THE EFFECTS PRODUCED BY THE INHALATION OF HAEMATITE AND IRON DUSTS IN GUINEA-PIGS.

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1. Introduction.

The production of a fibrosis due to inhalation of haematite in man has already been discussed by Goadby (1925). The following experiments were made to see what effects were produced by exposing guinea-pigs to the dusts of (*a*) haematite (Fe_2O_3) and (*b*) levigated iron.

Financial assistance towards these experiments was very kindly rendered by the Mines Department, to the Inspectors of which I am also indebted for the haematite powder from Cumberland.

2. Method.

The animals were exposed to the dusts in a machine of the same type as used in the previous experiments of Mavrogordato (1918) and Carleton (1924). In the case of the haematite, which was very finely ground, much trouble was experienced in getting the dust to fly. Accumulation of it used to occur chiefly on the sloping sides of the machine; nor could this deposit be moved except by scraping the dust down into the semicircular channel containing the fan after stopping the latter. To remedy this Mr C. J. O'Neill fitted a squeegee on each of the sloping surfaces leading down to the fan channel. A rod, attached to each squeegee, and passing out of the machine through a collar, enabled accumulated dust to be pushed back into the fans. The latter were four in number, centrifugal in type, and mounted on one shaft. The blades of each fan were set obliquely to the shaft so as to get a draught from each corner of the machine. The ordinary two-bladed fan used in experiments with ordinary dusts was not sufficiently powerful to disperse heavy metallic dusts. The rest of the dusting machine conformed to the types already used. The top was of plate glass so as to enable the animals to be observed by means of an electric bulb hung above the wire grid on which they were placed. As in other experiments the guinea-pigs were enclosed, except for their heads, in jaconet bags to prevent the dust being caught up in their fur.

3. The lung changes produced by the inhalation of haematite.

The animals were exposed on ten consecutive days to as thick a cloud as could be obtained without choking the bronchi. The duration of exposure was: on the first day, 15 minutes; on the second, 30 minutes; on the third,

45 minutes; on the fourth and all subsequent days, one hour. The reason for the graded exposures was to avoid killing any animals through bronchial obstruction with so cloying and fine a dust. The animals' health was good throughout the period of exposure: one died $8\frac{1}{2}$ months after the last exposure, but death was due to a fall from its cage on to a cement floor.

Animals examined 2 days after the last exposure.

At necropsy the lungs were stained deep pink by the haematite; here and there on their surface were small bright red spots. Other organs normal.

Animal A. Microscopically most of the lung is almost normal; there are occasional sub-pleural and peri-bronchial areas of thickened or collapsed alveolar epithelium. One very small patch of pulmonary tissue is consolidated. (N.B. Such small areas are often found in normal guinea-pigs; one cannot therefore implicate the haematite.) Dust is abundant, both free in the alveoli and inside dust cells. "Cuffing" of branches of the pulmonary veins with lymphocytes is marked. The bronchi are mildly catarrhal; dust is often found plastered on to the bronchial mucosa.

Animal B. The changes are of the same type as in *A*, but they are more marked. This is doubtless in relation to the amount of dust in the lungs, there being appreciably more of it in this animal.

$2\frac{1}{2}$ *months after last exposure.*

At necropsy the lungs show a faint, diffuse reddish discoloration; here and there the haematite is concentrated beneath the pleura to form dark brown spots. Other organs normal.

Animal A. Examined with the low power the general condition is much as in No. 1, *B*. Small areas of thickened and proliferated alveolar epithelium are still present. The changes are more marked in the apices and upper lobes than in the lower parts of the lung. The dust is now entirely intra-cellular, the cells being either attached to the alveolar walls, or in the inter-alveolar septa. Accumulation of dust beneath the pleura has begun and the total amount of dust in the lungs is rather less than in No. 1, *B*. Cuffing of the blood vessels with lymphocytes and plasma cells persists. The bronchi are normal. There is a definite increase in the dust-content of the tracheal lymph glands as compared with the first two animals.

Animal B. The changes are similar to those found in *A*, except that they are milder and that there is rather less dust.

