EXPERIMENTAL DUST INHALATION IN GUINEA-PIGS.

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(With 6 Text-figures.)

CONTENTS.

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Historical.

While the ill-effects produced in man by the inhalation of certain kinds of dust have been recognised since the seventeenth century, it is not until comparatively recently that experiments have been made to determine the nature of the processes leading to the pathological condition. The nature of the inhaled particles was also known to affect the course and result of the pneumoconiosis. In the case of some dusts large amounts were found *post-mortem* in the lungs of individuals who had not suffered from pulmonary disease. When siliceous dusts had been inhaled there was fibrosis of the lungs, very frequently with tuberculosis, which was the immediate case of death. It was further noted that very small concentrations of silica dust in the inhaled air were sufficient to produce fibrosis and tuberculosis, while very considerably larger amounts of other kinds of dust, including coal dust, had no such effect.

Experiments on the effects of dust on the lung, and the manner in which that organ disposes of the dust, have been conducted along three separate avenues: the earliest method was to expose the animals to a cloud of dust, while later methods were the injection of suspensions of dust intratracheally and intravascularly respectively. It will be obvious that the pulmonary reaction obtained by the first method will most closely approximate to that found in human pneumoconiosis.

Arnold (1885), who was the first worker to dust animals experimentally, used soot, sandstone and emery. He was more concerned with the question of

the elimination of the dusts than with that of the permanent lesions which might be caused by them, and found that much of the dust disappears from the lung in course of time. He described the phagocytic dust cells and their elimination either directly into the bronchi or *via* the lymphatics into the bronchial glands, and considered that the actual phagocytes were derived from the alveolar epithelium.

The next important paper on the pathology of various induced pneumoconioses is that of Beattie (1912), who used coal and various stone dusts. He was one of the first students of the problem to infer that the tendency of a dust to cause pulmonary fibrosis is the main key to its character. He stated that the more irritating the dust the more intense the fibrosis. This statement is only a half-truth, for many harmless dusts cause a marked irritative reaction immediately on their entry into the lung. If, however, a dust only develops its irritative properties after being phagocytosed within the lung, Beattie's statement holds good as a general rule.

Mavrogordato (1918), working on behalf of the Medical Research Council, conducted a series of experiments with guinea-pigs, using various dusts—coal, flue dust, shale, quartzite, precipitated silica and mixed flint and coal. It is unfortunate that in this paper he adopts as his principal criterion of pulmonary damage the formation of plaques of dust cells, and states that the plaques are the only site of fibrosis. This does not agree with the writer's findings. Further, he states that precipitated silica is rapidly eliminated. In the writer's series, while this dust was fairly rapidly removed from the lung, it was found to be very much the most apt to produce fibrosis of all the dusts examined (see p. 109).

To Mavrogordato is due the observation that the colourless dusts administered by him became blackened in the lung. This is also true in the wide range of dusts used by the author: some dusts become much darker than others, but all become pigmented some 6 months after inhalation. In this respect it may be of interest to mention the work of Granel and Hedon (1928), who state that the nucleated cells of the pulmonary epithelium may normally contain melanin. The haemoglobin of dead red blood corpuscles is taken up by cells of the reticulo-endothelial system, passing from the endothelium of the pulmonary capillaries to the alveolar epithelial cells, where it is converted to melanin, which passes away in desquamation of the cells. If this be true, the blackening of intracellular pigment may be due to the deposition of melanin on the particles.

Mavrogordato's most important experiment was the simultaneous administration of coal (notoriously the most easy of dusts for the lung to excrete) with flue dust and with crystalline silica, each of which causes permanent damage when administered alone. He found that the coal in the mixture tends markedly to assist the removal of the harmful ingredient before pulmonary damage has occurred: this if the latter be only in small proportion in the mixture. He also gave intense exposures of dust to guinea-pigs, keeping them for 36 hours in the dusting chamber, using six daily 6-hour exposures. While

only a few animals were used, he noted that "nearly all distinction between the behaviour of the different dusts disappeared" in the animals killed shortly after the experiments. There was much dust in all the lungs, though, in the case of coal and shale, considerable quantities had collected in the connective tissue at the roots of the lung. In animals killed 3 months after the last exposure he found that much more of the coal and shale dusts had been transported to the bronchial glands than was the case with flint and Transvaal dust (quartzite).

This author's conclusions are: Dusts that make mischief are dusts that accumulate. Dusts that are eliminated are dusts that produce a marked initial reaction, with much shedding of epithelium. Dusts that accumulate do not produce so marked an initial reaction, there being much less shedding of epithelium. Dusts that produce an initial reaction tend to carry out with them the more inert.

The same author (Mavrogordato, 1922) continued and amplified the coal-silica mixture experiments, and concluded that silica fixed in the lung is unaffected by subsequent inhalation of coal, but that prior or simultaneous exposure to coal appears to render the lung resistant to the action of silica.

Mavrogordato (1926) recounts the results of a long series of experiments carried out on the Witwatersrand. This paper is concerned with the relations of silicosis and tuberculosis in gold miners, and contains an elaborate summary, from which the following points are taken:

1. There are two types of disabling miners' phthisis: tuberculo-silicosis, where the fibrosis is dominant, and silico-tuberculosis, where the infection is dominant.

2. There are two factors in miners' phthisis: a phthisis-producing dust and the infection.

3. The reticulo-endothelial system is responsible for the phagocytosis of dust and of the tubercle bacillus. The cells of this system, with which he includes the vascular endothelium, though labile in function, may become determined towards their final form (fibrous tissue) under certain stimuli.

4. A phthisis-producing dust probably possesses a slight degree of solubility in the tissue fluids of the body.

5. The effective occupation of the lungs by a phthisis-producing dust may influence the course of infections other than those due to the tubercle bacillus.

6. It may need less accumulation of dust and less duration of exposure to lower resistance than to give rise to a simple clinical silicosis.

7. Coal dust does not appear to influence the resistance of the lungs of guinea-pigs or rats to pulmonary tuberculosis. This conclusion is in conflict with the findings of Wainwright and Nichols (1905), who found that guinea-pigs, after exposure to coal dust and subsequent inoculation of tubercle bacilli, died of tuberculosis but were free from pulmonary lesions. Mavrogordato's explanation is that he probably failed to select a suitable dose of bacteria. It is more likely that the discrepancy was due to the fact that Wainwright and

Nichols administered their tubercle bacilli per tracheam, whereas Mavrogordato injected his intraperitoneally. If the dust cells are derived from the reticulo-endothelial system one would expect the results of the two experiments to be the same, since it is admitted on all hands that the dust cell is responsible for the phagocytosis of the tubercle bacillus. If, on the other hand, the dust cell is derived from the alveolar epithelium, the results of the two experiments are what would be expected. Sewell (1918) studied this problem by the intravenous injection of carmine followed by the intratracheal injection of indian ink, pigeon's blood and other substances. He found that while the carmine particles were ingested by the macrophages of the blood, the carbon particles or foreign blood cells were taken up by phagocytes within the alveoli, which he states are definitely derived from the alveolar epithelium.

Permar (1920) disputes Sewell's findings, and claims that the dust cells are endothelial cells derived from the pulmonary capillaries, and that they invade the alveoli, phagocytose foreign particles, and make their way back through the alveolar walls into the lymphatics.

Westhues (1922) and Carleton (1927), using a technique similar to that of Permar, were unable to confirm his results, and each concludes that the dust cell is of alveolar epithelial origin. The former author washed the cells out of the vascular system of a rabbit and injected indian ink into the trachea. Two fragments of lung were then excised, one fixed immediately and the other placed at body temperature in saline for half-an-hour before fixation. In the former piece no phagocytosis was seen, but in the incubated piece of lung a considerable degree of ingestion of the carbon particles was noted. In supplementary experiments indian ink was injected into the blood vessels after removal of the blood cells. The lung was incubated in saline as before, but on fixation and sectioning no phagocytosis was seen.

Seeman (1925), after the intratracheal injection of iron lactate, demonstrated the presence of iron within cells free in the alveoli, and also attached to the alveolar wall, but after intravenous injection of the same salt was unable to find any iron in the pulmonary capillary endothelium. Further, Seeman showed that the staining reactions of reticulo-endothelial cells and of alveolar epithelial cells to vital dyes are different.

Binet and Champy (1926) and Carleton (1925) found that alveolar epithelial cells proliferate and are highly phagocytic in tissue culture, while endothelial cells are not.

Willson (1928) found that branching of the bronchial tree continues after birth, and new branches are formed both by centripetal and centrifugal processes, so that previous respiratory alveoli become incorporated into non-respiratory bronchioles. The endodermal origin of this highly phagocytic cell can hardly, then, be denied, and Guieyesse-Pellissier's statement that the alveolar epithelial cell is so little advanced in differentiation that it can evolve in divers ways according to the circumstances influencing it is probably true.

