EXPERIMENTAL ENDOGENOUS LIPOID PNEUMONIA*

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Discussions of lipoid pneumonia are generally concerned with chronic pulmonary inflammation due to lipids of exogenous origin. Robbins and Sniffen[1] recently described a significant deviation from this usual type in 11 instances of surgically excised pulmonary tissue in which a "chronic pneumonitis of cholesterol type" was found. Although bronchial obstruction was present in some of these cases, Mallory[2] indicated that such was not the case in all. It would seem that the associated lipids probably were endogenous even though the authors stated that the origin was unknown and that there was no evidence of formation locally.

Focal deposits of endogenous lipids are common in the walls of lung abscesses, or of bronchiectatic cavities. They also occur distal to bronchial obstruction and within macrophages in organizing pneumonia. A diffuse pneumonitis due to endogenous lipids has not been recognized heretofore, with the possible exception of the cases studied by Robbins and Sniffen[1] which were not associated with bronchial obstruction.

The purpose of this paper is to describe endogenous lipoid pneumonia which involved all lobes of both lungs and occurred with a very high incidence in experimental animals as an unexpected finding during an investigation into the toxicity of antimony trioxide.

Methods and Materials

An aqueous suspension of finely divided antimony trioxide was dispersed by a compressed air atomizer and the resulting suspension of droplets heat dried. The dust-laden air was then forced through an impinger to trap the aggregates and larger particles (greater than 1 μ) before being blown into an inhalation chamber housing 50 young male rats of the Sprague-Dawley strain. The chamber air was passed through an electrostatic precipitator before discharge to the outside. Electron microscope photographs of dust samples removed from the electrostatic precipitator indicated that the antimony trioxide dust had a mean particle size of 0.6 μ. The weight of the dust precipitated dur-

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ing a day's run divided by the total volume of air which passed through the chamber (determined by a flow meter) yielded 100 to 125 mg. of Sb_2O_3 per cubic meter as the average dust content of the air which the animals inhaled. The exposure averaged 25 hours per week and was continued for 14½ months.

As a second group, two control lots of 50 rats each were exposed simultaneously in identical inhalation chambers to dust in concentrations of approximately 25 mg. per cubic meter of air, and of the same particle size but containing only 1 per cent of antimony trioxide. The source of the compressed air and other experimental conditions were identical to those of the experimental group.

A third group of 30 rats was given intratracheal injections of aqueous suspensions of antimony trioxide (mean particle size, 1.5 μ) and then housed away from further contact with the dust. While the volume of each injection was 1 ml., the total weight of antimony trioxide injected varied from 75 to 125 mg. In a sense, this group constitutes another control in that it eliminated the inhalation apparatus.

Beginning at 2 months and at varying intervals thereafter, rats were removed from the inhalation chambers and sacrificed with ether, some immediately and others after holding periods which varied from 1 week to 12½ months. Some animals died of spontaneous pneumonia. The lungs of all rats were excised and expanded with formalin injected intratracheally under a head of 15 cm. Antimony determinations were made on formalin-fixed lung tissue by the method of Maren.[3]

Results

No significant difference in the gross appearances of the lungs from the various groups was noted until about the ninth month when the experimental group showed scattered, chalk-white, pleural foci about 2 mm. in diameter. With longer exposure this mottling increased in intensity and became associated with uniformly distributed fine depressions suggestive of orange skin. The cut surface showed similar mottling. When such a lung was stained *in toto* with Sudan IV, it took on a much deeper color than control tissue, and the white areas stained an even deeper red than the remainder. The cut surface was equally sudanophilic.

There were surprisingly few instances of grossly recognizable spontaneous pneumonia or bronchiectasis in the experimental group (9 deaths), whereas the control groups in the inhalation chambers showed a higher incidence of pneumonia and bronchiectasis with 14 and 18 deaths from each, respectively.

Microscopic Changes

The early microscopic changes in the lungs of the experimental group which inhaled the high concentration of antimony trioxide consisted of proliferation, swelling, and desquamation of alveolar lining cells. The dust was ingested rapidly by the alveolar macrophages. Similar cells were found in the interstitial tissue, forming dense pigment deposits in the peribronchial and perivascular stroma and also within some alveolar walls. These deposits were composed of densely aggregated pigment which was entirely intracellular. Such dense pigment also was seen in some of the macrophages lying in the alveolar spaces. Other nearby macrophages were filled with scattered, finely divided pigment granules. Pronounced proliferation and swelling of alveolar lining cells were accompanied by increasing tortuosity and branching of reticulum fibers in the thickened alveolar walls. These fibers tended to surround the lining cells as the disease progressed.