$11\frac{1}{2}$ *months after exposure.*

At the time of killing this guinea-pig had grown a great deal. Necropsy showed only slight pigmentation of the upper right and left lobes. The abdominal organs were normal.

Microscopically, small areas of thickened and proliferating alveolar epithelium are common. Some cuffing of blood vessels still persists. The general condition is rather like No. 1, *B*, though the dust has greatly diminished in amount as compared with animals Nos. 1, *A* and *B*. The dust particles are black instead of brownish-black as in the earlier specimens. Careful examination of sections stained with Heidenhain's iron haematoxylin and van Gieson (the latter used according to Schmorl's method) shows no traces of fibrosis. There is some catarrh of the smaller bronchi. The bronchial glands contain a little black dust.

Examination of sections of lung stained by the potassium ferrocyanide—hydrochloric acid method for iron [details of method in Carleton (1926)] gives a positive reaction around the haematite particles, as, of course, would be expected.

4. THE LUNG CHANGES PRODUCED BY THE INHALATION OF LEVIGATED IRON.

Two small groups of animals were exposed to levigated iron dust. The reason for the repetition of this experiment was the heavy mortality in the first group.

In Group 1, exposures were begun at the rate of two hours daily. After the third exposure two of the animals died (see note on p. 230). Consequently the exposures were cut down to half an hour per diem; 6 dustings of this duration were given. Finally, three more daily exposures, each of one hour, were administered. The total number of exposures was 12, the average duration of each thus working out at 1 hour. The exposures were spaced over a period of 20 days, because, after the occurrence of the first two casualties, it was judged better to suspend the dustings on days when the animals were apathetic and off their food.

Group 1.

Animals examined 24 *hours after the last exposure.*

At necropsy the lungs were covered by a dark grey mottling, especially over the apices and the upper aspects of the lobes. The other organs were normal.

Animal A. The alveoli are fairly normal except for the presence of plentiful dust cells. Here and there are small areas of broncho-pneumonia. The lungs contain much dust considering that the inhalations were spaced out and that the average duration of each dusting was short (60 minutes). There is cuffing of some of the blood vessels with lymphocytes. The bronchial epithelium is normal though the bronchi contain a good deal of dust—mostly free.

Animal B. Alveolar changes are widespread. They comprise general thickening and proliferation of the alveolar epithelium. Broncho-pneumonic patches are common. The more marked changes in this animal are obviously in relation to the amount of dust in the lungs, which is considerably greater than in animal *A*. The bronchi often contain much dust, and some are catarrhal.

3 *months after exposure.*

At necropsy the apices showed heavy grey pigmentation, this being less marked over the lobes. Other organs normal.

Microscopically the lungs show only a few small patches of thickened alveolar epithelium. The pleural drift of the dust cells is by now well established, though there is still a good deal of intra-cellular dust in the alveolar septa. No bronchitis, in spite of the presence of free dust in some of the bronchi. The amount of dust is less than in No. 1, *B*, but more than in *A*, 1. The appearances suggest that the initial amount of dust in the alveoli was large—probably akin to that found in No. 1, *B*. As usual, the apices are more heavily pigmented than the lobes.

6 *months after exposure.*

At necropsy the lungs were very heavily pigmented, especially the lobes. Other organs normal.

Microscopically there is very little normal lung substance left. The greater part of every section shows collapsed alveoli with proliferated and thickened epithelium; often there is frank consolidation, such areas being sometimes heavily infiltrated with pseudo-eosinophil cells. The connective tissue of the pleura is generally thickened, but the serous cell layer is normal. There is some cuffing of the blood vessels with lymphocytes. Bronchitis is frequent. Degenerative changes can be seen in many bronchi. In one the tissues have broken down as far as the fibrous sheath; the lumen is occupied by a dense mass of fibroblasts and giant cells. The dust is very abundant—more so than in any other animal of this series. Presumably, therefore, more dust found its way into the lungs in this than in the other animals. Plaque formation has occurred, especially beneath the pleura. There is an early fibrosis in the more damaged areas; the adventitia of some of the small veins and bronchi is hypertrophied.