The weight of evidence is in favour of the alveolar epithelial origin of the

dust cells, and in the course of the present research no phagocytosis by cells definitely not derived from the alveolar epithelium has been seen.

Carleton's publication (1924) is the most detailed study of experimental pneumoconiosis to date. This author investigated the action of mixtures of coal and flint, and found that one part of coal to two parts of flint by measure caused some degree of permanent damage, but no fibrosis, while in a series with the proportions of the dusts reversed the damage was less in degree, though still present. He concludes that "the early phagocytic response initiated by the coal is responsible for the rapid elimination of much of the flint."

The administration of china clay produced permanent lesions, which were also found in a group of animals exposed to half the usual concentration of dust. In a third group, exposed to half the usual amount of dust, but with the 24-hour exposures spread over 6 weeks instead of 14 days, the lesions were less marked, indicating that the lung may in some degree be trained to eliminate such a dust.

Felspar, ground pitcher (as used in the Potteries) and amorphous silica rendered insoluble by heating to 800° C. were all found to cause fibrosis. Flint, however, while causing lesions likely to lead to permanent pulmonary damage, was not found to cause fibrosis. Pure coal was administered to serve as an experimental control to the other dusts used, and was found to be rapidly eliminated without causing any trace of permanent damage. Shale gave results confirming those of Beattie (1912) and Mavrogordato (1918), being removed less rapidly than coal, but causing no permanent damage. To test the theory that coal and shale owe their innocuous properties to their contained organic matter stimulating phagocytosis, a group of animals was dusted with ignited shale, with results similar to those obtained with ordinary shale.

Dried garden earth was found to cause no permanent lesions, though it was but slowly phagocytosed, and ignited earth showed similar results in the early stages, though the experiment was not completed, as most of the animals were carried off by an epidemic of pneumonia. The present writer has found that all the dusts administered by him were rapidly ingested by phagocytes (see p. 104), although the speed of elimination of the dust cells varied considerably with the nature of the dust.

Carleton (1927) describes the effect of haematite and of levigated iron. Haematite was found to initiate a brisk reaction, and to be fairly rapidly removed without causing permanent damage (see p. 110), but iron produced greater inflammatory lesions, and caused a slight degree of fibrosis.

Landis (1925) states that there is no proof that organic dusts are harmful. Much of the appearance of damage from them comes from the large amount of silica and other inorganic dusts in them. Organic dusts at times produce protein intoxications, but they emphatically do not produce pneumoconiosis.

Methods of experiment.

The experiments were conducted as follows: twelve healthy guinea-pigs, usually not quite full grown, were chosen as a group. The animals were placed,

in cages, in the dusting chamber described by Mavrogordato (1918). Until recently each animal was placed in a jaconet bag, with only its head emerging, before being placed in the cage, this being done to prevent the dust cloud from being lessened by the animals urinating. In the later experiments performed at St Bartholomew's Hospital this has been found unnecessary, however. 140 c.c. of the dust was placed in the trough of the machine, and the animals exposed to the dust cloud for 2 hours. Towards the end of the dusting period the dust flocculated, and flew less well, as pointed out by Mavrogordato. This is in all probability due to its becoming damp. To allow for the dust thus out of circulation, 70 c.c. of dust was added to that already in the machine before the next dusting was commenced. In Group 19, however, where the object was to have the maximum amount of dust in the air, an unmeasured excess of dust was added each day.

Each group received 2 hours' dusting per weekday for a fortnight (24 hours in all) except in the case of Groups 1, 2, 3 and 19. The dust cloud in the machine was thick, but the animals appeared to suffer little, if any, discomfort. That the exposures to the dust were inadequate to affect the health of the experimental animals is shown by the fact that the percentage casualties in the epidemics of pneumonia, which periodically swept through the menagerie, were greater in the control than in the experimental animals. In this connection it may be of interest to mention that casualties have been less frequent since the animals have been kept in the City of London than was the case when they were housed at the Cherwell Laboratories, Oxford, which have definitely rural surroundings.

Two guinea-pigs were killed soon after the last exposure, two others 2 to 6 weeks afterwards, and the remainder at times varying with the indications found in the earlier animals.

In all cases where the survivors were not thinned out by casualties, two animals were killed at the same time, in order to minimise as far as possible the variations due to individual response of the guinea-pigs to dust.

Maitland, Cavon and Detweiler (1921) describe haemorrhagic lesions of the lungs in guinea-pigs, and ascribe them to the mode of killing. According to them, a blow on the head causes many of these lesions, as does also cutting the carotid arteries. They find the lungs most nearly normal in those animals killed by intensive chloroform inhalation. The writer's experience fully bears out their findings, and the animals were as a routine killed with chloroform, death occurring very rapidly. Immediately the animal was dead, the trachea was ligatured at the upper level of the thyroid, and the thoracic viscera removed *in toto*. In surprisingly few cases did autopsy reveal any pathological condition of the abdominal viscera. The haemorrhagic spots on the lung surface caused by the chloroform could almost invariably be distinguished from older ones by their difference in colour in the fresh specimens.

The fixative employed as a routine was mercuric-chloride-formol, experience having shown its superiority over other mixtures for all-round work on the lung. The tissues were immersion-fixed for 24 to 48 hours, and transferred to

70 per cent. alcohol containing iodine to remove the precipitate formed by the mercuric chloride. After 12 hours in this, they were dissected so as to give one section across both lungs at the level of the bifurcation of the trachea, another section being taken across the middle of each lobe. The tissues were dehydrated and cleared in the usual manner, and were freed from air in the vacuum thermostat when in paraffin of 48° C. melting point. Providing that the tissue is kept submerged in the paraffin by means of wire gauze, the reduction of pressure in the thermostat may be taken to the limits of a water-pump. The specimens were embedded in paraffin of 54° C. melting point, and the blocks allowed to set at room temperature, much better results being thus obtained than by cooling them in tap water.

As a routine, 8μ sections were studied, but 4μ, 25μ and 60μ sections were occasionally used. Where necessary, serial sections were made.

Delafield's haematoxylin and eosin was the standard staining method used, and sections from all animals were also stained in van Gieson's collagen stain. In cases where a nuclear stain was required to precede this, Heidenhain's iron haematoxylin was used. Curtis' substitute for van Gieson's stain (Ponceau S and picric acid) was found to be unreliable for guinea-pig lungs, as it does not pick out the youngest and finest fibres. Weigert's elastin stain and Ziehl-Neelsen's and Gram's methods were resorted to when necessary.

The staining in van Gieson must be most carefully done in order to get the correct degree of differentiation. This is obtained when the thin subserous layer of collagen in the pleura stands out brilliantly against the smooth muscle with which it is associated.

Controls.

Control animals were periodically examined. These were healthy beasts which had for some time lived under the same hutch conditions as the dusted animals. The controls were of all ages, so as to correspond more accurately with the experimental material. There was no constant variation in normal pulmonary condition with age.

The principal points to be mentioned in connection with the normal guinea-pig's lung are:

(1) There is very little connective tissue present except in the pleura and around the blood vessels and bronchi and their branches. This enables one to detect a very slight fibrosis—one which would pass unnoticed in the human lung.

(2) Small areas of congestion, with thickening and slight proliferation of the alveolar epithelium, are moderately frequent. Broncho-pneumonia, described by Carleton (1924) in some of his controls, was very seldom observed by the writer. It would appear by the present observations that broncho-pneumonia is rare either in normal or dusted animals.

(3) A very slight amount of intracellular dust is normally present in the guinea-pig's lung, in the alveolar wall, the pulmonary lymphoid tissue and the bronchial lymph glands. In the case of the controls to this paper, this dust was hardly likely to be carbon, since the hutches were in an exceptionally

smoke- and dust-free atmosphere. It is probable that they were derived from the animals' bedding, and became blackened after deposition in the lung. Although Willis (1922) describes a spontaneous pneumoconiosis in aged normal guinea-pig lungs, no trace of such a condition was observed by the writer. Indeed, in view of the efficiency of the guinea-pig's nasal filter, the occurrence of such a condition would seem to indicate that the animals had been housed under most unsuitable conditions.

(4) Small lymph nodules are fairly common, generally being perivascular or peribronchial, but sometimes occurring in the parenchyma.

(5) As first pointed out by Opie (1904) the lymph nodules and the connective tissue of blood vessels and bronchi are often infiltrated with eosinophils.

THE EXPERIMENTAL FINDINGS.

