

EXPERIMENTS ON THE EFFECTS OF DUST INHALATIONS.

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(Report to the Medical Research Committee.)

INTRODUCTION.

THIS investigation was commenced, and in the greater part carried through, in Dr Haldane's private laboratory at Cherwell, Oxford. The work was undertaken at his suggestion and I am indebted to him for help and advice both in planning experiments and in discussing results.

Owing to the war the work at Oxford had to be interrupted before its completion and some of the later experiments were carried out in the laboratories at St Thomas's Hospital, London, and I am indebted to the staff there for the facilities afforded to me: Dr L. S. Dudgeon especially who helped me in the microscopical work.

The work was done for, and expenses defrayed by, the Medical Research Committee under the National Insurance Act, with the object of throwing light on the dangers arising from dust inhalations in mining and many other forms of industrial work, and on the conditions under which precautions against dust inhalations are specially necessary.

So many people are, owing to the nature of their employment, compelled to pass a large portion of their time in dusty atmospheres, that this condition and its relation to health has attracted much attention. It may be regarded as established that all forms of dust enter the lungs and that some, such as coal, are relatively harmless, while others, such as flint, are deadly.

Dust rarely kills directly, but by predisposing to pulmonary tuberculosis. On the other hand uncomplicated dust appears capable of producing considerable fibrosis of lung.

There is a vast amount of clinical material bearing on this question, but it is lacking in precision owing to the fact that, under industrial conditions, workers are exposed to mixed dusts and it is difficult to be sure of the offender. There can be no doubt of the deadly character of dust rich in silica: experience in the Transvaal gold mines and in the

potteries is sufficient. But even with silica it is not all plain sailing, for under certain conditions dusts rich in silica appear no more harmful than coal. This comparative immunity might be due either to the conditions of work, physical state of the silica, or the presence of an antidote and, in the event of the latter hypothesis being correct, one might deal with flint by adding the protector. Coal dust on the other hand has its special risk—it is responsible for all the great coal mine explosions. This danger, however, can be reduced to a minimum by mixing coal with inert dusts. The ideal would be to stop colliery explosions by adding flint and to stop phthisis by adding coal, thus holding a tenace over providence.

The particular points selected for study in this investigation are these:

1. A comparison of the behaviour in the lungs of certain harmless and dangerous dusts with a view to deriving a practical means of recognizing what material could safely be used for stone dusting.

2. An attempt to account for the admitted fact that sometimes a dangerous dust may be relatively benevolent, with a view to devising a means of treating the dangerous dust so as to lessen its power for evil.

In addition to the clinical experience that has accumulated there is also much experimental work, of which Arnold's classical investigation is a good instance. Arnold showed that under experimental conditions a variety of dusts enter the lungs and there produced fibrosis. At the time when Arnold performed his experiments there was still some dispute as to whether dust actually entered the lungs; Virchow at first held that the appearances observed in these organs was due to pigmentation and not to inhaled foreign material. Following Arnold most of the workers along these lines have aimed at producing lesions, and the exposures were very severe. The experiments of Professor Beattie¹ were an exception to this rule. He showed that with moderate exposures the shale dust used for stone dusting in collieries is relatively harmless to animals as compared with dust from flint, etc.² In the investigation to be reported, the object has been to ascertain why in industrial practice some kinds of dust are harmless and some dangerous. Hence the exposures have been approximated to those found under working conditions—a running test as opposed to a test to destruction.

¹ *First Report of the Explosions in Mines Committee*, Parl. Paper, Cd 6307, 1912.

² *The Medical Journal of South Africa*, 1914.

The clinical and industrial experience culminating in Dr Watkins-Pitchford's investigations and experiments in South Africa, and the laboratory work bring out the following points:

1. A condition known as pneumokoniosis results owing to dust particles setting up cell proliferation which ends in the production of dense connective tissue. This connective tissue occurs first as nodules and these nodules may subsequently coalesce, obliterating large portions of air-containing vesicles.

2. Sufferers from this condition are peculiarly liable to pulmonary tuberculosis (miner's phthisis, potter's rot, etc.).

3. Some dusts, e.g. soot and coal, are comparatively harmless.

The introduction of the practice of "stone-dusting" into coal mines has made it specially desirable to attempt some classification of dusts into those which predispose to phthisis or other serious lung troubles and those which do not. Certain of these materials have been studied with a view to noting what variations there may be in their action and effects under experimental conditions.

As a start in this work it appeared sound to study the action of certain common dusts used pure and in moderate concentrations. Arnold and others have carried out experiments of this nature; but the severity of exposure was greater than occurs under industrial conditions, and the difference in the reaction of the lung to different kinds of dust was one of degree rather than of kind. In the investigation to be reported the exposures have been short and the thickness of the dust cloud moderate, allowing for the fact that the upper respiratory passages of laboratory animals such as guinea-pigs afford more protection than the corresponding parts in man.