Note on the casualties of this group. Of the four animals which died during the experiment, one (*A*) died after a single dusting; the second (*B*), after three exposures; the third (*C*), 2 days after the last dusting; the fourth (*D*), 1 month after the last exposure.

The cause of death is unknown for *B*, the pulmonary changes being very slight.

The findings in the other animals (A, B and D) point to an acute inflammatory process in the lungs. Bronchitis, general alveolar catarrh, areas of haemorrhage into the alveoli and hyperaemia are sufficiently widespread to account for death. Since the amount of dust in the lungs is no greater than in some of the animals which survived, one is forced to assume a greater susceptibility of these lungs to the dust.

Group 2.

The inhalations in the first series appeared to have been close to the lethal limit. So another series of experiments was made in which the exposures were graded, while dustings of longer than 1 hour per diem were avoided. Ten exposures were given: the first of 15 minutes; the second of 30 minutes; the remaining 8 of one hour each. The dusting was carried out on ten consecutive days. The average duration of exposure was 52·5 minutes.

Only one animal seemed unwell during the exposures, and it recovered.

24 *hours after last exposure, Animals A and B.*

The changes are like those in No. 1, *B* of the preceding group (see p. 229). The lungs are almost equally loaded with dust in both animals of the present group (2) and in No. 1, *B* of the first group.

3 *months after exposure. One animal.*

The dust has diminished. Sub-pleural accumulation is already well established. The changes are similar to those noted 3 months after exposure in Group 1, except that there an area of sub-pleural consolidation in which there is a slight fibrosis. A good deal of dust has found its way to the bronchial glands.

11½ months after exposure. One animal.

At necropsy there was marked pigmentation over the dorsal surfaces of the apices and upper lobes. Other organs normal.

Microscopically there are large areas of normal lung. Elsewhere there is thickening of the alveolar walls and, in one place, an area tending towards consolidation. Cuffing of the branches of the pulmonary vein with lymphocytes is fairly general. The amount of dust is much reduced as compared with the earlier animals of this series. Here and there are subpleural accumulations of dust; these are intra-cellular and suggestive of early plaque formation. There is no fibrosis. The bronchitis has almost completely disappeared. There is a moderate amount of dust in the bronchial glands.

5. THE DISTRIBUTION OF IRON AND HAEMATITE IN ORGANS OTHER THAN THE LUNGS.

Two facts must be kept in mind with regard to the above.

(1) That almost any dust inhaled into the lungs, and subsequently passed along to other organs, such as the lymph glands or spleen, will give a positive Prussian blue reaction for ferric salts when tested with potassium ferrocyanide. This is because iron is one of the most ubiquitous of the elements.

For instance, inhaled particles of calcium carbonate (B.P.), charcoal or flint, give a positive iron reaction. Again, a similar reaction is found around the intra-cellular particles in the lungs of monkeys kept in captivity in this country, as also in human and pit pony lungs. The positive reaction is found, in my experience, around particles which are generally regarded as carbon. Doubtless these particles are largely carbon, but the fact remains that with the carbon, or shale (pit pony lungs), or flint (a personal guinea-pig series (1)) there is also associated a trace of iron, which is sufficient to give the extremely sensitive Prussian blue reaction when this is properly applied.

(2) The picture is complicated by the tendency for iron compounds, if swallowed, to be absorbed in the intestine and stored in the spleen. Thence the iron is afterwards removed *via* the blood vessels to the liver, and finally excreted by the terminal portion of the intestine (cf. Cushny, 1918).

When animals, such as the guinea-pigs used in these experiments, are caused to inhale iron compounds, much of the dust is swallowed. These animals neither cough nor dribble during the inhalation, which shows that much of the dust in the mouth must pass down the oesophagus. And this is substantiated by the fact that examination of sections of the small intestine of animals, up to 2 days after exposure to the dusts of iron or haematite, give a strongly positive reaction to potassium ferrocyanide.

