally, the detachment of alveolar epithelial cells from the alveolar walls is especially noteworthy in the cat's lung subsequent to inhalation of "Mustard Gas" in high concentration. In this case the alveolar epithelial elements form mulberry-like masses of large clear cells, surrounded by naked alveolar wall composed chiefly of elastic fibres and beaded capillaries (Carleton, *Report of the Chemical Warfare Committee*, No. 2, April, 1918).

May 1924.

EXPLANATION OF PLATES VI–X.

Low power microphotographs taken with a Zeiss 50 mm. planar and projection ocular. Medium and high power photographs taken with Zeiss apochromatic objectives and compensating oculars.

All the microphotographs are untouched.

PLATE VI.

Fig. 1 (×17). *Control No.* 1. General view of normal guinea-pig lung. Note the scarcity of fibrous tissue.

Fig. 2 (×90). *Control No.* 4. Terminal bronchiole and adjacent alveoli of normal lung. Small branch of pulmonary artery above bronchiole. Note the flattened epithelial cells lining the alveolar cavities, and the absence of fibrous tissue except around the bronchiole and artery.

Fig. 3 (×17). *Control No.* 2. To right an area of thickened and proliferating alveolar epithelium —frequently present in the lungs of perfectly healthy guinea-pigs. Normal tissue on the left.

Fig. 4 (×17). *Control No.* 6. A small patch of chronic broncho-pneumonia; adjacent bronchi contain plugs of leucocytes and cell-débris. Normal tissue at the bottom. Small areas of broncho-pneumonia, surrounded by normal tissue, were noted in a few of the controls.

Fig. 5 (×90). *Inhalation of Flint and Coal* (*1st Series*). 24 days after last exposure. Section of unstained lung showing the abundant dust particles. Note the thickening of the alveolar epithelium in the dust-laden areas.

Fig. 6 (× 90). *Inhalation of Flint and Coal.* 10 months after exposure. Area of broncho-pneumonia; adjacent bronchi contain leucocytes and dust-cells; *LT* = nodule of lymphoid tissue in lung—normal in the guinea-pig.

Fig. 7 (× 550). *Inhalation of Flint and Coal.* 12½ months after exposure. High power view of dust particles within multinucleate dust-cells (*DC*). Alveoli nearly obliterated by the proliferation of the alveolar epithelium.

PLATE VII.

Fig. 8 (× 90). *Inhalation of Flint and Coal.* 16 months after exposure. Chronic broncho-pneumonia over the entire field. *C* = "cuffing" of branches of the pumonary vein by lymphocytes.

Fig. 9 (× 140). *Same specimen as Fig.* 8. The higher magnification shows an area of plaque-formation (*PF*).

Fig. 10 (× 90). *Inhalation of china clay.* 1 month after the last exposure. Lung nearly normal; some collapse at *X* (dark appearance of the microphotograph due to thickness of the section).

Fig. 11 (× 90). *Inhalation of china clay.* 6 months after exposure. Area of broncho-pneumonia with massive eosinophil infiltration.

Fig. 12 (× 17). *Inhalation of felspar.* 6 hours after the last exposure. Showing the early lesions. Most of the lung is normal; also area of thickened and proliferating alveolar epithelium.

Fig. 13 (× 90). *Inhalation of felspar.* 12 months after exposure. Later stage of the lesions showing a large sub-pleural plaque, thickened alveolar epithelium and (above) an area of compensatory emphysema.

Fig. 14 (× 550). *Inhalation of felspar.* 2 months after exposure. Showing an early stage of plaque-formation. *DC* = multinucleate dust-cells which have undergone concentration into plaques; *AE* = alveolar epithelial cells surrounding the plaques; *br* = a terminal bronchiole, its epithelium undergoing desquamation.

PLATE VIII.

Fig. 15 (× 35). *Inhalation of ground pitcher.* 24 hours after the last exposure. On the left; area of broncho-pneumonia. On the right; more normal tissue.

Fig. 16 (× 550). *Inhalation of ground pitcher.* 4 months after exposure. Incipient fibrosis in a dust-laden area. *FT* = strands of young fibrous tissue.

Fig. 17 (× 17). *Inhalation of ground pitcher.* 6 months after exposure. Note the extensive chronic broncho-pneumonia.

Fig. 18 (× 400). *Same specimen as Fig.* 17. Section of a bronchial gland. *M* = macrophages containing abundant dust particles.