With longer exposure, fatty degeneration became increasingly evident in the alveolar macrophages. This was indicated by foamy cytoplasm and sudanophilia. The fatty degeneration, accompanied by severe swelling of the cells, progressed to necrosis and culminated in cell rupture. The cellular débris was ingested by a fresh crop of macrophages which likewise underwent fatty degeneration, necrosis, and rupture, adding more débris and lipoid material to the alveolar content. This débris, with finely dispersed antimony trioxide dust, might be loosely distributed or it might form dense acidophilic masses containing varying amounts of structureless, deeply basophilic material.

One of the most striking features of this disease was the occurrence in frozen sections of many colorless, needle-shaped crystals within alveoli which were choked with débris and swollen, fat-containing macrophages. These crystals were soluble in fat solvents and occurred in large and small sheaves as well as singly. An occasional small crystal might also occur within a macrophage. Many, if not all, of the crystals gave a positive Liebermann-Burchard reaction for steroid (cholesterol?). In frozen sections abundant fine and coarse sudanophilic droplets were found within the macrophages as well as in the intra-alveolar débris.

Varying degrees of collagenous thickening of alveolar walls and interstitial tissue were noted. In the early stages this was mild and occurred in isolated and widely scattered small foci. Later the foci, becoming more numerous and larger, tended to coalesce so that little uninvolved lung tissue was found in the terminal stage. There was

particularly dense collagenization about crystal spaces where the connective tissue was frequently of acellular, hyaline character.

The changes in the reticulum pattern kept pace with the collagenization. There were irregular and heavy condensations of reticulum in the regions of the most dense collagen deposits, particularly about crystal spaces. In general, the simple pattern of the normal expanded alveolus was lost and was replaced by arborescent masses of fibers which tended occasionally to obliterate alveolar spaces.

It is of interest that the amount of the interstitial deposits of antimony trioxide in the lungs was not proportional to the duration of exposure. After 14 months of exposure the lungs actually exhibited less interstitial, but more intra-alveolar, pigment than the lungs of rats exposed 9 months. This suggests that the interstitial deposits are not static.

Although the morphologic and chemical changes in the lung tissue were frequently severe, a distinctive feature was that inflammatory cells other than macrophages were absent with one minor exception. In several animals which died spontaneously toward the close of the experiment a few polymorphonuclear leukocytes were found scattered in the massive alveolar débris. This was in striking contrast to the spontaneous pulmonary inflammations of many of the control animals and some of the experimental animals in which lymphocytic, plasma cell, and polymorphonuclear exudation was prominent.

The satellite lymph nodes from cases of outspoken lipoid pneumonia contained antimony trioxide dust in dense deposits, most abundant in the cortex but also present in the medulla. The bulk of the pigment was intracellular but isolated, coarse, extracellular granules also were seen. No fibrosis or increased reticulum was demonstrable in relation to the pigment here. Since in most lymph nodes the tissue elements frequently were separated by edema, the absence of fixed tissue reaction was easily and decisively demonstrated.

Controls

The microscopic alterations in the lungs of the inhalation controls were minimal and consisted of mild proliferation of alveolar lining cells. A few scattered alveolar macrophages containing fine, black pigment granules and an occasional small cluster of macrophages with foamy cytoplasm completed the picture of the experimentally induced changes in these control animals. Lacking were the sudanophilic débris, lipoid crystals, collagenization, and alteration in reticulum pattern. Many of these lungs showed the spontaneous infections common

to ageing rats and characterized by purulent bronchitis, bronchiectasis, bronchopneumonia, and atelectasis.

The intratracheal controls which received the coarser particles of antimony trioxide in aqueous suspension showed microscopic lesions which were qualitatively identical to those seen in the experimental group. They occurred, however, in widely separated, relatively small foci. It is of interest that the typical fusiform crystal spaces, delimited by dense, heavily pigmented collagen, were found as early as 2 months following the intratracheal injection of the dust.

The antimony content of some of the lungs of the experimental group is given in Table I.