The dusts used have been those arising from coal, shale, flint, quartzite from the Transvaal gold-bearing reef, and the dust obtained from the flues of furnaces driving mining plant, hereinafter known as flue dust. This latter variety has been employed to a considerable extent for preventing explosions in coal-mines. Coal has been selected as the "harmless dust," flint and Transvaal quartz as dangerous dust, and "flue dust" as the unknown for testing.

The dusts so far investigated are:

- (i) Coal dust.
- (ii) Shale dust.
- (iii) Quartz dust from the Transvaal.
- (iv) Flint dust.
- (v) "Flue dust."
- (vi) Pure precipitated silica.

Of these dusts, coal dust and shale dust are known from industrial experience and from the results of Professor Beattie's experiments to be relatively harmless, while the flint dust and that from the Transvaal gold-bearing reef are known to be deadly. Flue dust has not, so far, been studied, but is of interest as it is convenient for "stone dusting." Pure precipitated silica has been used to test the comparative importance of the chemical and physical factors in this undoubtedly dangerous material.

APPARATUS AND METHODS USED.

The materials so far used—the dusts of coal, shale, pure precipitated silica, quartzite from the Transvaal, flint, and that from flues—were studied in the following manner. The different dusts were mounted on slides in the same medium as the microscopical preparations and examined under the microscope directly and with crossed Nicols.

The coal dust, precipitated silica, and shale dust were found to be amorphous; they fractured without the formation of edges, and only isolated particles of shale dust exhibited double refraction. The other three dusts took the form of angular particles with sharp edges, and the overwhelming majority of the particles showed double refraction. It was readily noticed that very small angular particles, though easily visible under quite ordinary magnifications, did not light up on rotation and that particles of many kinds other than silica did light up if of suitable size. These points, of course, are common-places, but in this connection it is of importance to recognize that because a tissue contains particles which exhibit double refraction it does not follow that these particles are silica; and the absence of lighting up is no proof of the absence of small particles of silica. These facts have been insisted on and given great prominence by Dr Watkins-Pitchford in his Lecture on "Occupation Diseases in the Transvaal¹." I am indebted to Dr E. L. Collis for giving me the references to this work. Except for the coal all the dusts in a fine state of division were colourless. This is of importance in connection with their appearance in unstained sections where they ultimately blacken.

Guinea-pigs have been the animals used throughout, and they were exposed to dust in apparatus arranged thus:

A wooden box lined with zinc has in its lowest portion a two-bladed fan *A* driven by an electric motor. The dust to be studied is placed in the box and the fan keeps a cloud of it in suspension. This box has no

¹ *Loc. cit.*

lid but another wooden box fits closely over it. This box is floored with a few strands of wire upon which rest the cages and is closed above by a sheet of glass so that the animals and the thickness of the dust cloud can be observed. Into the side is fitted a small tube whence samples of dust can be withdrawn during the experiment, so that some idea may be formed of the comparative thickness of the cloud.

As it seemed advisable to test the thickness of the dust cloud obtaining in the experiments, a glass tube plugged with cotton wool was connected at one end with the inside of the dusting machine and at the other with a water aspirator containing some thirty litres. The same quantity of dust as that used in the experiments was placed in the machine, and the glass tube, weighed before and after running off the aspirator, gave the amount of dust in thirty litres.

The actual amounts were as follows:

Coal	1.367 grms., say 45 mg. per L.
Shale	1.294 grms., say 43 mg. per L.
Flue dust	0.957 grm., say 32 mg. per L.
Flint	0.827 grm., say 27 mg. per L.

Some rather interesting points came out, notably the great importance of the hygroscopic character of the dust. It had been noticed in the animal experiments that the glass top of the machine was frequently as it were splashed with dust clots which would not shake off, though it brushed off readily enough, and that some dust stuck to the zinc lining of the machine sufficiently firmly to resist shaking though it too would brush clean off. A soft brush or feather mop sufficed without scrubbing. The same facts were observed in some cases in these tests when no animals were used and moisture was reduced to a minimum. Shale dust for instance used fresh from the heater yielded 40 odd mg. per litre, but the same dust run again after being left a few hours in the machine only yielded 22 mg. per litre and spattered the roof and sides with more clots. Coal and shale then, the relatively harmless dusts, give—under these conditions—the thickest cloud. This however is not quite fair: flue dust and flint are less dependent on drying, and with several animals breathing into a confined space they maintain their cloud better. It is interesting to note that dusts which clot in the lungs also clot in the machine.

The concentrations given above are maximal; with several animals in the machine affording a large surface whereon dust can settle the cloud soon thins out unless maintained through a hopper as in the experiments where intense dusting was aimed at.

These clouds are of course far in excess of those experienced in mines and works; but the animals only had from 24 hours to 36 hours all told, and their nasal apparatus is better adapted to deal with the situation.

Sections of the lungs and films from the cut surfaces of the lobes were examined. Mr S. G. Shattock, Curator of the Museums at the Royal College of Surgeons and at St Thomas's Hospital, kindly put at my disposal museum specimens credited to various dusts so that I have been able to compare them with my experimental results.

