

ALUMINIUM PNEUMOCONIOSIS

II. EFFECT ON THE RAT LUNG OF INTRATRACHEAL INJECTIONS OF STAMPED ALUMINIUM POWDERS CONTAINING DIFFERENT LUBRICATING AGENTS AND OF A GRANULAR ALUMINIUM POWDER

BY

B. CORRIN

From the Department of Pathology, University of Manchester

(RECEIVED FOR PUBLICATION NOVEMBER 22, 1962)

Three stamped aluminium powders were injected into the lungs of rats. One powder contained stearine and another mineral oil, whilst the third had had its lubricant removed. The powders produced a rapid and marked fibrosis of equal severity. It is concluded that aluminium rather than any additive in the powders is the fibrogenic agent.

The protective action of stearine demonstrated *in vitro* was not confirmed *in vivo*, suggesting that pulmonary fibrosis may also occur in men handling stearine-containing powders. Such a case has recently been reported by McLaughlin *et al.* (1962), but this is exceptional to the general industrial experience.

A granular aluminium powder was also injected into the lungs of rats. In accordance with the results of *in vitro* experiments, this produced only minimal fibrosis, contrasting strongly with the action of the stamped powders.

Granular aluminium powders and stearine-containing stamped aluminium powders appear to be harmless. Only stamped powders containing a lubricant of mineral origin cause aluminium pneumoconiosis (Goralewski, 1947; Mitchell, Manning, Molyneux, and Lane, 1961; Swensson, Nordenfelt, Forssman, Lundgren, and Öhman, 1962). *In vitro* experiments (Jäger and Jäger, 1941; Corrin, 1963) suggest that this is due to the mineral lubricant allowing tissue fluids to attack metallic aluminium. An alternative explanation would be that the small amount of mineral oil in the powder, rather than the aluminium, is responsible for the disease. This paper reports the results of animal experiments in which stamped aluminium powders containing different lubricating substances, and a granular aluminium powder containing no lubricant, were used.

Materials and Methods

Three kinds of stamped aluminium powder were used. Except for their stearine and oil content, they conformed to the general description given by Mitchell *et al.* (1961), consisting of thin flake-like particles with the following

size distribution: over 5μ , 3%; 1- 5μ , 47%; below 1μ , 50%. One powder contained 0.2% stearine but no mineral oil, whilst another contained 0.2% oil but no stearine. A powder containing neither stearine nor oil was also required, but it is impossible to make a stamped powder without any lubricating agent, and the stearine was therefore removed from a little of the stearine-containing, oil-free powder by washing it with hot acetone until the washings were shown by evaporation to be fat-free. The stearine consists of 50% stearic acid and 50% palmitic acid. The oil is a conventionally refined light lubricating mineral oil consisting of aromatics, 31.3%; naphthenes, 63.1%; paraffins, 4.6%; and polar compounds, 1%. No additives are incorporated in the oil. The granular aluminium powder, 99.5% pure, had the following particle size distribution: 1 to 5μ , 11%; 5 to 10μ , 17%; over 10μ , 72%.

The animals used were female Wistar rats of 150 to 175 g. body weight.

The dusts were sterilized by autoclaving at 15 lb. per sq. in. pressure for 10 minutes. Immediately before administration, sterile physiological saline was added and the dusts were suspended by vigorous shaking in a final concentration of 100 mg. dust per ml. One millilitre of dust suspension was injected into the lungs of a rat by the

TABLE 1
NUMBER OF RATS INJECTED WITH EACH POWDER AND THEIR TIME OF SURVIVAL AFTER INJECTION

Time of Survival	Powder				Controls
	Stearine-containing	Oil-containing	Stearine- and Oil-free	Granular	
Up to 24 hours	3	2	5	1	—
2 months	7	2	2	5	1
4 months	5	6	2	4	4
6 months	3	5	2	3	4
8 months	4	4	1	2	3
12 months	3	2	1	3	5
	25	21	13	18	17

TABLE 2
INTERVALS UP TO 36 DAYS AFTER INJECTION AT WHICH RATS WERE EXAMINED, AND THE TYPE OF POWDER EACH RAT RECEIVED

Type of powder	Days after Injection									
	4	13	16	17	17	18	31	35	36	
	S	S	D	O	O	S	O	D	S	

S = Stearine-containing
O = Oil-containing
D = Defatted

intratracheal injection method of King, Harrison, Mohanty, and Yoganathan (1958). Occasionally there was reflux of a small amount of the suspension, but usually it all remained in the lower respiratory tract.