Fig. 19 (× 17). *Inhalation of ground pitcher.* 9 months after exposure. Showing a patch of broncho-pneumonia, small oval area of normal lung, and elsewhere thickened alveolar walls.

Fig. 20 (× 110). *Inhalation of flint, felspar and china clay.* A few hours after the last exposure. Showing bronchitis and thickened and proliferating alveolar epithelium.

Fig. 21 (× 35). *Same specimen as Fig.* 20. General view of the early lesions. A large area of dust-containing and proliferating alveolar epithelium.

Fig. 22 (× 152). *Inhalation of flint, felspar and china clay.* 4 months after exposure. Haemorrhagic areas (darkly stained) into lung substance; great thickening of the alveolar walls and capillary engorgement are also present.

PLATE IX.

Fig. 23 (× 600). *Same specimen as Fig.* 22. *FT* = fine strands of fibrous tissue in a dust-laden area.

Fig. 24 (× 79). *Inhalation of flint, felspar and china clay.* 12 months after exposure. Illustrates the chronic lesions. *PF* = areas of plaque-formation; general thickening of the alveolar epithelium.

Fig. 25 (× 35). *Inhalation of pure precipitated silica.* A few hours after the last exposure. Demonstration of the increased susceptibility of lobe as compared to apex. *Above*, portion of an apex showing a massive broncho-pneumonia; *below*, portion of a lobe of the same lung—some bronchitis and slight thickening of alveolar epithelium.

Fig. 26 (× 35). *Inhalation of pure flint.* 22 hours after last exposure. Patchy proliferation and thickening of the alveolar epithelium; some bronchitis.

Fig. 27 (× 17). *Inhalation of pure flint.* 12½ months after exposure. Section of apex; area of broncho-pneumonia near root of lung; elsewhere thickening of alveolar epithelium.

Fig. 28 (× 17). *Inhalation of pure flint.* 13 months after exposure. Section of lobe. Bronchitis at bottom of field. A few small areas of slightly thickened alveolar epithelium. Note the greater severity of the changes in the apex (see Fig. 27) than in the lobe (as in this figure).

PLATE X.

Fig. 29 (× 17). *Inhalation of coal.* Short exposure (two exposures of 2 hours each); animal killed immediately afterwards. Apart from some bronchitis the lung is normal.

Fig. 30 (× 17). *Inhalation of coal.* 6 hours after exposure (usual period). Patchy broncho-pneumonia. Black masses of coal dust in the bronchi.

Fig. 31 (× 17). *Inhalation of coal.* 19 months after exposure. More normal tissue than in the preceding photographs of the coal dust series. Broncho-pneumonia undergoing resolution. Still some bronchitis. Complete absence of plaque-formation.

Fig. 32 (× 17). *Inhalation of shale.* 1 month after the last exposure. An area of broncho-pneumonia; to the left of this, normal lung.

Fig. 33 (× 79). *Inhalation of shale.* 3 months after exposure. An area of compensatory emphysema—a common feature in the lungs of dusted guinea-pigs.

Fig. 34 (× 17). *Inhalation of shale.* 8½ months after exposure. Mild bronchitis and, in places, slight thickening of the alveolar epithelium.

REFERENCES.

ARNOLD (1885). *Unterschungen und Staubinhalation und Staubmetastase.* Leipzig.

BEATTIE (1912). A Comparative Study by Experiment of the Effect produced on the Lungs by the Inhalation of Coal Dust, etc. Appendix to: *Parl. Papers: Explosions in Mines.* Committee Reports, I.–VI. 12.

BRISCOE (1908). An Experimental Investigation of the Phagocytic Action of the Alveolar Cells of the Lung. *Jour. of Path. and Bact.* XII. 66.

CHANTEMESSE and PODWYSSOTSKY (1901). *Pathologie Générale et Expérimentale,* I.

CLAISSE et JOSUE (1897). Recherches expérimentales sur les Pneumoconioses. *Arch. de Méd. Expér. et d'Anat. Pathol.* IX. 205.

GYE and KETTLE (1922,1). Pathology of Miners' Phthisis. *Lancet,* CCIII. 855.

—— (1922, 2). Silicosis and Miners' Phthisis. *Brit. Jour. of Exp. Path.* III. 241.

HALDANE (1916). The Health of Old Colliers. *Trans. of the Inst. of Mining Engineers,* LI. 469.