The villi of such animals are literally distended by the iron-bearing phagocytes within them, while the columnar epithelial cells contain very fine blue particles. Inversely, examination of the intestine of control guinea-pigs reveals only occasional iron-containing phagocytes in the villi.

Distribution of iron a few days after the last exposure to iron dust.

The tips of the villi in the small intestine are enormously distended by phagocytes containing intra-cellular granules giving the Prussian blue reaction; material giving this reaction is also dispersed amongst the intestinal contents, while the columnar epithelial cells show a faint blue granulation beneath the striated membrane. The spleen and liver show an increase in the intra-cellular iron. In the former it is found in the sinusoids, in the latter in the hepatic cells, especially in those close to the portal sheaths.

2 *to* 3 *months after exposure.*

Although iron-containing phagocytes can be found in the villi, their numbers have greatly diminished. Such cells can also (but rarely) be picked out in the mucosa of the large intestine. Iron-containing phagocytes can be seen in a mesenteric lymph gland, some of them in the afferent lymphatic vessels themselves. The liver shows a marked diminution in intra-cellular iron; the spleen still contains plenty.

6 *months after exposure.*

Phagocytes charged with iron are now extremely rare in both duodenum and large intestine. The liver cells themselves are negative to the Prussian blue test; but an occasional phagocyte containing blue granules can be seen compressed between the hepatic cells. The spleen still contains plenty of intra-cellular iron.

$11\frac{1}{2}$ *months after exposure.*

The liver and intestine are negative to the Prussian blue, potassium ferricyanide and ammonium sulphide tests. The spleen is still strongly positive. Intra-cellular iron is abundant not only in the pulp but also in the fatty connective tissue at the hilum of the organ. Here it can be seen (i) in a small lymphoid nodule, and (ii) in cells which lie compressed between the adipose tissue cells.

In *control guinea-pigs* the liver and large intestine are negative on testing with potassium ferrocyanide. Nor can any pigment be seen in unstained sections. The upper part of the small intestine, on the other hand, shows intra-cellular particles in the villi. These particles are yellowish in unstained material, and give a typical Prussian blue reaction; they are fairly frequent. The spleen only shows very rarely intra-cellular ferric compounds on testing with potassium ferrocyanide.

6. Discussion and Summary.

A. *Haematite.* The immediate reaction produced by the inhalation of this dust is a proliferation of the alveolar epithelium. A mild bronchitis is also caused. Such changes are usual after the inhalation of any dust that elicits a brisk phagocytic response, such as coal or shale (Haynes, 1926; Carleton, 1924). Later ($2\frac{1}{2}$ months after exposure), a perivascular inflammatory change (*i.e.* the "cuffing" of branches of the pulmonary vein) appears. The dust by this time has been carried along the lymphatics towards the pleura and bronchial lymph glands. The bronchi, no longer irritated by the inhaled dust, have become normal. Finally ($11\frac{1}{2}$ months after exposure), the dust is greatly diminished in amount, though "cuffing" of the blood vessels and areas of thickened alveolar epithelium are still common. There is no fibrosis.

Under the conditions of these experiments, it would seem then that haematite, as compared with flint, ground pitcher, felspar or china clay,

(Mavrogordato, 1918; Carleton, 1924), is a comparatively harmless dust. But it does not follow from these observations that haematite, if inhaled over long periods, might not promote a fibrosis upon which tuberculosis might become grafted. The two human cases recently reported by Goadby (1925) are suggestive in this respect, though further evidence seems necessary to prove conclusively that haematite when inhaled can promote a fibrosis in iron miners. What the guinea-pig experiments described above do appear definitely to show is that haematite is a dust (i) which is eliminated more rapidly from the lungs, and (ii) produces far less serious lesions, than felspar, china clay, ground pitcher or flint when administered experimentally to guinea-pigs. Under experimental conditions it is certainly a relatively harmless dust; it stimulates phagocytosis and is removed from the lungs[1]. Doubtless it is also removed by solution, as suggested by Goadby (1925). The histological reaction of the lung towards haematite is rather like that towards shale or coal dust.