The reaction of the guinea-pig's lung to inhaled dust may be briefly summarised as follows. Immediately after the last inhalation there is usually a fair amount of dust free in the bronchi, and to a less degree in the alveoli. The response of the lung to the first entry of dust is described in the first three groups of animals dealt with below. After the normal exposures, dust cells are numerous, both forming part of the alveolar wall and free in the alveoli and bronchi. Little or no dust is to be found in the pulmonary lymphoid tissue, or in the tracheo-bronchial lymph glands. There is thickening of the alveolar epithelium to a degree varying with the dust used, while there may be epithelial proliferation. In animals killed 3 or 4 weeks after the last exposure there is usually a less marked reaction to the dust than in the first animals. The thickening and proliferation of the epithelium is less general, but is often intense in patches. In such patches there is congestion. To the naked eye the lung may begin to show pigmentation, and there may be one or two haemorrhagic spots. After 3 or 4 months large areas of lung may be normal, except for the presence of dust cells in the alveolar walls. Some degree of thickening is always present, and incipient fibrosis may be found in the areas of most marked epithelial thickening. No matter if the inhaled dust were colourless, it has become blackened by this time, and the naked eye pigmentation of the lung surface has increased. The dust has commenced to enter the lymphatics in fair amount, and the dust cells are found beneath the pleura and in the pulmonary lymphoid tissue. The tendency of dusts to undergo lymphatic elimination varies, and from this time on the lungs of different groups of animals vary in appearance according to the nature of the dust. If this be harmless the number of dust cells in the lung slowly but gradually decreases, while the thickening of the epithelium decreases in intensity and extent. More and more dust cells appear in the lymphoid tissue, which may increase in amount, while others are constantly found on their way up the bronchi. In the case of a "harmful" dust (one which produces permanent pulmonary lesions) the dust appears to undergo removal in only a slight degree, free dust cells being comparatively rare after the first few weeks. Plaque formation may occur, either with or without

fibrosis, while fibrosis in areas of marked thickening may exist without the presence of plaques. The fibrosis is very seldom of the nodular variety so generally found in the human lung. In the later stages of a lung containing an irritating dust an eosinophil infiltration is often found; it may be taken as an indication of the likelihood of fibrosis supervening.

The following experiment was performed to discover the nature of the immediate response to a dust known to be one of the "harmless" class. Six animals were exposed to a dense cloud of shale dust for 2 hours. One was killed immediately. Dust was found freely plastered on the epithelium of the trachea and the larger bronchi, while the smaller bronchi contained plugs of mucin with a good deal of entangled dust. Even so early as this, occasional dust cells were found free in the bronchi, while there was very little free dust in the alveoli. A considerable amount of dust had obtained access to the lung. The larger particles were found entangled in mucin in the trachea and bronchi, while the alveoli, especially those immediately beneath the pleura, contained the finest particles, often in large numbers. Many dust cells were found in the alveolar walls, often in process of detachment. The dust cells were most numerous near the pleura, where there was the most dust (see Figs. 1, 2 and 3).

This experiment shows that within a maximum period of 2 hours after the entry of dust into the lung it may not only be phagocytosed, but the phagocytic cells may be detaching themselves from the alveolar walls. No sign of phagocytosis by cells not derived from the alveolar epithelium was seen.

The second animal was killed $1\frac{1}{2}$ hours after the dusting. There was very little free dust in the trachea, but a moderate amount plastered against the bronchial epithelium. Dust cells were lying free in bronchi and alveoli, the walls of the latter containing many dust cells. There was a very slight amount of dust free in the alveoli. Occasional ciliated columnar cells of the bronchial epithelium contained phagocytosed particles.

There was a marked though patchy thickening of the alveolar epithelium $4\frac{1}{2}$ hours after the dusting, with some proliferation and a certain amount of congestion. The alveoli were practically free from extracellular dust, but many dust cells were present in the alveolar walls, while some were detaching and yet others were free in the alveoli and bronchi (see Fig. 4).

After $22\frac{1}{2}$ hours the only free dust seen was in the medium-sized bronchi. Dust cells were actively becoming detached from the alveolar walls. Numbers of dust cells were to be seen free in the alveoli and bronchi.

The last animal, killed $44\frac{1}{2}$ hours after the dusting, showed no free dust anywhere in the lung. The thickening of the alveolar walls was still to be noticed, while the shedding of dust cells into the alveoli and bronchi continued.

In all the animals the detached dust cells were smaller than those usually met with in animals killed a longer time after the last exposure. They were spherical, with fairly well-staining nuclei. In the animals killed after $22\frac{1}{2}$, $27\frac{1}{2}$ and $44\frac{1}{2}$ hours there were many polymorphs in the blood vessels and the

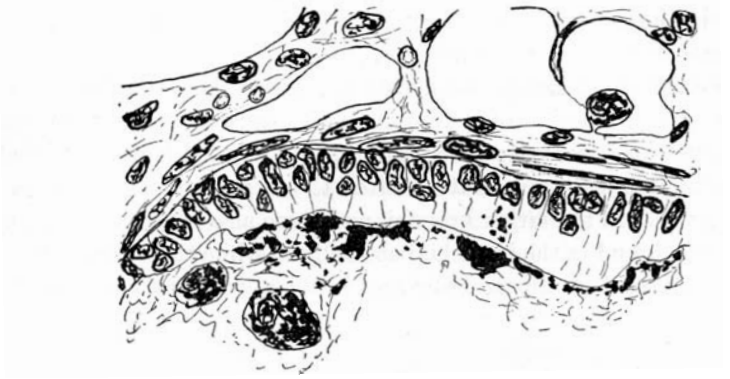

Fig. 1. Animal killed immediately after a single 2-hourly exposure to shale dust. Dust cells and free dust in a bronchus. Dust particles within a ciliated columnar epithelial cell. Sessile dust cell about to detach.

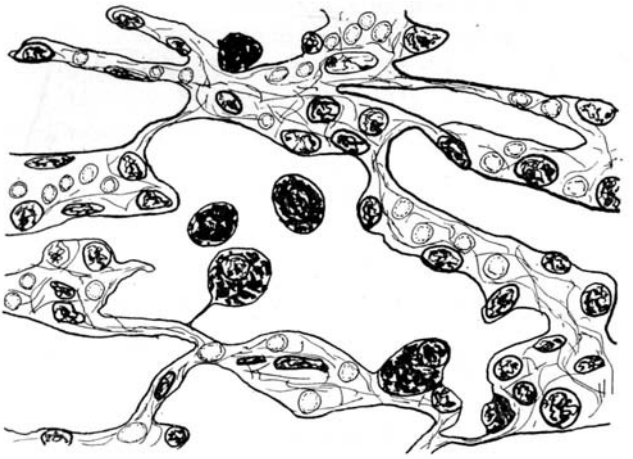

Fig. 2. Animal killed immediately after a single 2-hourly exposure to shale dust. Heavily loaded dust cells free in alveolus, detaching and attached to alveolar wall.

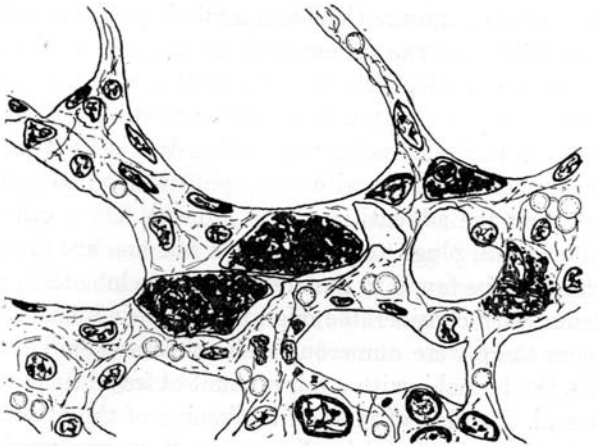

Fig. 3. Animal killed immediately after a single 2-hourly exposure to shale dust. Two very heavily loaded cells filling an alveolus.

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parenchyma in those areas where the reaction to the dust was most marked, but no polymorph contained any dust.

A similar experiment was performed to determine the immediate response of the lung to the inhalation of a dust known to cause rapid and permanent lesions. The dust used was pure precipitated silica, as used for Group 5 below.