The "raw" dusts were first passed through a No. 90 sieve to remove coarse particles, and then dried on an electric heater to facilitate their suspension in the air. The readiness with which fine dust takes up moisture and "dumps" varies. All the dusts studied were more or less affected, coal dust to the greatest extent, flue dust to the least. Dust readily deposits on the sides of the box and on the animals and, if a thick cloud is required, it must be frequently renewed or shaken down within range of the fan.

The guinea-pigs were kept in separate cages during the actual dusting and it was necessary to place them in sponge bags as they proceeded to lay the dust by passing incredible quantities of water. It was of interest to note that they rarely passed water in the bags.

REPORTS OF EXPERIMENTS.

1. CONTROL STUDY OF NORMAL GUINEA-PIG LUNGS.

There is considerable variation in, presumably, normal guinea-pig lungs¹.

- (i) Some have a much more congested appearance than others.
- (ii) The amount of fibrous tissue at the root of the lung varies.
- (iii) Pigment may be found under the pleura and in the bronchial glands of young animals born at the laboratory, which is practically in the country.
- (iv) While there is much lymphoid tissue scattered through the lungs of normal guinea-pigs, the amount appears to vary and there is no pigment in this situation.
- (v) The fibrous tissue is practically confined to the root of the lung and large bronchial tubes and arteries; the general framework of the lobes is very slight and composed almost entirely of elastic tissue.
- (vi) Owing to the slightness of the framework the lungs tend to collapse with some completeness when the chest is opened; and for the

¹ Cp. Beattie, *loc. cit.*

purpose of these experiments it was desirable to reduce this collapse to a minimum.

2. TECHNIQUE ADOPTED.

The trachea was tied low down before opening the chest and the "pluck" was removed bodily. One lobe was immediately tied off and used for films and the rest of the tissue placed bodily in the fixative. After two or three hours the lobes were cut in slices and left for three hours more in the fixing solution.

The following preparations were usually made:

- (i) From trachea.
- (ii) At bifurcation of trachea and arranged to include the glands and connective tissue in this region.
- (iii) The lobes were cut so that the sections should pass through the apices.

The routine staining was performed with logwood and eosin and logwood and Van Gieson's fibrous tissue stain. Numerous preparations were treated with Weigert's stain for elastic tissue and with Pappenheim's stain for plasma cells. The typical granular leucocyte in the guinea-pig is acidophilic and I found Biebrich's scarlet very useful for showing up these cells both in the lungs and glands. I used this stain on the recommendation of Dr S. G. Scott, to whom I am indebted for much kind assistance and for putting the resources of the histological department at the Oxford University Museum at my disposal. The films were stained either with Giemsa's stain or some other of the eosinates of methylene blue.

3. PRELIMINARY EXPERIMENTS.

Nine animals were used for preliminary experiments. The object of these was to ascertain the general rate at which dust was taken up, the extent and rapidity of its removal after brief exposures, and the behaviour of the upper respiratory passages. Information was also required as to thickness of the cloud of the various dusts which could be maintained by the apparatus.

Animals were killed with coal gas immediately after two hours' exposure and the heads divided in the sagittal plane. In these cases dust was thickly deposited over the turbinate bones and in all the recesses of the naso pharynx. From this region however it was removed in 36 hours. A certain amount was to be found in the mouth and in the upper part of the oesophagus, but not in the stomach. The efficiency

of the "nasal filter" was shown by the comparatively small quantity to be found in the trachea on naked eye examination.

On microscopical examination hardly any dust was to be found in the lungs after two hours' exposure to a small amount; but after three hours' exposure to a fairly thick cloud it was readily to be seen in trachea, bronchi and alveoli. In these cases coal dust and dust from the Transvaal gold-bearing reef were used. Both dusts were tested with each type of exposure.

In these early cases the dust appeared in free clumps for the most part. In fact only in the coal dust cases was there shedding of epithelium to be made out, though in one of the Transvaal dust cases, killed after 36 hours, there were a few typical dust laden cells to be seen. The lungs were congested, but there was no evidence of broncho-pneumonia. None of the animals appeared any the worse. The glands were free.

4. FIRST SERIES OF EXPERIMENTS.

Six guinea-pigs were exposed for two hours per diem to a moderately thick cloud of dust on twelve consecutive week days, giving 24 hours' exposure in all. Coal dust, shale dust, and Transvaal dust were studied, six animals being used in each case.

Two animals from each series were killed on the completion of the experiment, and one animal at intervals subsequently, so as to provide specimens after 7 days, 3 weeks, 3 months and a year. It was impossible to carry out this plan completely in all cases, as some of the guinea-pigs died. None of the animals appeared to suffer any ill effects as the result of the experiments; and considering the condition of the lungs in many cases this was rather astonishing. As to whether these guinea-pigs had the same capacity for doing work as untreated animals it is impossible to say, but they seemed fully equal to the exigences of a hutch life such as eating and breeding. A few died, but the mortality was considerably less than that which occurred in a Mendelian experiment on a considerable scale which was being carried on simultaneously with animals from a similar source.