Groups of 37, 32, 15, and 18 rats received the stearine-containing, oil-containing, defatted, and granular powders respectively, and 17 rats were injected with 1 ml. of sterile physiological saline containing no dust. Eight animals in each of the first two groups died of asphyxiation immediately after the injection; the injected fluid appeared to be added to by marked pulmonary oedema. A few more animals, which apparently recovered from the injection, died within the next 24 hours. Most of the remaining animals were killed at two-monthly intervals (Table 1) but a few died spontaneously and a few were killed within the first month to study the early development of the lesions (Table 2).

The animals were killed with ether, and the abdomen, thorax, and neck were opened in the midline, cutting through the sternum and snapping open the shoulder girdle. After opening the trachea the lungs were distended gently by the intratracheal injection of about 10 ml. of 10% formalin. The trachea was then tied off and the entire thoracic contents removed and placed in an excess of fixative. After about four days blocks were taken from the lungs and hilar lymph nodes and embedded in paraffin. Sever-micron sections were cut and stained with haematoxylin and eosin, Masson's trichrome stain, and aurine tricarboxylic acid (for aluminium). Certain sections were also stained with the periodic acid-Schiff, Unna-Pappenheim's methyl green-pyronine, and Weigert's elastin stains. In some cases frozen sections were stained for fat, and tissues from some animals were sectioned serially to study the distribution of dust and lung lesions.

Results

Controls.—No abnormalities were found in the animals injected with dust-free saline.

Stearine-containing Powder

Appearances within 24 hours of the Injection.—One animal in this group was killed 4 hours after the injection and two others died within 24 hours. Naked-eye examination of the lungs showed numerous small, dark areas scattered uniformly throughout all lobes. Microscopically, these areas were seen to consist of focal collections of dust particles of unequal size. Occasionally it could be seen in a single section that the collections of dust were related to respiratory bronchioles, and serial sections provided general confirmation of this. The respiratory bronchioles were mainly those of the first or second order. Most of the dust lay in alveoli which opened directly off the bronchioles or lined the walls of the respiratory bronchioles themselves. A small amount of dust reached the alveoli of the peripheral parts of the secondary lung lobules, but the dust was distributed mainly to the central parts of the lobules.

The individual dust particles were black, irregular, and jagged. Many were separate but collections of particles forming large aggregates up to 70μ in length were frequent. At this stage the particles did not stain for aluminium with aurine, presumably because there had been insufficient time for a high enough concentration of dissolved aluminium to be reached.

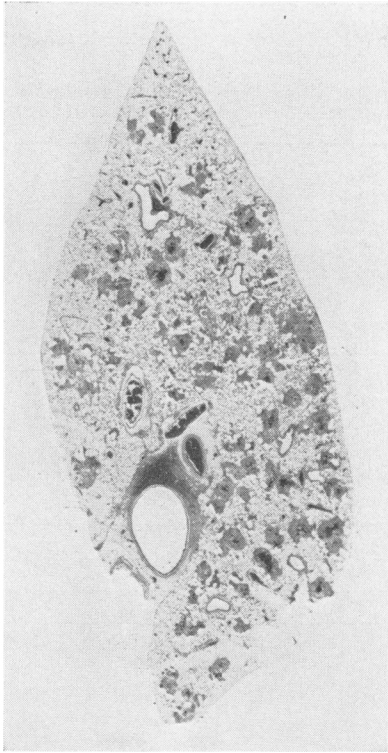


FIG. 1.

FIG. 1.—Rat lung injected with 100 mg. of stearine-containing aluminum powder 2 months previously, showing a regular distribution of fibrous nodules. (H. and E. \times 5).

FIG. 2.—Same animal as in Fig. 1 showing well demarcated, cellular, dust-containing fibrous nodules. (H. and E. \times 50).