Discussion

In searching for an artefact or technical defect which might be responsible for the lipoid pneumonia, the inhalation of lubricating oil droplets from the air compressor was considered. The following facts eliminate the possibility of such an artefact and establish that the lipids are endogenous and intimately related to the inhaled or injected antimony trioxide:

1. No oily substance could be demonstrated, following prolonged exposure of white filter paper to the blast of air from the compressor.

2. The lipoid changes could not be demonstrated in the lungs of the inhalation control groups which were kept under the same exposure conditions as the experimental group except for a greatly reduced antimony content of the dust.

3. Lesions similar to those of the experimental group were produced by intratracheal injections of antimony trioxide.

4. The Liebermann-Burchard reaction establishes that some of the lipid is a steroid similar to, or identical with, cholesterol. (The recent work by Engle[4] suggests the need for caution in the interpretation of a positive Liebermann-Burchard reaction as definitely indicative of cholesterol.)

The absence of lipids and of fibrosis in lymph nodes containing deposits of antimony is intriguing. It is obvious that the alveolar macrophages, as living cells, can obtain nutrient material and can discharge waste products only by contact with the alveolar surface unless edema fluid is present. Failing such contact, the cellular metabolism suffers. One gains the first impression from a study of lung sections that many macrophages are suspended in air within the alveoli. This, of course, is not so since such cells could be kept in suspension only in edema fluid. Otherwise they will maintain contact with the alveolar wall due

to adhesiveness of the moist surfaces. If there are very many macrophages within an alveolus, however, some or many of them will have no continuous contact with the alveolar wall. Instead, many of the crowded macrophages will have contact with each other or, at best, only intermittent contact with the alveolar wall. On the other hand, if only relatively few macrophages are present within an alveolus, much of the free surface of the macrophages is brought in contact with the alveolar surface by the alternate collapse and expansion of the alveolar space. The phagocytic cells are thereby actively moved about and

TABLE I

*Antimony Content of Formalin-Fixed Lungs (Wet Weight)**

Rat no.	Exposure	Holding period	Weight of sample	Sb content	Average Sb content
	months	*months*	*gm.*	*γ/gm.*	*γ/gm.*
323	2	3	0.0708	1,306	1,306
329	2	4	0.1377	1,135	1,135
334	2	5	0.1978	367	367
343	2	5	0.0902	388	388
339	2	5½	0.1382	461	461
335	2	11	0.0902	292	292
345	2	12½	0.1305	393	393
363	9	0	0.1736	3,399	4,034
363	9	0	0.1659	4,665	
366	9	0	0.1911	3,807	
366	9	0	0.0902	6,430	
366	9	0	0.1014	1,430	2,795
366	9	0	0.1700	544	
366	9	0	0.2024	1,766	
369	9	0	0.0165	4,030	
369	9	0	0.0218	1,950	
369	9	0	0.0364	1,670	2,718
369	9	0	0.0282	3,220	
328	12½	0	0.3938	2,730	2,730
332	12½	0	0.2959	2,954	2,954
356	12½	0	0.1815	3,815	3,815
336	14	0	2.134	4,616	4,616
338	14	0	2.2746	7,237	7,237
346	14	0	0.0189	4,050	
346	14	0	0.0223	3,630	3,864
346	14	0	0.0209	2,970	
346	14	0	0.0180	4,805	
350	14	0	2.119	5,061	5,061
333	14	¼	3.229	3,107	3,107
354	14	¼	1.868	3,921	3,921
331	14	½	4.334	3,562	3,562
351	14	½	2.301	2,464	2,464
358	14	¾	1.6899	4,390	
358	14	¾	0.0122	5,150	
358	14	¾	0.0102	8,725	5,513
358	14	¾	0.0222	4,180	
358	14	¾	0.0170	5,120	
365	14	¾	1.5830	5,942	5,942

* The conversion factor for dry lung weight was found to be 4.85.

different portions of their surfaces are alternately brought into intimate contact with their source of nutrition and with inhaled particulate matter which is adherent to the alveolar surface. Thus, it is reasoned, the alveolar macrophage is more vulnerable to degenerative influences than is the macrophage within a lymph node which is continuously and entirely surrounded by its nutrient tissue fluid. A second point of difference, but of unknown significance, is the higher oxygen tension in the milieu of the alveolar macrophages as compared to that of macrophages within lymph nodes.