(a) *The coal dust series.*

Animals killed immediately at close of experiment. The upper air passages were dusty as in the preliminary experiments but the trachea and bronchi were no more affected to the naked eye than after brief exposures. The lungs were grossly pigmented, and the glands were swollen and more conspicuous than in untreated animals; but though

they showed pigment it was not very marked. On microscopical examination no dust was made out in the epithelium or sub-mucous tissue of either trachea or bronchi. Loose dust and eosinophil cells were snared in the cilia of the cells lining the large air passages; but these cells appeared healthy, and the evidence of bronchitis in the way of increase in goblet cells and infiltration by leucocytes was trifling. The bronchi however were often plugged by masses of cell débris containing dust. There was dust to be made out in the lymphatics but round blood vessels rather than round bronchi. The dust in the lungs was largely intra-cellular, comparatively little being free. Many cells—of large and small endothelial type and dust laden—were to be made out free in the alveoli, but there was also much dust in the fixed cells. There was some cell proliferation but no broncho-pneumonic patches. Comparatively little dust had reached the lymph glands at the root of the lungs and it was mostly in large endothelial cells. Eosinophil cells were not conspicuous.

It was rather surprising to note that the lymphoid tissue scattered through the lung was practically free.

Specimen examined a week after close of experiment.

The differences between this case and the previous one were as follows:

1. The upper air passages were quite clear of dust.
2. Free dust had disappeared from the trachea and bronchi, though plugs of dust-loaded débris were still conspicuous in the latter.
3. There were fewer free dust cells in the alveoli though plenty of dust in fixed cells on the alveolar walls throughout the lung: presumably the continued shedding of these is keeping up the supply of free cells.
4. There are numerous masses suggesting coal dust "dumped" on the alveolar walls, since, on focussing, these masses appear on the surface as opposed to within the cells. The cells seem to take up coal dust with such avidity that they burst and deposit the dust which is moistened and clumped by the pulmonary secretion. This probably facilitates its exit via the bronchi.
5. More dust has reached the bronchial glands, where again one gets this impression of "dumping," though dust cells are present also.

Specimen after three weeks.

There appears to be less dust, the dumps seen in the earlier specimen are less numerous and tend to concentrate round the vestibules. Fewer plugs are to be made out in the bronchi, but dust is now appearing in

the connective tissue round the larger branches at the root of the lung. Eosinophil cells are increased in number in this region and appear in the bronchial epithelium.

The glands are similar to the last case. The dust there does not appear to have increased in amount.

The cell proliferation in the lung has not progressed.

Specimen after three months.

The lung is undoubtedly clearing up. Pigment is to be made out under the pleura, but the lung on section is no longer conspicuously black.

There are now hardly any free dust cells, but a good deal is present in fixed cells scattered about, while concentrations appear round the vestibules.

There are no areas of conspicuous proliferation, though there is some general proliferation.

Dust laden plugs are to be found in the bronchi.

The dust is now plentiful in the connective tissue at the root of the lung and is also to be seen between and probably in the epithelial cells lining the bronchi. It is, presumably, on its way out as it has not been observed in this region before and has only appeared since the accumulation in the connective tissue. It can be traced in the muscular and sub-mucous coats.

The glands at the root of the lung are now less pigmented.

Specimen after ten months.

The lung might almost pass for normal. It is not pigmented to the naked eye save one or two patches under the pleura.

Under the microscope very little dust is to be made out. Even that round the bronchi has almost gone, and the cell proliferation and general richness in nuclei is not marked. Such dust as remains is to be seen in fixed cells, mostly isolated, but there are one or two patches to be made out where cells carrying dust appear to have coalesced and lost their staining qualities.

There is no evidence of increase in fibrous tissue and not the slightest sign of the appearance of nodules in the lung substance.

Summary of coal dust series.

(i) The dust cells, mostly cells derived from pulmonary epithelium, take up coal dust with great avidity and are very readily shed. Dust is seen in fixed cells as well as in free cells but the former are for the most

part isolated and one does not meet islets of dust-loaded cells to any extent.

(ii) The free dust cells appear to disintegrate readily and deposit their contents, which are seen as clumps on the surface of fixed cells.

(iii) There is a good deal of proliferation of cell nuclei, but no inflammatory foci and no fibrosis.

(iv) In cases examined some time after the cessation of dusting the coal dust is most conspicuous in two situations: round the vestibules and in the connective tissue at the root of the lung.

(v) Dust leaves the lung via bronchial tubes as plugs of mucin-containing dust and dust-laden cells. The clumps of dust mentioned are most conspicuous round the vestibules and contribute largely to the plugs.

Dust also leaves via lymphatics. It does not accumulate indefinitely in the lymphoid tissue but passes to the connective tissue at the root of the lung and is thence excreted through the large bronchial tubes.