FIG. 3.—Same animal as in Figs. 1 and 2, showing details of a nodule. Collagen fibres can be seen between the dust particles. (H. and E. \times 250).

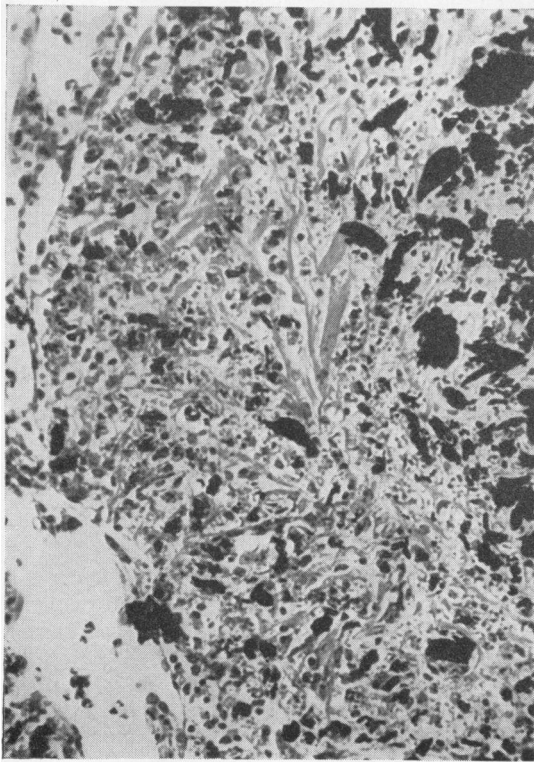


FIG. 3.



FIG. 2.

Apart from the presence of dust, pathological changes were observed only in the animals dying spontaneously. These showed areas of pulmonary congestion, oedema, and haemorrhage.

Appearances at 2 months.—Seven rats were killed 2 months after the injection. The lungs of these animals showed numerous firm dark nodules distributed equally throughout all lobes, and the hilar lymph nodes were enlarged and dark grey. Microscopically, the even distribution of the nodules throughout the lungs was confirmed (Fig. 1). The nodules were of varying size, well defined, and sharply demarcated from the surrounding lung tissue (Fig. 2). They contained many macrophages and dust particles and a few plasma cells and fibroblasts. Many of the dust particles had been ingested by the macrophages. Also present in the nodules was a rich network of hyaline collagen fibres, passing haphazardly between the cells and the dust particles (Fig. 3). The collagen fibres formed an irregular pattern and showed neither a concentric nor a radial arrangement. Within the nodules, the lung tissue was entirely destroyed and replaced by fibrous tissue. Frequently the nodules were situated close to a respiratory or terminal bronchiole, showing that the dust had maintained its original distribution except for local concentration into more compact collections.

Outside the nodules there was only a small amount of dust, and most of this was intracellular. The dust-containing macrophages lay mainly within the lumen of alveoli. Occasional dust cells appeared to be inside alveolar walls, but it was difficult to exclude the possibility that they were merely applied closely to the walls. Apart from the presence of dust and macrophages, the lung tissue between the nodules appeared normal.

The dust-containing cells all had a slightly foamy cytoplasm. Fat stains were negative, but the macrophages were strongly Schiff-positive. Plasma cells were fairly numerous at the periphery of the nodules. Many of the dust particles gave a strongly positive reaction with aurine, indicating that aluminium was entering into solution.

The hilar lymph nodes contained numerous dust particles, mainly intracellular. The lymphatic sinuses were distended by dust-laden macrophages but there was no fibrosis.