In the animal killed immediately after the exposure there was a fair amount of dust free in the larger bronchi, and some also in the alveoli. Intracellular dust was found in the bronchial epithelium, while there were numerous dust cells in the alveolar walls and also free in the alveoli and bronchi. It was

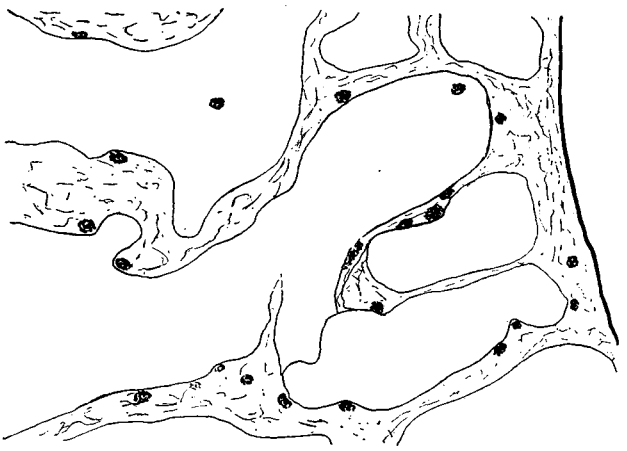

Fig. 4. Animal killed 4½ hours after a single 2-hourly exposure to shale dust. Low-power view of juxtapleural alveoli (unstained) showing amount and distribution of the dust.

obvious that the entry of the dust had elicited a brisk phagocytic response within the lung, and that it was already being eliminated by the bronchial route.

Two hours after the exposure there was a fair degree of oedema in patches, with some congestion. In the oedematous areas there was some degree of eosinophil infiltration, while, although the total number of dust-containing cells in such areas was not great, a fair number of swollen dust cells were becoming detached from the alveolar wall. Free dust cells were found in the bronchi in fair numbers, together with eosinophils which had certainly in some cases migrated from the adventitia of the bronchi. These cells were usually entangled within mucin plugs, together with a fair amount of free dust. Very little free dust was to be found in the alveoli. In one lobe there was a marked degree of oedema, with considerable migration of eosinophils.

After 6 hours there were numerous eosinophils and fair numbers of dust cells free within the bronchi, with a fair amount of free dust in mucin plugs in the larger bronchi. Patches of marked thickening of the alveolar epithelium were frequent in the apices, and in these areas there was much intracellular dust, while the nuclei of many of the cells were pyknotic. Large numbers of

eosinophils were present in the parenchyma, in the areas of thickening most of them being in a state of degeneration. Many dust cells were found in the alveolar walls, and a fair number free in the alveoli and bronchi. The lesions as a whole were much less marked in the lobes than in the apices.

In the animal killed 24 hours after the exposure there was marked and almost universal thickening of the alveolar epithelium, with a massive eosinophil infiltration not only of the parenchyma but of the adventitia of the bronchi. Numerous eosinophils were seen migrating through the mucosa of the bronchi to become free within the lumen. There was some free dust in the bronchi, but very little in the alveoli. Pyknotic nuclei were common in those areas where there was any great amount of dust. Dust cells were free both in the alveoli and the bronchi.

After 48 hours the condition of the lungs was less severe than at 24 hours, the intensity of the reaction obviously having passed its maximum. No free dust was seen. Dust cells were free in the alveoli and the bronchi. There were patches of marked thickening in the lobes. The dust was by now of a golden colour.

The lung condition was much more nearly normal in the last animal, killed after 96 hours. Fair numbers of dust cells were found free in the bronchi, but not many within the alveoli. The eosinophil infiltration was by now only slight except in the areas of most marked thickening, where there was a good deal of dust. There was a certain amount of bronchitis.

It is obvious from this experiment that there may be very little difference in the phagocytic reaction of the lung to a definitely harmful dust and to a harmless one. The whole of the dust within the lung had been phagocytosed within 48 hours, and the dust-containing cells were being shed into the alveoli within 2 hours after the first moment of exposure. The subsidence of the lung towards a normal condition 96 hours after the exposure, after a marked reaction to the entry of the dust, was most striking, as was also the eosinophil infiltration. This is never seen in the guinea-pig except in the case of an irritating and potentially harmful dust. Another point worthy of notice is the pyknosis of the nuclei in areas where there was much dust. This phenomenon has very seldom been seen in the course of this research.

A group of animals was dusted in the same manner, using flint dust. The reaction of the lung was in all ways similar to that in the previous experiment, but was less intense. No free dust particles were to be found 24 hours after the exposure, but while dust cells were detaching from the alveolar walls, they were doing so in smaller numbers than in the preceding experiments.

Group 1. Dust used: equal parts of flint and shale, administered as follows: once weekly for 2 weeks, twice weekly for 2 weeks, and once each alternate day for 12 days, making twelve 2-hourly exposures in all, spaced out over 6 weeks. The aims of this series were twofold: firstly to see if the administration of a rapidly and easily eliminated dust such as shale along with such a dangerous dust as flint would result in the dust cells carrying away the flint along with the

shale, and secondly to see if the lung could be trained to remove a noxious dust more rapidly (and therefore with less eventual damage) than is the case when daily exposures are given. Carleton (1924) used the acclimatisation method of dusting with china clay, and found that with that dust the lung could in some degree be trained to remove the inhaled particles with greater rapidity.

In the earlier animals there was the usual reaction to dust: dust cells in the alveolar walls and free in the alveoli and bronchi, thickening and proliferation of the alveolar epithelium, some congestion, and a gradually increasing amount of dust in the bronchial lymph glands. Indications of the irritating nature of the dust were found in the later animals in the lymphoid infiltration of the blood vessels, and, in the last animal killed (28 weeks after the last exposure), the presence of patches of incipient plaque formation.

The conclusions to be drawn from this series are that the lung may be in some measure trained to cope with a dust, and that shale, administered along with flint, and in at least equal amount, facilitates the removal of the flint and so reduces the liability to pulmonary damage. The greater the proportion of shale in the mixture the less would be the likelihood of damage.

Group 2. Dust used: china clay, administered as for Group 1. This group was a repetition of a series examined by Carleton, but using the normal amount of dust in the dusting machine, instead of the reduced amounts used in Carleton's series (1924). The writer's findings differ in some degree from those of that author, but are rather more conclusive. This is no doubt due to the larger amount of dust used in the present series.

There was not a very marked response to the entry of the dust, but some emphysema was noted in an animal killed one month after the last exposure. Incipient plaque formation, which is never caused other than by potentially harmful dusts, was found in two animals killed 14 weeks after the last exposure, incipient patchy fibrosis in two animals at 39 weeks, and definite fibrosis in areas of chronic inflammatory change in animals examined at 60 and 64 weeks respectively.

These facts indicate slow elimination and a slowly developing but progressive damage to the lung. Acclimatisation with this dust in the amounts used is not sufficient to have any appreciable effect on the course and degree of the damage.

In clinical experience, china clay is not a harmful dust, and the lesions found in these experiments may be due to the large amount of dust entering the lungs in a short time. It would appear, however, that the danger of pulmonary lesions must be expected in the case of human beings exposed to heavy concentrations of china clay.

Group 3. Dust used: flint, administered as for Group 1. Not much dust entered the lungs of any animal of this series. The early response was not very brisk, but a certain amount of the dust was removed *via* the bronchi. In the later stages the lungs settled down to definite and progressive fibrosis. Plaque formation was not noted in any animal of this series, but incipient fibrosis

was found at 12 weeks, and had become definite at 30 and 54 weeks. The fibrosis was always found in areas of chronic proliferation resulting from the presence of a good deal of dust. Even in the case of flint, one may find areas which contain little dust and are histologically normal, while neighbouring patches containing many dust cells are fibrosed. Acclimatisation was definitely unsuccessful with flint, probably on account of the toxic nature of the silica which is constantly being dissolved away in minute quantities from the ingested particles.

Group 4. Dust used: slate. Two hours' exposure each week day for a fortnight, making 24 hours' exposure in all. ("Rapid" method.) In no case did the lungs of animals of this group contain much dust, as it flies badly in the machine. The dust particles are very fine. Slate was tested on account of the anxiety felt as to the liability to pulmonary damage of workers in slate, who are exposed to moderate concentrations of the dust.

The initial reaction was moderately severe, but of the usual type, and, while the dust undergoes progressive bronchial and lymphatic removal, fibrosis was noted at 15 weeks, and was constant in all animals examined thereafter. Since there was little dust in the lungs of any animal, the fact of permanent damage being caused within 15 weeks under these circumstances indicates that slate must be ranked among the definitely harmful dusts, although the decreasing amount of damage in the last animals (31 and 52 weeks) suggests that the harmful action is more transient than is the case with flint. The fact that guinea-pigs of a group often show some slight degree of variation in response to a dust must, however, be kept in mind.