(vi) With moderate dusting all dust is probably eliminated, and lungs of animals thus exposed might pass for normal after about a year.

(b) *The flue dust series.*

Flue dust, as mentioned before, is a dust composed of colourless particles of angular character, the majority of which show double refraction on examination with crossed nicols.

On examination of the animals killed immediately at the close of the experiment it was at once noticed that the lungs were grossly pigmented to the naked eye, though not as intensely black as in animals exposed to coal dust.

When studied under the microscope the particles of dust showed all variations between colourless and black as coal.

Cell proliferation is much more marked than in the cases exposed to coal.

Free dust cells in the alveoli are not nearly so conspicuous as in the corresponding coal cases, and while individual cells do not carry as great a load the invasion of the fixed cells is far more widespread. As far as can be judged the actual amount of dust that gets into the lung is about the same in the two cases, but as the epithelial cells are less readily shed in the animals exposed to this dust, more is to be found in fixed cells and less in the alveoli and in mucinoid plugs in the bronchi.

As with coal dust, but little dust is present in the large bronchial tubes and none is found in the bronchial epithelium or the sub-mucous tissue.

The connective tissue is practically clear and very little dust has reached the bronchial glands.

Specimen examined a week after close of experiment.

The lung is blacker to the naked eye than in the previous cases.

The cell proliferation has progressed, and is more marked than in the animals examined at the close of the experiment. There is the same scarcity of free cells, and most of the dust is now nearly coal black.

There is no tendency for masses of dust to be deposited as was the case with the coal dust. On the other hand the dust cells tend to aggregate and plaques composed of dust-laden cells are a conspicuous feature.

The connective tissue round the blood vessels contains a little dust and some is leaving in the mucinoid plugs.

The glands are very dark on section: they contain plenty of dust, but it is all intra-cellular.

Specimen after three weeks.

This lung is as dark to the eye as the corresponding coal lung.

Under the microscope the cell proliferation has, if anything, progressed: the dust is black, and there is far more of it remaining in the lung than in the corresponding coal case. The plaques of dust-laden cells are a very conspicuous feature. In the connective tissue the dust is still remote from the root of the lung, and is round the smaller blood vessels.

The glands are dark on section, and contain dust. Plaques are now to be seen in this situation.

Specimen after ten months.

The lung is still dark on section and under the microscope there is still plenty of dust, and cell proliferation is marked; in fact in parts it suggests a chronic broncho-pneumonia. The plaques are still conspicuous, and plasma cells occur in their neighbourhood. No young fibroblasts were definitely made out, and there was no pretence to the formation of fibroid nodules. Dust is scattered about the connective tissue, but it has not collected round the main bronchi and does not appear in the muscular or sub-mucous tissue of the tubes. Plasma cells occur.

The glands are dark and dust-laden. Plaques of dust-laden non-staining cells are numerous.

Considering the state of the lungs it was astonishing that the animals appeared in such robust health.

Summary of the flue dust series.

1. Flue dust blackens in the lung.
2. Cell proliferation progresses for some time after close of experiment.
3. While dust enters to about the same extent as with coal dust it leaves far more slowly.
4. Plaques of dust-laden non-staining cells are a conspicuous feature.
5. The dust cells are less readily shed than with coal dust.
6. The animals suffered no direct ill-effects.

(c) The shale dust series.

This dust gave results intermediate between the coal dust and the flue dust. On the one hand the initial catarrhal reaction was not as intense as with the coal, and the rate of elimination was not so rapid: on the other hand there was no plaque formation and the latest lungs examined were almost normal in appearance, though a little dust was still to be found.

This dust also blackened in the lung.

(d) The Transvaal dust series.

This dust is notoriously deadly, and it was a matter of some surprise to find that the animals appeared to suffer but little inconvenience. The general course was similar to that with the flue dust. The differences noted were two. Firstly the dust did not collect so readily in the bronchial glands; and secondly, though it turned a pale yellow, it did not blacken so quickly. The lungs were obviously abnormal up to the end: cell proliferation was conspicuous, and the dust was still abundantly present. The lungs however did not appear more damaged than those of the animals exposed to flue dust. No fibrosis occurred.

As far as this set of experiments goes the lung behaves as other tissues exposed to the action of irritants. Cell proliferation occurs; large endothelial and mono-nuclear hyaline cells are conspicuous; and active phagocytosis takes place. Where the dust accumulates the large cells coalesce and plasma cells occur. In no case did this pass on to fibrosis, and polynuclear cells were neither an obvious nor a consistent feature. This last is the only difference made out between the behaviour of the

lung and the behaviour of other tissues in the long list of experiments that have been made on inflammatory reactions.

*Examination of films*¹.