Appearances at 4 months.—Five animals were killed at this stage. Their lungs contained small firm nodules of similar distribution to those seen previously. In addition to the dark dust pigment, pale grey fibrous tissue in the nodules could now be seen by the naked eye. Microscopically, more collagen was apparent in the nodules, and macrophages were fewer than at 2 months. The centres of the nodules consisted of dense hyaline collagen containing dust

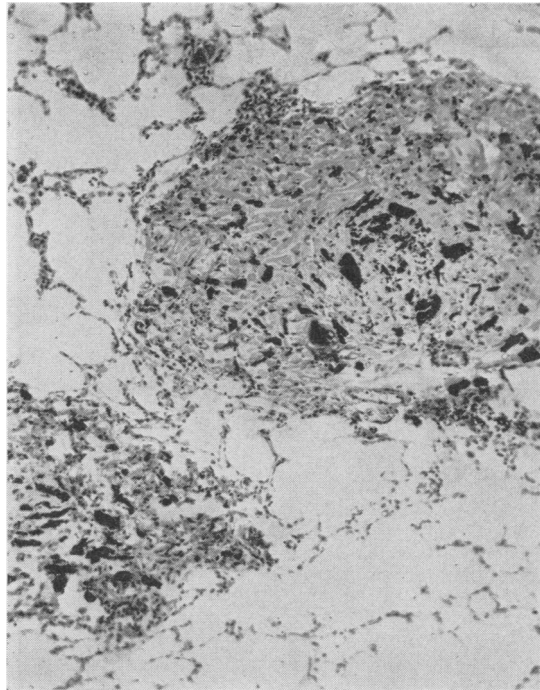


FIG. 4.—Rat lung showing the reaction to 100 mg. of stearine-containing powder injected 4 months previously. The dust particles are enmeshed by poorly cellular collagen. (H. and E. $\times 100$).

particles, but very few cells (Fig. 4). However, at the periphery of the nodules, fibroblasts and dust-laden macrophages were numerous, indicating that active fibrosis was proceeding at that site. Plasma cells were also present at the periphery of the nodules. Dust cells were still Schiff-positive, and many dust particles gave a positive aurine reaction for aluminium. Between the nodules there was considerably less dust than had been seen previously. Dust had been removed from this position, presumably to the fibrous nodules or to sites outside the lung. The lymph nodes at the hilum of the lung contained much intracellular dust but showed no fibrosis.

Appearances at 6, 8, and 12 months.—Three, four and three animals were killed at 6, 8, and 12 months respectively. In all these animals, small hard nodules were palpable in the lungs, and occasionally the visceral pleura was wrinkled over the more superficial nodules. On the cut surfaces of the lungs the nodules showed a dark and pale grey mottled appearance due to intermixed dust and fibrous tissue.

Microscopically, the fibrosis consisted of thick hyaline fibres which surrounded the dust particles in an irregular manner. As before, the centres of the nodules consisted almost entirely of dust and collagen

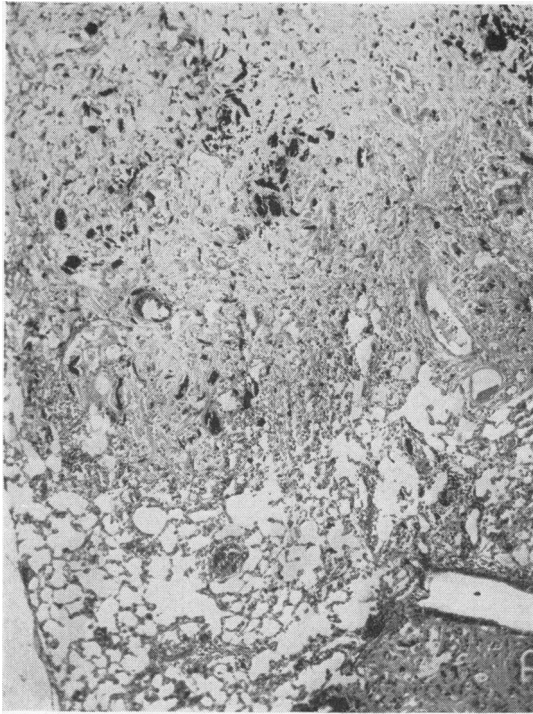


FIG. 5.—Rat lung 12 months after intratracheal injection of 100 mg. stearine-containing powder, showing densely hyaline fibrous tissue. (H. and E. $\times 50$).

and contained few cells. By 6 months a reduction in the cellularity of the periphery of the nodules was noticeable, but even at 12 months some active fibrosis appeared to be taking place at the periphery; small numbers of dust-laden macrophages and fibroblasts persisted around the central collagen, and plasma cells were still present. Between 6 and 12 months the fibrous tissue in the middle of the nodules became progressively more dense and hyaline (Fig. 5). The lung tissue between the nodules contained very little dust and, apart from this dust, was of normal appearance. There was no appreciable reduction in the dust content of the nodules, but the aurine stain was persistently positive, indicating continuous dissolution of the aluminium particles. The hilar lymph nodes contained many dust particles, all within macrophages. Some of the dust cells had penetrated the lymph follicles, but there was no evidence of fibrosis in the lymph nodes.