Group 5. Dust used: pure precipitated silica, administered by the rapid method, *i.e.* twelve 2-hourly exposures spread over a fortnight. There was not much dust in the lungs of any animal of this group. This dust was used because it seemed difficult to reconcile the conclusion of Mavrogordato (1918), that the dust was eliminated rapidly and without causing damage, with the observations on flint. There was a rapid and marked response to the dust, in the form of thickening and proliferation of the alveolar epithelium, but only 13 days after the last exposure an early but definite fibrosis was noted. With no other dust has such an early onset of fibrosis been described, and the finding is thus at variance with Mavrogordato's observations. The dust certainly tends to disappear from the lung fairly rapidly, but this is only partly due to elimination *via* the bronchi or lymphatics, a considerable amount of it being dissolved. Indeed, it is most probably due to this dissolved silica that the fibrosis occurs so early. The fibrosis was found in patches in the lungs of all animals examined from 13 days to 44 weeks, while in the later specimens there was a good deal of eosinophil infiltration, always a sign in the guinea-pig lung of an irritative process.

Precipitated silica is the most deadly dust investigated, and the utmost care should be taken to avoid its being inhaled in industry.

Group 6. Dust used: haematite, obtained from the Cumberland mines by H.M. Mines Department, administered by the rapid method. There was a

considerable amount of dust in the lungs of the animals of this group. There was a marked initial reaction to the entry of the dust, but the thickening and proliferation of the alveolar epithelium gradually diminished in intensity, while the dust was rapidly eliminated, both by the bronchi and the lymphatics. In the last animals examined (34 weeks after the last exposure), while a good deal of dust remained in the lung, all the signs of progressive elimination were present, and there appeared no likelihood of permanent damage being caused. This finding has been borne out by clinical experience. In this series, lymphatic removal played a greater part than was usually the case with a harmless dust, the probable explanation being the unusually large amount of dust which entered the lung (see Group 19, p. 116).

Haematite, inhaled in small amounts, may be regarded as a harmless dust, but in large amounts the irritation produced might, apparently, cause definite pulmonary symptoms.

Group 7. Dust used: carborundum, administered by the rapid method. This dust was chosen as an example of an almost completely insoluble material. In the first animals killed, 4 hours after the last exposure, there was very little response to the presence of the dust. Although the majority of the particles had been ingested by dust cells, there was little thickening of the alveolar epithelium, and only a slight amount of proliferation. At 24 days, however, the reaction was intense in patches, where there was occasional plaque formation and incipient fibrosis. At 19 weeks the removal of the dust, which had hitherto been very slow, was much hastened, while at 52 weeks large amounts of dust had been carried to the pulmonary and bronchial lymphoid tissue. The fibrosis never became more than very slight.

Carborundum is an important dust as illustrating the influence of the dissolved portion of an inhaled dust on the rate of elimination of the dust-containing phagocytes. In this case, the dust being so slightly soluble, elimination was very slow in onset, the particles acting at first as inert foreign bodies, but at 4 months a slight but sufficient amount of solute has perhaps been liberated materially to hasten the removal of the phagocytes.

This dust is one which would in human experience probably cause little damage if inhaled only in small amounts, but would undoubtedly be dangerous if much were present in the air.

Groups 8, 8 *a*. Dust used: wood charcoal, administered by the rapid method. Since the remaining animals of Group 8 died 4 months after the last exposure, Group 8 *a* was dusted to study the later stages of the pulmonary reaction.

While coal is the least harmful of all dusts, and although it has been so much investigated, the reason for its harmlessness is not at all clear. It has been suggested that the soluble calcium salts present stimulate phagocytosis and elimination, but this is no adequate explanation, since calcium carbonate itself is a harmful dust (see Group 10). It was thought that the carbon in coal might be the cause of the rapid elimination and the freedom from damage, and therefore wood charcoal was chosen as a reasonably pure form of carbon which yet

contained mineral salts which might be thought to stimulate the elimination of the dust.

Under the experimental conditions much dust entered the lungs in both groups. In the first animals there was a surprisingly slow response to the presence of the dust, free particles being found both in the alveoli and bronchi 24 hours after the last exposure. This is a somewhat uncommon condition in the course of these experiments, and may usually be taken as an indication that the dust functions rather as an inert foreign body than by virtue of its solutes. A further suggestion as to the potentially harmful character of the dust was given by the lymphocyte infiltration ("cuffing") of the blood vessels in both the 24 hours' and 26 days' specimens. At 12 weeks there was a drift of the dust towards the pleura. The drift had become marked at 17 weeks, while in both cases an incipient patchy fibrosis was noted. Neither animal killed at 26 weeks showed any fibrosis, and the general pulmonary condition had much improved, though an eosinophil infiltration of the parenchyma indicates that there was considerable chronic irritation. These specimens showed very well that the dust was largely removed by the lymphatic route. In an animal killed 53 weeks after the last exposure there was some plaque formation, and a very slight patchy fibrosis.

Group 9. As it was thought that the permanent damage inflicted on the lung by wood charcoal in the last group might have been due to the very large amount of dust which entered the lungs, a batch of animals was exposed to the same dust for 12 days, but only one-third of the usual amount of dust was put in the machine for each dusting. Since wood charcoal easily forms a dense cloud, however, a considerable amount of dust entered the lungs of the animals of this group also.

The early stages showed the same picture as in Group 8, with the dust being largely removed by the lymphatic route. Animals killed at 19 and 49 weeks showed a slight patchy fibrosis in areas of chronic thickening. The damage caused was not very marked, and since the animals were exposed to a concentration of dust unlikely to be met with industrially except under the most unusual circumstances, wood charcoal should be regarded as a border-line dust, in that it would, if inhaled in large amount, be very likely to cause permanent damage, while smaller doses would be comparatively harmless.

Group 10. Dust used: precipitated chalk, administered by the rapid method. This dust was used to test the effect of a fairly soluble dust, in order that comparison might be made with the soluble silica series. At this stage of the research a theory as to the causation of damage had been formed, and the majority of the later experiments were carried out to test it. It was thought that the degree of solubility of inhaled substances materially affects both the degree of damage caused in the case of harmful dusts, where the solute is noxious to the tissues, and the rate of elimination of the more harmless ones.

In Group 10 there was a widespread though not marked early reaction, which at 9 weeks had proceeded to a patchy thickening and proliferation of

the alveolar epithelium, sometimes intense, and there was incipient fibrosis in some of these areas in both animals. At 28 weeks the thickening and proliferation persisted, and was often intense in degree. Fibrosis was slight but definite, although there was much normal tissue, and the dust particles were being eliminated by the bronchial route. At 55 weeks there was more normal lung, and the dust was still being passed out of the lung both *via* lymphatics and bronchi, but nevertheless there was a slight patchy fibrosis in both animals, while eosinophil infiltration of areas of thickening showed the presence of an irritant.

Clearly this dust is to be regarded as a potentially harmful one, although it is removed to a considerable degree by the lung. Precipitated chalk is fairly soluble, and it would seem logical to attribute at least part of the damage to the solute's acting as an irritant.

Group 11. Aluminium hydroxide, administered by the rapid method. This group was dusted as an additional experiment on solubility factors, and also with a view to Group 14, which is a logical advance on the evidence obtained.

There was not a very marked early reaction, but at 9 weeks the response was much more marked (cf. Group 10). There was an incipient patchy fibrosis in one animal. At 30 weeks there was a considerable increase in the rate of removal, both lymphatic and bronchial, but both animals showed fibrosis. At 72 weeks the condition was more serious, plaques being present as well as fibrosis, and the dust was largely to be found in the interalveolar septa, while at 106 weeks the lesions were yet more marked.

Aluminium hydroxide is obviously a progressively harmful dust, causing only a slight early response, which later becomes more brisk and then falls off again to a largely lymphatic removal.

Group 12. Dust used: calcspar, administered by the rapid method. This substance is crystalline calcium carbonate, and, being crystalline, is not so soluble as the amorphous precipitated chalk. It might, therefore, be expected to offer some comparison with the latter.

More dust was found in the lungs of this series than of Group 10, which would tend to intensify the degree of damage. There was a brisk reaction to the entry of the dust, and at 48 hours a good number of dust cells were found free in alveoli and bronchi. At 10 weeks the reaction was still lively, and dust cells were seen migrating from the peribronchial lymphatics to become free within the lumen of the bronchus. It is of interest to note that the dust was blackened by now. At 20 weeks there was some setback, and a very slight fibrosis was found in both animals. At 66 weeks, however, there was a largely normal lung, with no fibrosis, and the dust undergoing drift towards the pleura. At 74 weeks there was widespread but slight thickening and proliferation of the alveolar epithelium, patches of marked plaque formation, and a slight patchy fibrosis.

Dust cells were free in the bronchi and alveoli in all the animals of this group, but the pleural drift and (in the last animal) plaque formation indicate that lymphatic removal also plays a not unimportant part in the elimination

of the dust. This dust is, therefore, to be regarded with some suspicion, as are all dusts tending to cause plaque formation.