—— (1918). The Effects of Dust Inhalation. *Trans. of the Inst. of Mining Engineers,* LV, 264.

HAYTHORN (1913). Some Histological Evidence of the Disease Importance of Pulmonary Anthracosis. *Jour. Med. Res.* XXIX. 259.

JORDAN (1920). *Textbook of Histology,* 2nd ed.

MAVROGORDATO (1918). Experiments on the Effects of Dust Inhalation. *Jour. of Hygiene,* XVII. 439.

—— (1922). Studies in Experimental Silicosis and other Pneumokonioses. *Publicn. of S. African Inst. for Med. Res.* No. XV.

MELLOR (1914). *Modern Inorganic Chemistry.*

METCHINKOFF (1901). *Immunity in Infectious Diseases* (Eng. Trans.).

OLIVER (1903). Discussion on Miners' Phthisis. *Brit. Med. Jour.* II. 568.

OPIE (1904). The Occurrence of Cells with Eosinophil Granulation and their relation to nutrition. *Am. Jour. Med. Sci.,* Phila. CXXVII. 217.

PARKES and KENWOOD (1920). *Hygiene and Public Health.*

PERMAR (1920, 1). An Experimental Study of the Mononuclear Phagocytes of the Lung. *Jour. of Med. Res.* XLII. 9.

—— (1920, 2). The Development of the Mononuclear Phagocyte of the Lung. *Jour. of Med. Res.* XLII. 147.

—— (1920, 3). Migration and Fate of the Mononuclear Phagocyte of the Lung. *Ibid.* XLII. 209.

SEWELL (1918). The Phagocytic Properties of the Alveolar Cells of the Lung. *Jour. of Path. and Bact.* XXII. 40.

SUMMONS (1907). *Report of an Investigation at Bendigo into the Prevalence, Nature, Causes and Prevention of Miners' Phthisis.* Melbourne.

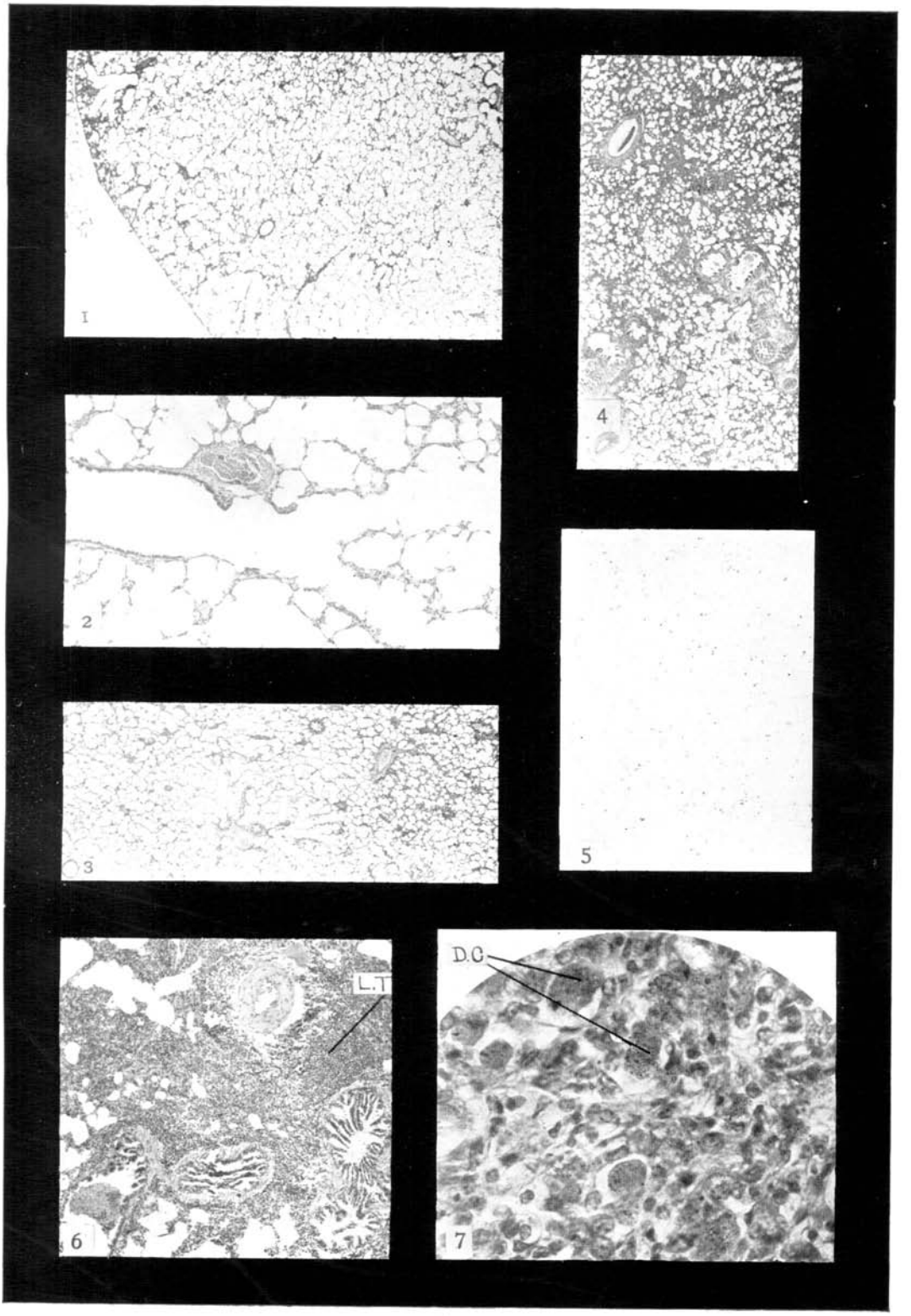
TCHISTOVITCH (1889). Des phénomènes de phagocytose dans les poumons. *An. de l'Inst. Pasteur*, III. 337.