Oil-containing Powder

Appearances within 24 hours of the Injection.—Two animals injected with a suspension of this

powder died within 24 hours. The naked-eye and histological appearances of the lungs were the same as those described at this stage in rats given the stearine-coated powder. The dust particles were of similar appearance to those made with stearine, and there was no difference in the distribution of the dust or in its negative staining reaction with aurine.

Appearances at 2 months.—Two rats were killed 2 months after the injection. Their lungs contained many well-defined, dust-containing nodules of varying size, distributed equally throughout all lobes. A small amount of dust was also present in the hilar lymph nodes. Microscopically, the lung nodules consisted of macrophages, dust particles, many of which were intracellular, and small numbers of fibroblasts and plasma cells. Collagen fibres were also present, passing between the cells and dust particles in an irregular manner. Many macrophages were Schiff-positive, and many dust particles stained positively for aluminium. A comparison between these animals and those killed two months after receiving the stearine-coated powder showed no differences whatsoever (Fig. 6). The amounts of

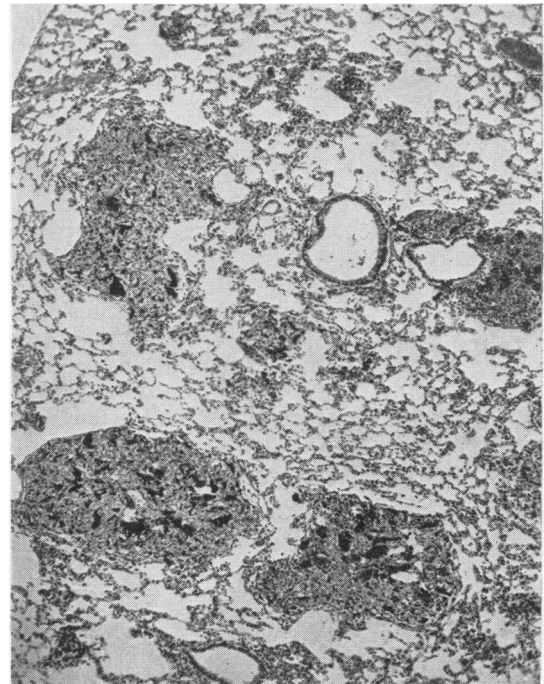


FIG. 6.—Rat lung injected with 100 mg. of oil containing aluminium powder 2 months previously. For comparison with Fig. 2. (H. and E. $\times 50$).



FIG. 7.—Rat lung injected with 100 mg. of oil-containing powder 12 months previously. For comparison with Fig. 5. (H. and E. \times 50).

collagen present and the histological appearances in the two groups of animals were identical in all respects.

Appearances at 4, 6, 8, and 12 months.—Six rats were killed at 4 months and five at 6 months. At 8 months one died of intercurrent extrapulmonary infection and three others were killed. Two animals were killed at 12 months. In all these animals the pathological appearances corresponded to those observed at these stages in the rats given the stearine-containing powder (Fig. 7). Collagen continued to be formed, and the fibrous tissue at the centres of the nodules became more hyaline and less cellular, whilst young fibrous tissue was continuously produced at the margins of the nodules. Dust was cleared from the intervening lung tissue and there was considerable transport of dust to the hilar lymph nodes, but no fibrosis occurred in the lymphatic tissue. No differences at all were detectable between rats given stearine- and oil-containing powders.

Stearine- and Oil-free Powder.—Five rats died within 24 hours of the injection, and at 2, 4, 6, and 8 months 2, 2, 2, and 1, respectively, were killed. One rat died of extrapulmonary infection at 12 months.