The apparently contradictory states of animals at 20, 66 and 74 weeks is indicative that the dust is a border-line one, and that the variation in response depends on individual susceptibility.

Correlating the results of Groups 10 and 12, it is seen that the precipitated chalk produces definitely more harmful results, especially when it is remembered that Group 12 received much more dust than Group 10. This was not due to variations in dusting technique, but to the fact that chalk becomes more easily damped in the dusting chamber and, therefore, flies less well than calcspar. Since the two substances are identical except for their solubilities, the solute can be the only explanation of the variation in reaction of the lung. It is suggested that on the inhalation and phagocytosis (which follows very rapidly) of a dust a certain amount of solute is formed, which stimulates not only the phagocyte but the neighbouring tissues. If the stimulus be a mild one, the response is the detaching of a cell and its passage into alveolus or lymphatic, but if the solute be a cell poison such as silica, not only may the phagocyte be killed by the toxin but the solute diffuses out into the neighbouring tissues and affects them. It would appear to the writer that only on this hypothesis can be explained the onset of fibrosis in an area where there are very few dust cells and very little dust.

Group 13. Dust used: emery, administered by the rapid method. The variation in solubility between aluminium hydroxide and emery is very much greater than that between chalk and calcspar, emery being almost completely insoluble. Emery consists of crystalline corundum (Al_2O_3) associated with small and varying amounts of magnetite, tourmaline, garnet, etc. (Geikie, *Text-book of Geology*, London).

The initial reaction was fairly marked, and dust cells were free in alveoli and bronchi. At 8 weeks the usual lesions were found, but in no marked degree, while at 31 weeks there was some plaque formation in one animal and incipient patchy fibrosis in both. In a 106-weeks' casualty there was a slight patchy juxtapleural fibrosis.

The indications are that emery behaves as do all approximately insoluble dusts, being largely (after the initial few weeks) removed *via* the lymphatics, with the tendency to damage that this method of removal implies. The fibrosis which is caused by such dusts is the result of inert foreign-body irritation, the distribution of fibrosis in the 106-weeks' specimen being indicative of this.

The soluble hydrated alumina is a more dangerous dust than the insoluble anhydrous crystalline emery, and, as in the case of chalk and calcspar, the causative factor must be the solute.

Irving Clark (1929) states that continued inhalation of the dust of artificial abrasives, consisting of emery or carborundum, did not produce *disabling* pulmonary lesions in men exposed for from 10 to 41 years.

Group 14. Dust used: mixed aluminium hydroxide and precipitated silica.

In this experiment the dusts were mixed in the proportions in which they occur in shale. Shale (*U.S. Geol. Survey Bull.*) contains on an average 59 per cent. SiO_2, 15 per cent. Al_2O_3, 4 per cent. Fe_2O_3, 2·5 per cent. FeO and 1 to 3 per cent. each of CaO, Mg, K and Na, with traces of other metals.

There was a lively reaction to the mixture and the dust was progressively removed. At 24 weeks there was much lung normal except for the presence of dust, and no permanent lesions (nor any indication of their supervening) were found in any animal.

The mixture was composed of the actual dusts used in Groups 5 and 11, which caused fibrosis in 2 and 9 weeks respectively. The conception underlying the experiment was that the dusts when phagocytosed would produce their solutes within the cell, and that these would combine to form silicate. Since the mixture proved harmless in practice it would appear that this happened and, therefore, that not only are many natural silicates harmless if at all soluble, but that similar compounds formed within the body are themselves harmless. The liberation of a solute into the cytoplasm of a phagocyte, and therefore by diffusion into the surrounding tissues, renders the phagocyte obnoxious to those tissues and so causes it to be cast off. Some solutes may be toxic and yet not provide an adequate stimulus for the casting off of the cell, which is thus enabled to diffuse more of the toxic substance. Silica is a type of dust whose solute is toxic, while many natural silicates are not. Here it is not possible to speak more definitely about silicates, as they are very varied in chemical constitution, and this may be the explanation of the harmful nature of china clay, where the silica is present as a disilicate, and of felspar, where it is a trisilicate, while the harmless shale largely consists of monosilicate.

Group 15. Dust used: light magnesium carbonate, administered by the rapid method. This dust was used in view partly of its solubility and partly since naturally occurring magnesium silicates are common, and a series similar to Groups 5, 11 and 15 was thought likely to furnish a useful control to the results obtained from those groups.

Although very little dust was present in the lungs of this group, there was a very marked initial reaction to its inhalation. The violence of the reaction continued, and the dust was predominantly eliminated by the bronchial route. Sub-acute irritation was none the less shown by the presence of eosinophil infiltration at 4 weeks, and at 16 weeks fibrosis was noted. At 31 weeks much of the dust had been removed, yet very few dust cells were found in the tracheobronchial lymph glands, indicating that the majority of the dust cells had been removed from the lung through the bronchi. There was still, however, a good deal of thickening and proliferation, and in these areas a slight fibrosis was to be found.

The indications are that here is a dust which, save for its somewhat undue solubility, would be rapidly eliminated by the lung without the intervention in any marked degree of lymphatic removal, but the dissolved substance is of such a nature as to cause a fibrosis.

The dust, therefore, if inhaled in quantity by man, would cause permanent pulmonary damage, though whether tuberculosis would supervene upon such damage is a moot point.

Group 16. Dust used: one part of precipitated silica to three parts of light magnesium carbonate by weight, administered by the rapid method. These proportions are those in which the constituents occur in talc. The majority of the dusted animals died during an epidemic of pneumonia, but 24-hour specimens showed a marked general reaction, although not much dust was present. Animals killed as long as 54 weeks after the last exposure had a large amount of normal lung, with some eosinophil infiltration of the parenchyma, but no fibrosis. While eosinophil infiltration in 3- or 4-month specimens is generally an indication of a later fibrosis it may be taken when occurring as much as a year after the original exposure to indicate a degree of irritation short of that necessary to produce a fibrosis. The fact that no fibrosis was found after 54 weeks is indicative that the mixture is, to say the least, much less harmful than is the case with either constituent alone.

Group 17. Dust used: talc, administered by the rapid method. Talc is a naturally occurring magnesium silicate. In the case of the natural dust, the irritation and course of the pulmonary reaction are somewhat different from those with the mixture of silica and magnesium carbonate. The early reaction is not so marked, and initial phagocytosis is not so ready, but the later specimens show that the solute merely stimulates elimination of the dust cells, and in no way acts as a toxic agent, even although lymphatic removal plays a not inconsiderable part in the removal of the dust. Even at 60 weeks, both bronchial and lymphatic removal are occurring moderately briskly, and with no signs of pulmonary damage. This degree of lymphatic removal without pulmonary damage has not been met with in any other dusts investigated, and must be ascribed to the very low degree of toxicity of the substances dissolved from talc, which yet are sufficiently obnoxious to the neighbouring tissues to cause the removal of the cells containing the particles.

Group 18. Dust used: "colloidal" coal, administered by the rapid method. This experiment was performed to determine the effect of very finely divided particles. The coal was ground wet in a colloid mill, but the particles were not as fine as might be desired, on account of the leaf-like structure of coal, so that the particles were largely in the form of very thin plates. Coal dust is generally in this form, which disposes of the theory that dusts cause damage by the irregularity of their particles. Nothing sharper than a thin plate with irregular edges can be imagined, yet coal is the least harmful of dusts.

A large amount of dust entered the lungs, and plugs of dust cells were found in the bronchi at 1½ hours. The lung was largely unaffected except for some thickening and proliferation of the alveolar epithelium. At 5 weeks a good deal of dust was found in the bronchial glands, and much more at 12 weeks. No permanent damage appeared likely to be caused, even though there was a parietal drift of the dust. This is probably because the small amount of sub-

stance dissolved from coal is non-toxic, and yet briskly stimulates elimination. The parietal drift and the removal of dust to the bronchial glands indicate that the lymphatic route is employed as well as the bronchial, but this is only likely to lead to trouble when the dust is inert or toxic, or is present in the lung in large amount (see Group 19). In this respect the writer's investigation of the pit pony's lung (Haynes, 1926) showed that dust aggregations are always found in the perivascular, peribronchial and subpleural lymphatics, and in any lymph nodes in the parenchyma, although the bronchi always contain shed dust cells. In some ponies the writer found massive lymphatic aggregations, yet in no case was fibrosis found.

In the present case coal dust, as finely divided as possible, and in fairly heavy concentration, is not harmful.