WAINWRIGHT and NICHOLS (1905). The Relation between Anthracosis and Pulmonary Tuberculosis. *Am. Jour. Med. Sci.* CXXX. 403.

WASHBOURNE and EYRE (1902). Croonian Lectures. *Lancet*, II. 1528.

WATT, IRVINE, JOHNSON and STEUART (1916). *Silicosis on the Witwatersrand.* Pretoria.

WESTHUES (1922). Herkunft der Phagocyten in der Lunge. *Beitr. z. Path. Anat.* LXX. 223.



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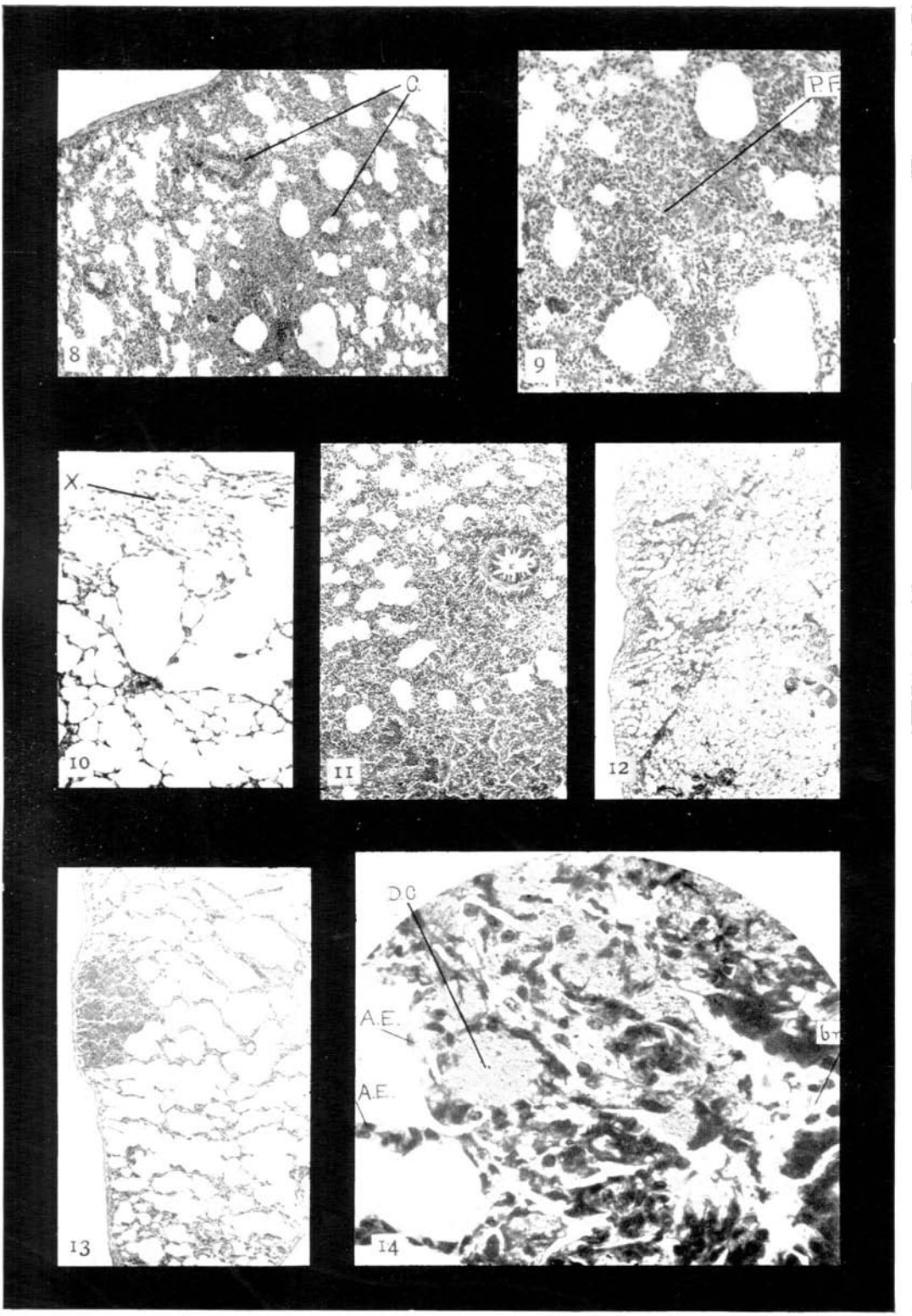