These were prepared by rubbing the fresh cut surface of lung on a clean slide. It was thought that there might be some difference in the cytological reaction to the different dusts. Nothing of the kind was made out. The films were like those which would be obtained from peritoneum under similar circumstances, with the exception that there was no consistent invasion by polynuclear cells. In some cases they were marked; but this had no relation either to the type of dust or the period of the experiment. Dust was found in these cells, but it was not common, and on slides where they were numerous crowds of empty eosinophil cells were to be found surrounding loaded cells of endothelial type. There were numerous un-nucleated fragments containing dust, presumably broken off from the large cells. The blackening of colourless dust is well shown by this method.

5. REPETITION OF THE EXPERIMENTS WITH FLUE DUST, SHALE DUST, AND TRANSVAAL DUST.

As flue dust had not been studied before and there is a question of using it for "stone-dusting" it was desirable to confirm the previous result. The behaviour of the animals was identical with that in the previous set of experiments.

6. SERIES OF EXPERIMENTS AS TO THE RATE AT WHICH DUST ACCUMULATED IN THE LUNGS.

Groups of six animals were exposed to the dust as before for two hours per diem for a fortnight and an animal was killed every second day. The results were as follows:

With any dust accumulation begins at once and increases with each dusting; but all the dusts do not behave alike. More dust appears to get into the lungs when coal, flue dust or shale are used than with Transvaal dust or flint.

The fact that the two latter dusts are less readily seen than the former is not sufficient to account for the difference.

With coal dust and shale dust the increase is at first rapid but after about the sixth exposure the rate of elimination appeared to approach the rate of invasion; and lungs after 24 hours were not much more laden

¹ Compare Briscoe, *Journal of Pathology and Bacteriology*, 1907, "Experiments on phagocytic action of alveolar cells."

than after 12 hours. This was not the case with the flue dust or the two crystalline silicas. With these the dust increased steadily, though less rapidly as the experiments went on. These experiments were all carried out with dust clouds of quite moderate intensity.

7. EFFECTS OF INTENSE EXPOSURE TO DUST.

As the work advanced very slowly owing to the length of time occupied by each experiment it was decided to compare the effect of intense dusting for brief periods with the previous experiments. A funnel was arranged to act as a hopper and deliver dust continuously to the box from above and each exposure lasted six hours instead of two hours. Even under these severe conditions the guinea-pigs suffered no permanent ill-effects and began to run about the cage and eat as soon as they were released. In one or two cases where the animals seemed at first to be upset this was probably due to the moist heat of the rubber bags in which they were enclosed. The preliminary experiments showed, as was to be expected, that much more dust entered the lungs under these circumstances; and a series of experiments was carried out with all five dusts, in which groups of animals were exposed to the thick cloud for about 36 hours, spread over six days.

The result was rather unexpected. Nearly all distinction between the behaviour of the different dusts disappeared. At this time the supply of guinea-pigs was rather short and it was only possible to use three animals for each experiment. In each case one animal was killed immediately at the close of the experiment, one was killed after about ten days if available, and a group of five were examined after three months. All the lungs contained great quantities of dust, although, in the case of the coal and shale, and to a lesser extent in the case of the flue dust, considerable quantities had collected in the connective tissue at the roots. There was much congestion, and cell proliferation was marked and widespread. In all cases cell aggregates containing dust were to be seen, and the cell proliferation was especially marked around them, where too the plasma cells could be demonstrated. Definite emphysema could be made out, areas of widely distended alveoli contrasting with the areas of cell proliferations. Even in these cases no formation of new fibrous tissue was to be demonstrated, though as the tissues rather suggested an early diffuse granulomatous condition it might have developed had the animals been preserved longer. While the bronchial glands contained quantities of dust in the case of the coal, shale, and flue specimens, there was not nearly as much to be found where flint

and Transvaal dust had been used. Considering the condition of the lungs it was matter of some remark that the animals should have appeared normal and shown no symptoms of illness.

8. AN EXPERIMENT WITH PURE PRECIPITATED SILICA.

This silica is amorphous when examined under the microscope: there is no double refraction, and it is comparatively soft when chewed. It was of interest to test this dust as it afforded an opportunity of comparing the same material in a crystalline and non-crystalline form. It is known too that in some industries dusts containing a high percentage of silica are harmless; and, as has been suggested, this difference in behaviour may be due to difference in physical state.

The animals were exposed to the dust for two hours per diem on twelve days; and when at the end of that time an animal was killed the lungs were found to be practically dust free. The dust forms a fine powder and, when dry, flies well. I can only account for its rapid disappearance on the assumption that it is soluble.

Mr T. F. Winmill, chemist to the Doncaster Coal Owners, has been good enough to make some tests for me in this direction and finds that 100 cc. of N/5 Na_2CO_3 dissolve 90 mg. in 48 hours at 30° C. The solubility rate depends largely on the fineness of the SiO_2 .