Group 19. In order to examine the effect of such finely-divided coal dust when inhaled in massive amounts, a group of animals was exposed to "colloidal" coal dust for twenty-four 2-hourly periods extending over a month, double the usual quantity of dust being placed in the machine for each exposure. The experiment is really an enquiry into the effect of the entry of immense numbers of particles into the lung, with the consequent shock to the pulmonary tissues.

At 24 hours (*i.e.* 29 days after the *first* exposure) enormous amounts of dust were present, and in so little time a well-marked parietal drift was found. The general reaction of the lung was intense, but the dust was being dealt with in an orderly and normal manner, no lesion other than the inevitable thickening and proliferation of the alveolar epithelium being found. At 39 days there was an improvement in the pulmonary condition, and the dust was being progressively removed. Mechanical interference with the functioning of the lung, however, was indicated by some emphysema in one animal. The other animal, killed 39 days after the last exposure, had a good deal of broncho-pneumonia with red hepatisation, but in view of the rarity of this condition in the experimental animals it is probable that this was not directly due to the dust, though the interference with lymph drainage due to the intralymphatic dust cell masses probably lowered the resistance of the lung to what was in all likelihood a bacterial infection. No bacteria were seen in the sections, however, either in this animal or in casualties dying of pneumonia.

At 26 weeks intralymphatic aggregations of dust were macroscopically easily visible, and the bronchial lymph glands were black with dust. A fair amount was also found in the spleen. Dust cells were still being actively shed from the alveoli, but in places where the dust was present in large amount the parenchyma was infiltrated with eosinophils.

The condition of the last specimen is rather disturbing, as eosinophil infiltration must be taken as indicative of a chronic sub-acute irritation when found not too long after the last exposure, as has been already mentioned. This irritation may in time provoke a fibrosis.

The important deductions to be drawn from this experiment are that the

lung responds nobly to massive exposures to a harmless dust, but with the passage of time the large entry of dust into the pulmonary lymphatic system seriously deranges that system by mechanical occlusion, with a consequent lowering of local resistance to infection. Further, this occluded dust holds back other dust cells, which must therefore lie in the alveolar wall for bronchial elimination, which is harmless, or pass into the periatrial lymphatics, where their continued presence acts as an irritant and may in time lead to a fibrosis.

Unfortunately the later animals of this group were wiped out in a serious epidemic which killed 85 per cent. of the animals in the menagerie, and a definite statement as to the very late effects of such massive exposures to coal dust cannot be made. Sufficient evidence has been gathered, however, to state that any dust, no matter how harmless it may be in small doses, is dangerous to health if inhaled in large quantity. Definite and serious impairment of pulmonary function occurs fairly rapidly. These conclusions are of the utmost importance in view of modern mechanical methods of coal-getting, in which unless precautions are taken to diminish the amount of dust in the air (*e.g.* by wet-working) the miner is exposed to very much heavier concentrations of dust than is the case where the older method of mining obtains.

In this connection it is of importance to mention the work of Collis and Gilchrist (1928), who state that clinical observation by X-rays shows that, after many years of work, the lungs of coal trimmers are not normal, and exhibit signs similar to those widely regarded as characteristic of silicotic fibrosis. In a personal communication, Haldane informs me that similar X-ray appearances have recently been found, in the course of investigations under the direction of Prof. Cummins, in numbers of South Wales colliers. In the case of colliers the matter is somewhat complicated, since such workers always inhale stone dust along with coal dust. It is occasionally the case that colliers have to work through stone containing a large proportion of free silica in order to reach the coal-seam. Middleton (1929) states that in such cases, once enough silica has been inhaled to produce fibrosis, the subsequent inhalation of coal dust into a lung in which the lymph drainage has been disorganised has the effect of increasing the fibrosis and producing disability. It must be borne in mind, however, that, as the preceding experiment demonstrates, disorganisation of the lymph drainage may be produced by the inhalation of coal dust alone, or, indeed, by the inhalation of any dust in massive amounts. Middleton (*loc. cit.*) states that coal dust may (without any evident exposure to silica) produce a condition in the lungs which radiographically resembles the appearances produced by silicosis. Here again the above experiment shows that such appearances are only likely to be produced in the case of exposure to very heavy concentrations of dust. There is no evidence that such a pure anthracotic condition carries with it any increased liability to tuberculosis, although naturally the pulmonary efficiency of the affected individual will be decreased.

Group 20. Dust used: shale, administered by the massive method, as follows. An excess of dust was placed in the dusting machine, so that the

densest possible cloud was obtained. Two hours' exposures were given on each of the first 3 days and 4 hours' exposures on the remaining 9 weekdays, so that it might be imagined that the animals would inhale almost as much dust in the 12-day exposures as did the animals in the 24-day exposures of the last experiment. In practice this was found not to be so, since not nearly so much dust was found in the lungs of the majority of the animals of this group as in those of Group 19. This may be of importance when considering human experience, where the exposures are seldom less than 4 hours' duration.

Fig. 5. Lateral aspect of right lung of animal killed 12 months after massive exposure to shale dust.

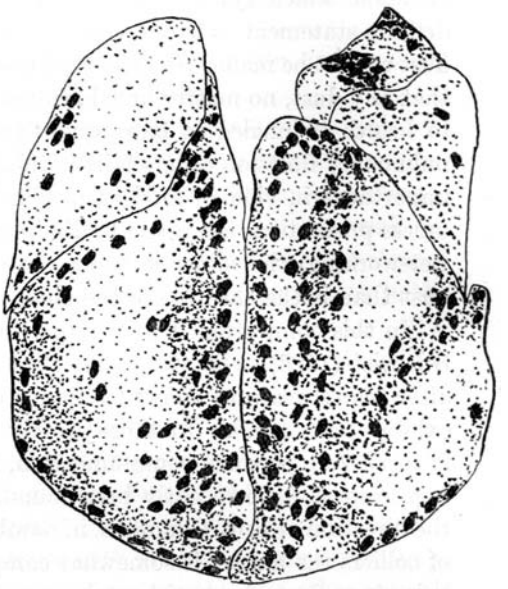

Fig. 6. Dorsal aspect of lungs of animal killed 12 months after massive exposure to shale dust.

In the first animals, killed 4 days after the last exposure, there was a good deal of dust present, with general thickening of the alveolar epithelium, and patchy intense thickening and proliferation. Comparatively few free dust cells were present, but in one animal there were peribronchiolar dust aggregations.

At 11 weeks there was little sign of aggregation of the dust, but in the azygos lobe of one animal there was some giant cell formation. The reaction to the dust was general, but only marked in patches. In one animal there was much normal lung, but a comparatively small amount of dust.

At 8 months there was much approximately normal lung, but many free dust cells were present, although the dust was undergoing lymphoid aggregation, particularly in the lobes.

After 10 months there was a fair amount of dust present, with numbers of dust cells free in the bronchi, while some dust cells were seen passing through the bronchial wall from the lymphatics. Plaque formation was present, especially subpleurally. Dust was present in considerable amount in these areas, and

also in the subpleural, peribronchial and perivascular lymphatics in the apices. A good deal of dust was present in the tracheo-bronchial lymph glands in both animals.

In the last animals, killed 65 weeks after the last exposure, there was a good deal of dust in the lung, but much of the parenchyma was approximately normal. There were numbers of subpleural plaques, however, and a general lymphatic distribution of the dust. There was an incipient fibrosis in one or two areas of plaque formation. The naked-eye appearances of the lungs at 12 months are indicated in Figs. 5 and 6.

The dust used in this experiment was the same as that used for stone-dusting in collieries. It would appear that even such a comparatively harmless dust as shale cannot be inhaled in large amounts with impunity, and the results confirm the deductions made from Group 19 above.

RESULTS OF EXPERIMENTS.

The guinea-pig is the animal which has been most widely used for experimental pneumoconiosis, and it has been objected by some critics that findings in these animals cannot logically be applied to human experience in the naturally occurring condition, on account of the considerable difference in structure between the lungs of experimental animals and of man.

The same objection has been raised against all forms of animal experiment, with as little justification. It is sufficient to know the variation in response between the experimental animal and man to normal stimuli, and with that criterion to be able to estimate very accurately the condition in man comparable with the effects produced in the experimental animal.

Certain dusts produce certain effects in guinea-pigs. These dusts have well-known effects on man, and it may definitely be taken that, notwithstanding the comparatively very delicate structure of the lung in guinea-pigs and the consequently apparently slight permanent effects caused by dusts dangerous to man, the unvarying response of the guinea-pig's lung to dangerous dusts renders this animal a very suitable subject for pneumoconiosis experiments. The fibrosis obtained in guinea-pigs is, under the circumstances of these experiments, of the diffuse variety, while that usually seen in human fibrosis is of the nodular variety. Experiments are now proceeding in an attempt to produce a nodular fibrosis by means of dust-inhalation in guinea-pigs, when the response of the lung to bacterial infection will, it is hoped, offer a better comparison with that of man.