At the various stages the pathological appearances in these animals were exactly the same as those already described for the two previous groups (Fig. 8). The amount of fibrosis produced and its time of onset were identical with those observed with the stearine- and oil-containing powders.

Early Development of the Lung Lesions.—As the three powders appeared to be equally fibrogenic, it was considered justifiable, in studying the early development of the experimental lesions, to examine animals which had received any of the powders. Nine rats were available for study of the changes occurring within 36 days of the injection. These included three animals which had received 100 mg. of the oil-containing powder and died of extrapulmonary infection, and four and two animals killed after receiving 100 mg. of the stearine-containing and defatted powders respectively (Table 2).

In all these animals, macroscopic examination of the lungs showed numerous dark grey areas scattered equally throughout all lobes. Histological examination at 4 days showed a similar distribution of dust to that seen immediately after the injection. There was

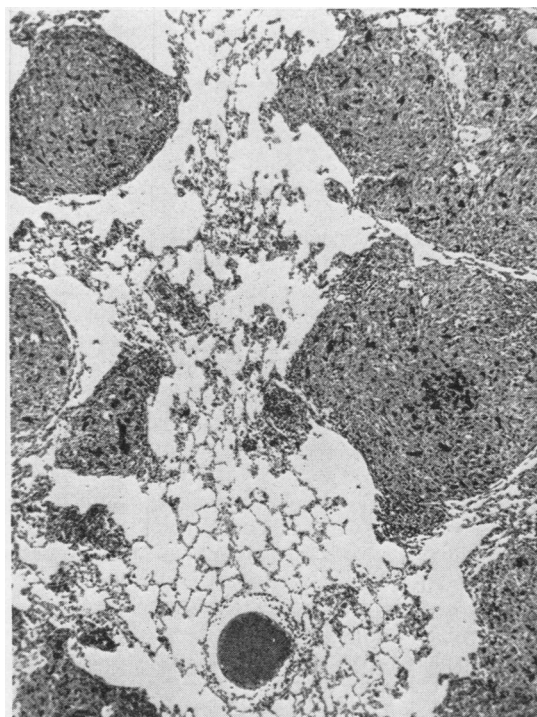


FIG. 8.—Rat lung injected with 100 mg. of hot acetone-washed powder 2 months previously. For comparison with Figs. 2 and 6. (H. and E. \times 50.)

also a considerable macrophage response to the dust, and many of the smaller particles had already been ingested by the macrophages.

By 13 days many macrophages had a swollen foamy cytoplasm, and many of the dust particles and phagocytic cells were concentrated in well-defined nodular collections about the respiratory bronchioles. These collections filled several contiguous air sacs. Both the dust particles and the macrophages were intra-alveolar, and elastin stains showed that there was no destruction of alveolar walls at this stage. Occasional elongated cells resembling fibroblasts were present in the nodules but no fibrosis was detectable.

At 16, 17, and 18 days, Masson's trichrome stain showed a small amount of fine collagen fibre formation between the macrophages and dust particles comprising the nodules. This initial fibrosis was not interstitial but intra-alveolar. Apart from the fibrous tissue there was little change from the appearances seen at 13 days. No plasma cells were present.

At 31, 35, and 36 days, there was a considerable amount of fibrosis. Thick collagen fibres were arranged in an irregular manner between the dust particles and phagocytic cells of the nodules, completely replacing the lung tissue within the nodules so that alveolar walls could no longer be recognized. Fibroblasts were numerous and plasma cells were also present in the nodules. The macrophages, which up to now had been Schiff-negative, gave a positive Schiff reaction.

Granular Powder

Appearance 24 hours after the Injection.—One rat was killed at this stage. The lungs had a uniformly faint grey appearance. No patchiness in the distribution of the dust was apparent. Histological examination showed considerably fewer dust particles than were present in the animals receiving stamped powders; the larger size of the granular particles was also noticeable. The particles were scattered equally throughout the lungs and were situated within alveolar sacs. Occasionally a few particles were found close together but generally the particles were quite separate and there were no focal collections or aggregates. Small numbers of macrophages were present in the alveoli but there was no other abnormality.