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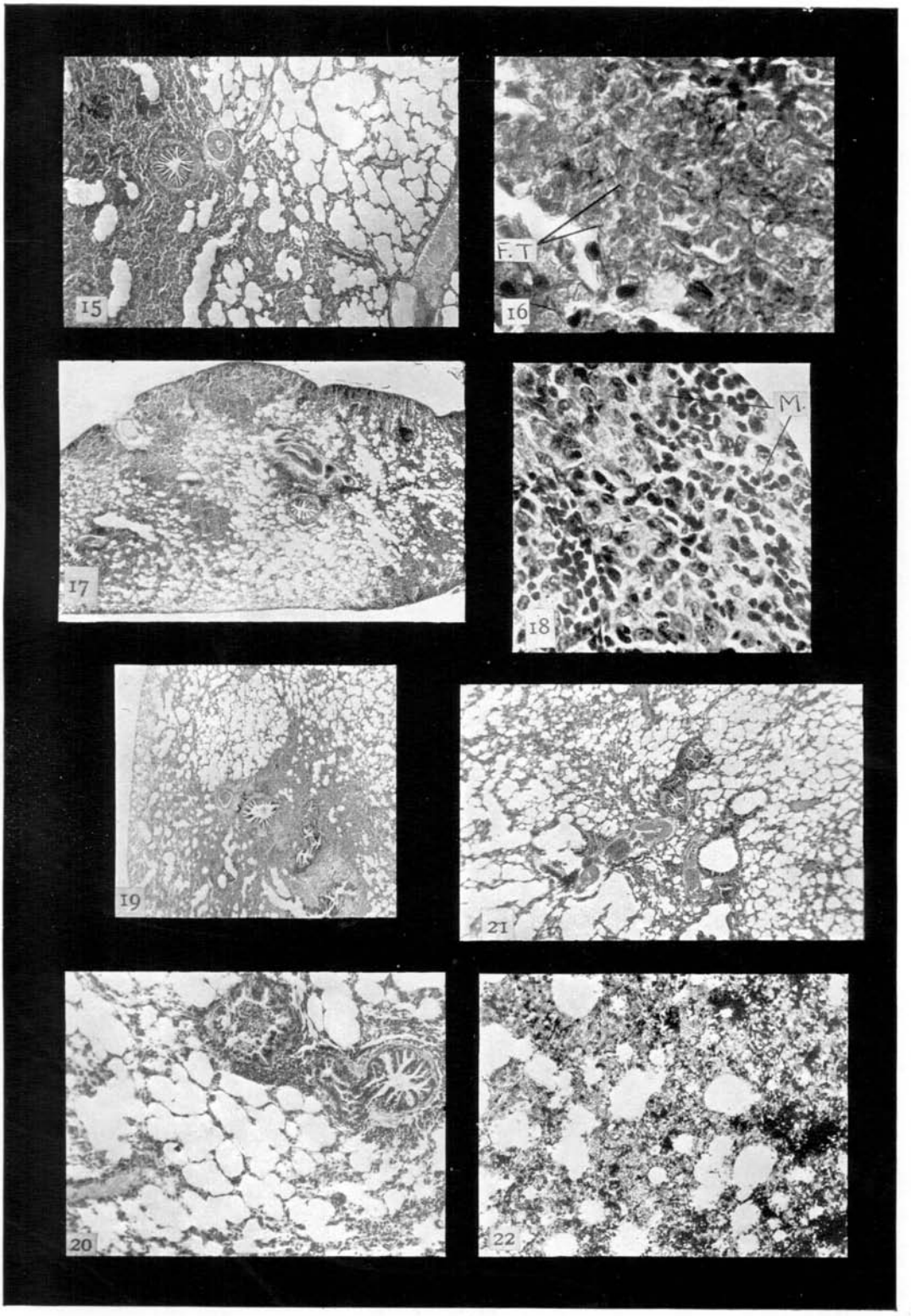