9. ANIMALS WHICH DIED.

A group of three animals may be described which died during the course of experiments, one during an experiment with quartzite, one with powdered flint, and one with flue dust. The first two died on the fourth day, and the flue dust case on the sixth. In each case the appearance of the lung was similar. A considerable amount of dust had reached the alveoli, but not an undue quantity for the exposure. Some of it was free, i.e. resting on the surface of cells as judged by focussing, but most of it was intra-cellular. Very many of the laden dust cells were shed free in the alveoli, and comparatively few were embedded. Much of the dust was blackened. Coarsely granular eosinophil cells were conspicuous in each case; but the outstanding feature was acute oedema of lungs, the alveoli being loaded with an albuminous exudate. The damage appeared to be concentrated on the blood vessels, the connective tissue around them being infiltrated with cells. The muscle was vacuolated and did not stain well, while there were numerous extravasations of red cells through the damaged walls. At the time these sections were being studied occasion had arisen to examine materials from animals

killed by exposure to irritating gases such as N_2O_4 , and the similarity was striking. The outstanding difference was that the lungs from the gassed animals showed gross emphysema with a good deal of ruptured lung tissue; and, of course, dust cells were not conspicuous. One of the dust cases however does show marked emphysema of a portion of the lobe, the rest of which is fluid-laden.

One hesitates to relate these deaths direct to the dust for the following reasons:

1. In each case the animal was one of a batch exposed under precisely similar conditions, and none of the others showed symptoms.
2. Out of the large number of animals dusted only these three cases occurred, and their lungs were not excessively dust-laden.

The appearance of the lungs, however, was not that of the ordinary guinea-pig pneumonia which carries off so many laboratory animals; and it is possible that these were cases of pneumonic infections in lungs irritated by dust. It is hoped to examine the next case from the bacteriological point of view; but at the time these deaths occurred facilities for this work were not available.

It is interesting in this connection to again quote Dr Watkins-Pitchford, who describes under the heading "Acute Pulmonary Silicosis" a type of case reported among the coloured workmen. "The lungs are congested, oedematous and mottled with islands of pigmentation. There is no fibrosis. Microscopically the connective tissue is laden with siliceous particles and the alveoli are distended with serous exudate and catarrhal cells. Such cases are rare and due to inhalation of large quantities of dust over a short period."

10. EFFECTS OF VERY SLIGHT EXPOSURES TO DANGEROUS DUST FOR SHORT PERIODS.

The following experiment was devised to test the ability of the lung to deal with a small but definite invasion by dangerous dust.

Groups of three animals were exposed to small quantities of flint dust, Transvaal dust and flue dust for one hour a day on six occasions. One animal from each series was then killed and examined. In each case dust was readily seen in the lungs, though in small quantities. A second batch was examined at the end of a fortnight. Dust was still present, but there was no general reaction. The remaining animals were killed after six weeks, and the lungs might have passed for normal, though dust could be found on careful search.

11. EFFECTS OF SLIGHT EXPOSURES OVER A LONG PERIOD.

The following experiment was devised to test the influence of extending the period over which a given total exposure was spread. The dusts used were coal, flint, and flue dust, the concentration aimed at being that used in the moderate dusting series. The animals were dusted for half an hour a day for eight weeks, Sundays excepted, thus making up the 24 hours total exposure but in smaller units.

The coal dust animals behaved as might have been expected from series 6. In the animal killed two days after the close of the experiment the lung contained very much less dust than in animals who had received similar total exposure within a briefer period—the last dusting being half an hour instead of two hours was bound to influence the character of the lung in this case. In the animal killed after a fortnight the lung was obviously clearing very rapidly and the rate of elimination must have been not far short of rate of invasion.

In the animals exposed to flint and flue dust no such marked difference was observed as compared with those who had a similar dose within a fortnight, but as far as any contrast was observed the lungs were less affected in this experiment than in the earlier series.

12. EXPOSURE TO MIXED DUSTS.

Two fairly distinct types of reaction having been obtained under the experimental conditions, it was then decided to test the reaction to the two types of dust when mixed.

Two lots of six guinea-pigs were taken. One lot was exposed to flint dust, and the other to a similar quantity of flint dust with coal dust added. Exposures two hours a day for twelve days. The same concentration of flint dust was aimed at as in the earlier unmixed experiments. These experiments gave interesting results: the unmixed flint corresponded to earlier experiments, and went on to plaque formation; while in the mixed dust set the coal produced its usual catarrhal reaction and intense pigmentation of lung, this pigmentation gradually passing off, though not so rapidly as with pure coal. In the latest specimens examined, after nine months, there was still naked eye pigmentation, though slight. The fact that these experiments were carried out in London, while the earlier series were performed at Oxford, was not enough to account for retention of pigment, the unmixed flint series serving as a control. The general condition of the mixed dust lung was different to that of the pure flint. On the one hand the dust

was more conspicuous in the earlier stages, and on the other there was no plaque formation and no signs of fibrous tissue in the later cases. The nine month flint lungs showed plaques, and these plaques showed invasion with fibroblasts as judged by the reaction to Van Gieson's stain. Although the mixed dusts animals exhibited a good deal of dust up to the last the lungs were less loaded than those of animals which had received a smaller total quantity of unmixed dust and, in time, might have practically cleared. In addition to the dust there was sufficient cell proliferation to catch the eye up to the last.