Appearances at 2 months.—Five rats were killed 2 months after the injection. Their lungs all had a uniformly faint grey appearance. Microscopically little change was noticed from the appearances seen immediately following the injection. There was no alteration in the distribution of the dust. Macrophages were slightly increased in number, and small collections of them, together with a few lymphocytes,

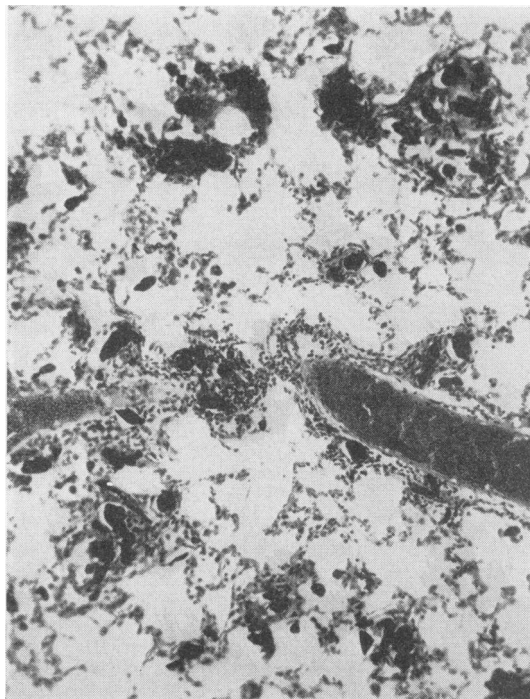


FIG. 9.—Rat lung 12 months after the injection of 100 mg. of granular aluminium powder. The dust particles are surrounded by a few macrophages and lymphocytes only. For comparison with Figs. 5 and 7. (H. and E. $\times 100$.)

were found about occasional dust particles. However, the cellular exudate was not great and many dust particles lay free in the alveolar lumina. No fibrosis was detectable and the dust particles did not stain with aurine tricarboxylic acid, in contrast to the particles of all three stamped powders which by this stage stained intensely with aurine. The hilar lymph nodes showed slight endothelial proliferation and contained a few dust particles, but there was no evidence of fibrosis.

Appearances at 4, 6, 8, and 12 months.—Four, three, two, and three rats were killed at 4, 6, 8, and 12 months, respectively. Changes from the appearances seen at 2 months were few. There were a few small collections of macrophages, lymphocytes, and dust particles, and after 4 months Masson's stain showed an occasional thin collagen fibre in a few of these collections. However, the fibrosis did not progress and even up to a year after the injection such an appearance was exceptional. Most of the dust particles lay free in the alveoli and excited no cellular or fibrotic response (Fig. 9). The appearances amounted to no more than a mild foreign body reaction to an inert dust. After 4 months some of the aluminium particles stained weakly with aurine.

Discussion

The stamped aluminium powders are highly fibrogenic and contrast strongly with the granular powder. The difference in particle size and shape between the granular and stamped aluminium powders does not allow a close comparison of these two types of dust, but the difference in tissue response is so marked that it can hardly be accounted for solely by differences in the dimensions of the dust particles. Atmospheric oxidation of the surface of the granular particles, due to the absence of a lubricant in the manufacturing process, may protect this dust against solution in the lung. The granular powder appears to be almost inert *in vivo* and is considerably less active than the stamped powders.

The fibrous reaction to the stamped powder is present as early as 16 days after the injection and is well established by 4 months. This corresponds to the clinical experience, which is that aluminium pneumoconiosis occurs after relatively short exposures and is rapidly progressive. (In the two fatal cases of Mitchell *et al.*, symptoms commenced 2 years 6 months and 3 years after the first exposure to aluminium dust, and death occurred 1 year 2 months and 2 years 4 months after the onset of symptoms.) The experimental lesions do not have the diffuse distribution of the human disease, but this is attributable to the intratracheal injection technique which distributes the dust in a patchy fashion.

There is no evidence that the fibrosis is due to pulmonary infection or that infection is a necessary part of the fibrogenic process, as it was in the experiments of Jötten and Eickhoff (1943). Sterile apparatus was used throughout the experiments, and at the end of a series of injections the remaining dust suspension was expelled through the cannula which had been introduced into the rats' tracheas, plated on culture media, and shown to be sterile. The histological appearances were not those of pulmonary infection.