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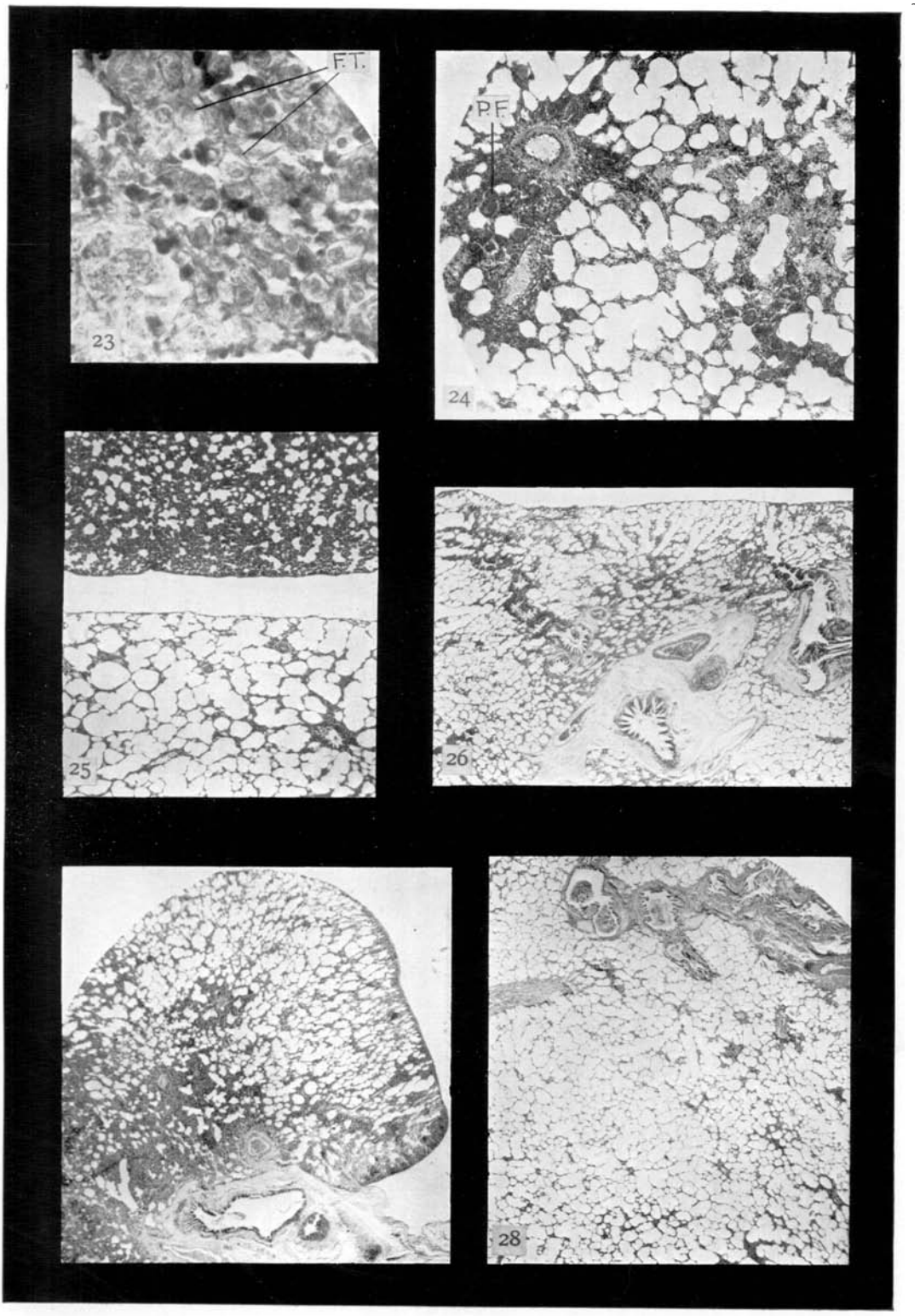