Mr F. Haynes has shown me results—obtained after similar experiments to mine with haematite—and which he is duly publishing. His observations agree with the ones recorded here.

B. *Iron.* A number of animals in Group 1, in which two-hourly exposures to iron dust were attempted, died. There was no epidemic amongst these or the other guinea-pigs. Neither the symptoms nor the findings at necropsy bear out the hypothesis that death was due to mechanical blockage of the air passages; therefore some toxic effect must be supposed[2]. It is significant also, that Group 2, in which carefully graded exposures were employed, sustained no casualties during the period of dusting.

Toxic symptoms have apparently not been noted among workers in the shops where iron castings are drilled. But the probable reason for this is that human beings are never exposed to the massive inhalations used in these experiments.

The immediate effects produced by iron dust in the lung are (i) proliferation and thickening of the alveolar epithelium, (ii) a varying degree of bronchitis. Phagocytosis is brisk. The pleural drift of the dust is established by the third month after exposure; the bronchitis has abated; the dust is markedly reduced in amount. But one small area of fibrosis was found beneath the pleura. Later (6 and 11½ months after exposure), varying lesions were found. In the former specimen widespread damage had been caused. (Plaque formation, early and localised fibrosis, much consolidation, bronchitis.) In the latter, large areas of lung were normal; there was neither fibrosis nor bronchitis

[1] John Tatham (1903), in Oliver's well-known treatise on "Dangerous Trades," states that the vital statistics of haematite miners are rather like those of coal miners; that they are, on the whole, a healthy lot of men, and that the mortality from tuberculosis is beneath the average.

[2] To make sure that the dust was at least as pure as that breathed under industrial conditions, it was analysed.

Dr B. Lambert, of the Inorganic Chemistry Department, Oxford, very kindly undertook this. He reported that the iron dust contained "only the merest trace of manganese." Also that the amount of arsenic was "very little (if any)." The Marsh test had to be applied to elicit a reaction. It follows therefore that the dust was far purer than that inhaled by workers under industrial conditions.

though cuffing of the pulmonary veins still persisted. Beneath the pleura were accumulations of intra-cellular dust suggestive of early plaque formation. The key to the very different lesions in these two specimens is afforded by the amount of dust present. In the first case, the amount of dust was greatly in excess of what was found in the second, or in any other animals used in these experiments.

Under the conditions of these experiments, iron dust was found to be more harmful than haematite. For whereas no permanent lesions were caused by the latter, iron dust produced greater inflammatory changes, and some permanent alterations (*e.g.* a slight fibrosis) in the lung.

C. *The distribution of iron dust in organs other than the lungs.* The interpretation of the findings already noted would seem to be that iron inhaled into the lungs is also swallowed in sufficient quantity to be absorbed by phagocytic cells in the villi of the small intestine. Both liver and spleen are heavily charged with a pigment, giving the reactions of a ferric compound, in specimens taken in the early days during or after the dustings. A few months later, the absorption from the gut would seem to cease; the intra-cellular ferric compounds in the liver disappear, though the spleen and mesenteric lymph glands still contain them in abundance. From 6 to 11 months after exposure the spleen was found to be the only one of the organs examined which still contained an iron pigment in quantities above the normal.

D. *The distribution of haematite in organs other than the lungs.* The reaction with potassium ferrocyanide is less marked than with iron dust. Such specimens as were examined suggest that far less haematite was taken up in the intestine. Furthermore, the spleen appears to be the only organ in which a ferric compound can be detected in quantities above the normal in the later months.

E. *The need for caution in interpreting a positive reaction for iron compounds with the potassium ferrocyanide* (*Prussian blue*) *reaction has been emphasized.* Practically any dust will give a positive reaction with this very sensitive reagent, presumably because any substance inhaled contains traces of iron compounds. In assessing the amount of iron or haematite in a lung it is necessary to compare it with control lungs also subjected to the standard histo-chemical tests for iron, since the dust in normal lungs—no matter what it may be—will give a positive reaction for ferric salts with the Prussian blue reaction.

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