In identifying the fibrogenic agent in the powders, spectrographic analysis (Corrin, 1963) has shown that dangerous trace elements such as beryllium are absent. As well as aluminium and its compounds, the powders contain stearine, mineral oil, and carbon. Carbon may produce slight fibrosis if present in large amounts, but the main feature of coal-workers' pneumoconiosis is emphysema and not fibrosis (Heppleston, 1953); the carbon content of the stamped aluminium powders is small (1 to 2%) and this component has therefore been disregarded.

Fatty substances are known to be capable of producing severe lung damage (Pinkerton, 1928) but here the histological appearances are quite characteristic, being those of a granulomatous foreign body

reaction to accumulated globules of the lipid. Nothing resembling this has been seen in either the human or animal material. Nevertheless there is little experimental evidence of the effect on the lung of repeated small amounts of finely divided lipid, the state in which stearine and mineral oil are introduced into the lung on the surface of stamped aluminium powder. If one of the lubricants is responsible for the fibrosis it is more likely to be the mineral oil than the stearine for, except for the case of McLaughlin *et al.* (1962), the disease has only appeared at times when stearine has been totally or partially replaced by a mineral wax or oil (Goralewski, 1947; Mitchell *et al.*, 1961; Swensson *et al.*, 1962). However, the fact that all three powders produced severe fibrosis indicates that both stearine and mineral oil may be discounted as possible fibrogenic factors for neither is common to all three powders; indeed, one of these powders has had its lubricant removed. This provides indirect evidence that it is neither of the lubricants which is responsible for the fibrosis, leaving only aluminium and its compounds as possible fibrogenic agents.

The expected difference in the effects of the two lubricating agents has not been realized in these experiments. Stearine was shown to prevent contact between water and aluminium *in vitro* and was expected to prevent fibrosis *in vivo*, but the stearine-containing powder was found to be just as fibrogenic as that containing oil. An explanation for this must be sought.

It might be postulated that the disease was due to an increase in dust concentration following the introduction of mineral oil. No dust measurements had been made before this change, but the management emphasize that there was no alteration in the process other than the introduction of mineral oil and that there was no apparent change in its dustiness. It is unlikely that an alteration in such a small part of the powder as the lubricants (less than 1%) would affect its behaviour as a dust, particularly since particle size and shape in stearine- and oil-containing powders are identical.

In the injection experiments, freshly made powders have been used exclusively, but in the *in vitro* experiments the powders were not fresh. If the protective action of stearine took some time to develop, as it does in aluminium paint powder (Edwards and Wray, 1955), this would explain the anomaly between the results of the *in vivo* and *in vitro* experiments. This explanation, however, is at variance with the occupational experience for the stearine-coated powder has apparently been free of harmful effects for many years, although it is encountered by the workmen during its manufacture, *i.e.* in its freshest possible state.

Although it was shown *in vitro* that stearine completely prevents wetting of aluminium for at least three days, over a longer period *in vivo* the stearine may be slowly removed from the surfaces of the particles. The large amounts of dust administered to the rats may permit a concentration of aluminium, high enough to cause fibrosis, to dissolve from even the stearine-coated powder. In man, a relatively smaller amount of dust enters the lungs over a long period and removal of the stearine may be too slow to permit a fibrogenic level of aluminium to be reached in the surrounding tissues, so that pulmonary fibrosis occurs mainly with the oil-coated powder. The large amounts of powder used in these experiments may have swamped the effects of the two lubricants. Most cases of aluminium pneumoconiosis have been caused by powders containing a mineral wax or oil, but recently McLaughlin *et al.* (1962) have reported a case associated with a stearine-containing powder. The difference between the

lubricants may therefore be only relative and not absolute.

I am grateful to Professor R. E. Lane of the Department of Occupational Health for introducing me to this problem, to Professor A. C. P. Campbell for helpful advice, to Mr. N. Mowat for the photomicrographs, and to the management of the stamped aluminium powder factory for their co-operation and financial aid.

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