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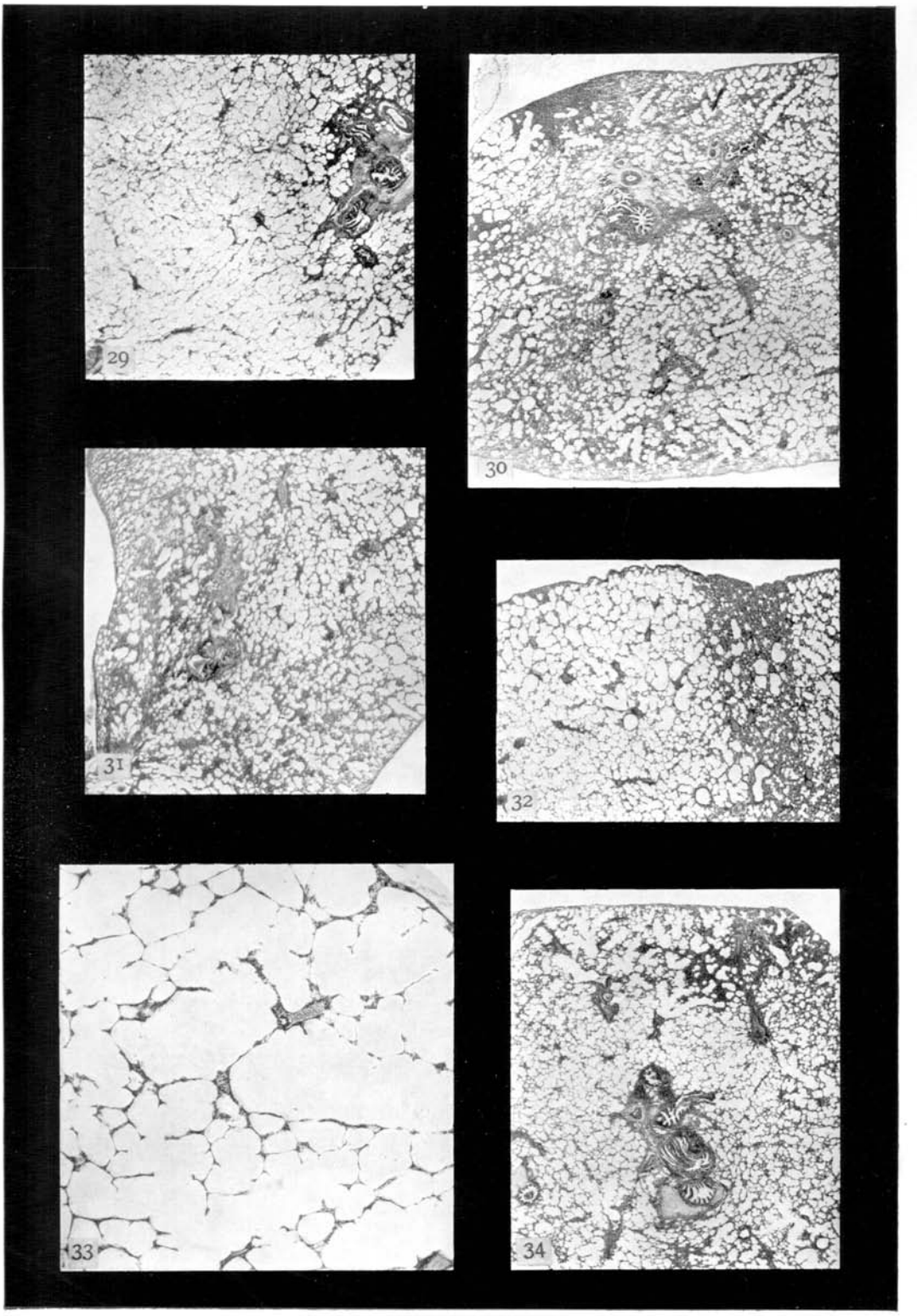

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