These experiments have shown that fibrosis may be caused by many apparently innocent dusts, that is, dusts whose inhalation in man is not followed by increased mortality from pulmonary disease. What is lacking in the experiments is any correlation between fibrosis and liability to tuberculosis. Since, in human experience, some of these dusts do not appear to predispose to tuberculosis, it would not seem that there is any necessary relation between fibrosis and a liability to tuberculosis. It must be borne in mind, however, that

a fibrosed lung must reduce the efficiency of the worker, and that, whether predisposing to tuberculosis or not, the inhalation in considerable amount of any dust tending to produce fibrosis should be avoided.

As the weight of clinical evidence accumulates, silica-containing dusts stand first on the indictment of causation of pulmonary tuberculosis. According to the work of Kettle this is due to the fact that silica is not only a cell poison, but also forms a medium favourable not only to the survival but to the multiplication of the tubercle bacillus. Policard, Doubrow and Boucharlat (1929) found that the addition to tissue cultures of embryonic chick lung of suspensions in Tyrode's solution of crystalline silica obtained from mine rock did not interfere with the development of the culture. The silica was phagocytosed, however, and had a slow toxic action on the engulfing cells. It appears likely, therefore, that the hypothesis of Heffernan (1929) that silica exerts its deleterious action by means of its adsorption on the surface of cells is not well founded. Again, Middleton (1929) mentions that silicosis, while insidious in onset, may proceed after cessation of inhalation if the dose were heavy. Occasionally, very rapid forms of silicosis are found, particularly among those employed in the manufacture of dry soap powders and cleansers, where alkali is present in the dust along with silica.

As mentioned on p. 114, silicates in general are not particularly harmful, but Badham (1927) reports a rapidly fatal case of silicatosis resulting from the inhalation of orthoclase (potash felspar, a trisilicate) dust containing no free silica. He further states that a fine diffuse fibrosis is found in Broken Hill miners inhaling dust containing less than 10 per cent. of quartz but much iron and manganese silicates. The classification of silicates according to their pulmonary effect would be of considerable value in the elucidation of the problem of silicosis.

While, on account of the delicacy of the guinea-pig's lung, it is easy to recognise a slight degree of damage, it is necessary for experimental purposes to expose the animals to concentrations of dust much heavier than those usually met with in industrial experience. This is in order that a sufficient amount may be introduced into the lung. That the amount inhaled was not sufficient to interfere with the health of the dusted animals is shown by the facts that no animal died in the dusting chamber, and that the dusted animals suffered rather less mortality during the pneumonia epidemics which swept through the menagerie than did control animals.

It must be borne in mind, however, that the "onset-mass" of the dust was considerable, and might tend to produce lesions which would not occur were the animal more gradually acclimatised to the dust. In this respect the findings in Group 19 are of interest.

In most of the animals killed immediately after the last exposure to the dust, occasional ciliated columnar cells of the bronchi contained ingested particles. To the writer's knowledge, phagocytosis by such highly specialised cells as functional ciliated columnar epithelium has not previously been described.

Reference has been made (p. 103) to the occurrence of eosinophils in the guinea-pig's lung. When the dust inhaled was such as to cause a chronic irritation short of the degree necessary to produce fibrosis, there was generally found a considerable increase in the number of eosinophils present in the parenchyma. In some cases the eosinophils were so concentrated in a chronically proliferated area that the tissue was solidly infiltrated with them.

In many animals it was found that there was a considerable increase in the number and size of the lymph nodules in the lung. This increase can be taken (on the results of this work) to be indicative of the irritation caused to the lung and its lymph drainage by the dust.

Plaque formation is a criterion first adopted by Mavrogordato as an indication of permanent damage to the lung. In the writer's opinion, the term is misleading, as the so-called plaques, when studied in thick or serial sections, consist of more or less solid aggregations of the cells whose individual nature is so well described by Carleton (1924, p. 445).

The writer, while agreeing that plaque formation indicates some degree of permanent damage, would suggest that the appearance of such structures may reasonably be ascribed to the fact that the atria have become obstructed either by the accumulation of dust cells within their surrounding lymphatics or by direct obstruction by engorged dust cells, so that other dust cells becoming free are unable to pass from their alveoli, and therefore remain free within them. The picture eventually produced is not unlike that of a small area of grey hepatisation, except that the cells filling the alveoli are dust cells and not polymorphs. As the dust cells degenerate and disintegrate their contained particles are liberated, to be phagocytosed again, with further irritation to the alveolar wall. Unless the obstruction to bronchiolar elimination be removed this continued irritation will result in fibrosis, while if free passage be allowed to the plaque cells—which are merely ordinary dust cells—they will pass out *via* the bronchi and cause no fibrosis.

This explanation appears to be the only one which will cover the facts of plaque formation occurring in some animals of a group and not in the later ones, even allowing for the variation in individual response.

It may be mentioned that when an insoluble dust has been inhaled a large part of it is present in the lung after the lapse of over a year. When inhalation is continuous there is a tendency with a benign dust such as coal or shale for a balance to be reached, and the daily removal of dust to become equal to the intake (Haynes, 1926).

SUMMARY.

The following dusts produce a fibrosis in the guinea-pig's lung, and are therefore to be classed as dusts whose inhalation in industry would be attended by risks of pneumoconiosis. The most deadly of all dusts examined was precipitated silica. Less dangerous, but all producing fibrosis, were the following, arranged in order of decreasing toxicity: flint, slate, aluminium hydroxide, pre-

cipitated chalk, magnesium carbonate and carborundum. In the concentrations used in the experiments calcspar and emery were border-line dusts, indicating that their inhalation in any considerable quantity would cause fibrosis. Wood charcoal inhaled in large amount produces a slight fibrosis, and must, therefore, be placed on the "dangerous" list. Colloidal coal, when inhaled in massive amounts, is potentially dangerous, while shale under similar conditions is rather more dangerous.

Haematite, talc, and molecular mixtures of soluble silica with aluminium hydroxide and magnesium carbonate respectively were not found to cause any permanent lesions in the lung.

The deductions to be drawn from this work are:

1. *All* inhaled particles are rapidly ingested by certain individual cells belonging to the alveolar epithelium.

2. These cells (dust cells or phagocytes) remain in the lung parenchyma until they have ingested an amount of dust constituting the cell's saturation load. This load varies with different dusts.

3. A cell having attained its saturation load becomes sooner or later detached from the alveolar wall and either migrates into the lymphatics or becomes free in the alveolus. In the former case it passes into the pulmonary lymphoid tissue and thence to the bronchial lymph glands. In the latter case it passes up the bronchial tree to be either coughed out or swallowed.

4. Dust cells which speedily leave the alveolar wall are principally eliminated by the bronchi.

5. In the case of a dust cell being eliminated from the lung *via* the lymphatics, it may be arrested in the periatrial lymphatics on account of its bulk. The dam thus produced offers obstruction to the passage of other dust cells shed into the alveoli. Groups of free dust cells in the obstructed alveoli form plaques, which degenerate and liberate their dust. This is again ingested, and the irritation caused by such a process may lead to fibrosis.

6. The continued presence of dust-laden cells in the lymphatics may set up a foreign body irritation, with resulting fibrosis.

7. Most inhaled particles contain soluble matter to at least a very small extent. The solute may be either harmlessly active or toxic. If the former, the cell is stimulated to detach itself from the alveolar wall, and so remove the dust. If the latter, the solute effects the viability of the phagocyte, which becomes less able to detach itself. At the same time the solute diffuses into the neighbouring tissues, with irritation to them, and consequent fibrosis.

8. The more soluble form of a substance causes greater pulmonary damage than the less soluble. The solute, therefore, plays a large part in the determination of damage.

9. While many dusts cause pulmonary fibrosis, silica is the dust par excellence predisposing to tuberculosis. This is doubtless due to its influence in forming a medium suitable not only for the survival but the proliferation of the tubercle bacillus in the lung (Kettle, private communication). The harmful

effects of soluble silica may be neutralised by simultaneous administration of basic dusts such as aluminium hydroxide or magnesium carbonate, though the latter are themselves harmful when inhaled alone. It is suggested that their respective solutes combine to form monosilicate. Monosilicates do not appear to have any harmful effect on the lung.

10. Heavy inhalations of any dust are liable to cause pulmonary damage.

11. The intensity of the initial pulmonary reaction to a dust is very generally in inverse ratio to the degree of eventual damage caused by the dust.

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