When it was seen that the mixed dusts were behaving somewhat differently to the unmixed another series were started. Mixed flint and coal were given, but the total dust was kept equal to the total flint in the control: half the quantity of flint was taken and the balance was made up with coal. It was thought that under these circumstances as complete an elimination might be produced as with the mild dusting of series 10, although the amount of the flint was much in excess of that used for the earlier animals, and the duration of exposure four times as great.

Neither this experiment nor a repetition was conclusive, as none of the animals lived out the necessary number of months. It was only possible to use four for one set and three for another, and in each case the last animal died before the lungs could have been expected to clear. In two animals, one of which survived for five months and the other for nearly six, the lungs were in each case pigmented; but there was no plaque formation: the cell proliferation was extremely moderate; and the amount of dust small. The disappearance of coal was more rapid than in animals when a greater quantity of flint was used, and, as was only to be expected, there was less flint retained with the reduced dosage.

13. SOME FEEDING EXPERIMENTS.

From time to time various organs other than lung and lymph glands were examined. As already found by Arnold, dust was seen in the cardiac muscle, thymus, spleen and bone-marrow. None was found in the alimentary canal wall, salivary glands, liver, kidney, or pancreas.

With a view to testing the effect of dust by the mouth three animals were fed with Transvaal dust, coal dust, and flue dust mixed with their food. None of the animals showed any ill-effects in the course of an experiment lasting four weeks. At the end of this time they were all killed and the alimentary canal and abdominal lymph glands examined.

No dust was found either in the intestinal mucous membrane or in the abdominal lymph glands.

SUMMARY AND DISCUSSION.

With intense dusting all the dusts used produced much the same effects, both immediate and remote.

With moderate exposures some dusts are much more readily eliminated than others. While coal dust and shale dust enter the lung with great readiness they do not produce, under these conditions, permanent lesions; and the lung might pass for normal after a twelve-month.

Flue dust and crystalline silica are not eliminated with such readiness.

Two methods are available for distinguishing the dusts—perhaps three.

1. Coal and shale are taken up by cells which are quickly shed and consequently do not set up processes which block lymphatics. These cells frequently break down, and masses of dust are to be seen on the surface of the alveolar walls.

Flue dust and the crystalline silica are taken up by cells which tend to remain *in situ* and form plaques, which appear early and persist. They are the only site of fibrosis made out in this investigation.

Dusts which form plaques are not readily eliminated.

2. Rate of elimination is more important than rate of invasion. Dusts which are not readily eliminated after moderate exposures are to be avoided.

3. There are two ways out of the lung: direct via the bronchi in the form of plugs of mucin, dust cells and dust; and indirectly via the lymphatics. These ultimately bring some of the dust to the large bronchi at the root of the lung, and the dust can be traced through the connective tissue, muscular, and mucous coats into the epithelium, whence it is excreted. Colourless dusts become pigmented, and to judge from the data as yet available, the more marked the pigmentation the readier the elimination. It is possible that pigment plays a part in this bronchial excretory process, on the analogy of the lipochromes and the deposit of particles in the skin. The different rates at which dusts reach the mucous coats of large bronchi illustrates their relation to lymphatic drainage.

It is of importance to make out whether dusts observe an "all or none rule" or whether they exhibit gradation.

Flue dust for instance in all these experiments produced permanent

lesions; but a great deal entered the lung and a great deal was eliminated. Specimens taken from animals immediately after exposure to flue dust, Transvaal quartz, and flint, show more flue dust than crystalline silica; but in cases taken later, owing to flue dust elimination being more rapid, the dust remaining is nearly the same in quantity in all three.

Experiments were carried out (series 10) to determine whether these three dusts can be eliminated like coal and shale if the amount of dust in the air be slight and the invasion of the lung moderate. It is worth making great efforts to diminish the dust cloud if there are reasonable grounds for thinking that under these circumstances rate of elimination might approach rate of invasion. If, on the other hand, a considerable proportion of any dust that enters remains, then other methods must be sought.

The result of these experiments was not very encouraging although it was fairly clear that dosage was an important factor. While it is certainly not the case that concentration of dust in the air \times duration of exposure is a constant; still with flue and silica the dose must be very small if accumulation is to be avoided.

More encouraging results were obtained by adding coal. The vigorous catarrhal reaction produced results in the relatively inert dust being carried out in the plugs along with the coal; and these experiments have certainly left the impression that under the conditions described the lungs would practically free themselves from flue or crystalline silica dust if these dusts enter in small quantities only and with coal. The fact that crystalline silica—even when present in considerable quantities—does not always tend to produce grave pulmonary disease, may perhaps be accounted for by assuming that when silica is relatively benevolent there is inhaled together with it some substance that provokes a reaction in the lung with shedding of epithelium and expectoration of débris. If flue dust has been used for stone dusting with impunity it may be due to the presence of coal, since, experimentally, it behaves like the silica group when used pure.

Under the conditions of these experiments:

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.