Regardless of whether this explanation is correct or not, the fact remains that the ingestion by alveolar macrophages of the relatively inert (chemically) and relatively insoluble antimony trioxide results in the accumulation of intracellular lipids and ultimate cell rupture. Much, if not most, of the fibrosis within the lung appears to be secondary to the lipids so liberated and acting as irritants. The absence of fibrosis in lymph nodes where heavy deposits of antimony trioxide are present may be considered evidence for the assumption that antimony trioxide does not directly cause pulmonary fibrosis.

This interpretation of the origin of the fibrosis is similar to the suggestion advanced by Fallon,[5] who explained the resemblance between the structure of a tubercle and that of a silicotic nodule on the basis that both are fibroblastic reactions evoked by phospholipids. In the first instance the lipid is derived from the capsule of the tubercle bacillus and in the second, from alterations in tissue metabolism induced by silica. Fallon demonstrated by chemical extraction that the phospholipid content of silicotic rabbit lungs increased in proportion to the severity of the experimentally induced silicosis.

Further consideration of these observations suggests certain interesting clinical implications. There is the possibility that lipidic substances may be associated with other forms of pneumoconiosis which are characterized by pulmonary fibrosis. (In lung sections from a case of pneumoconiosis due to bauxite fumes* we recently found much sudanophilic material associated with abundant needle-shaped crystals, soluble in fat solvents.) Another possibility is that some of the cases of clinical pneumonia not now fully explained[2] may be related to endogenous lipids.

Summary and Conclusions

The inhalation by rats of finely divided antimony trioxide particles (100 to 125 mg. per cubic meter of air) for periods of 2 to 14 months produced a lipoid pneumonia in a high percentage of these animals.

* We are indebted to Dr. G. R. Finlay for this tissue.

Antimony trioxide, finely divided (1 μ or less), in contact with the alveolar lining induced proliferation, swelling, and desquamation of alveolar lining cells. The antimony trioxide particles cause metabolic disturbances within macrophages which lead to their fatty degeneration and necrosis. Lipids derived from such cells are demonstrable as sudanophilic droplets and as crystals. The latter are soluble in fat solvents and give a positive reaction for steroid (cholesterol?). It is these lipids from the disintegrated macrophages which cause varying degrees of pulmonary fibrosis. The disease in rats is further characterized by a paucity or absence of inflammatory cells other than macrophages. The absence of fibrosis from lymph nodes which contain heavy deposits of antimony trioxide is considered evidence for the assumption that antimony trioxide does not directly cause pulmonary fibrosis.

Similar pulmonary lesions, though much less severe, were produced in rats by intratracheal injections of finely divided antimony trioxide (75 to 125 mg.) as aqueous suspensions.

Possible clinical implications are: (1) that other pneumoconioses may be associated with lipidic substances of endogenous origin; and (2) that certain clinical lipoid pneumonias not now fully explained may be related to endogenous lipids.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

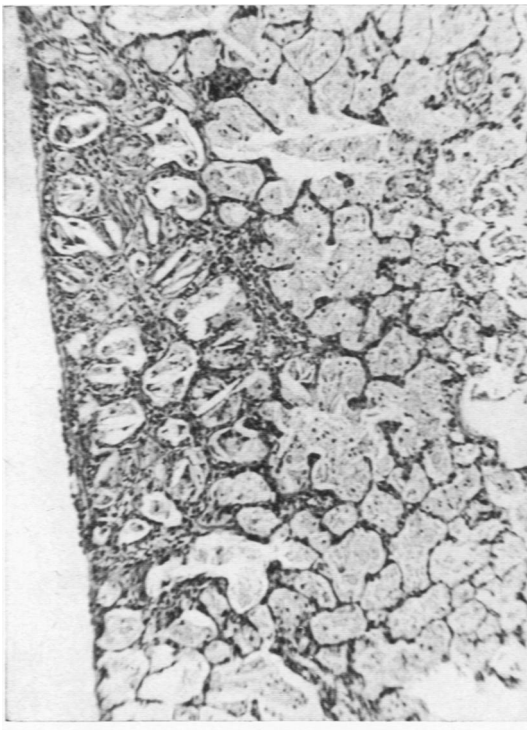
PLATE 27

FIG. 1. Subpleural region of fibrosis with obliteration of some alveoli. There is irregular fibrous thickening of alveolar walls associated with the presence of many crystal clefts. The alveolar spaces are filled with foam cells and their débris, so that few or no air-containing alveoli remain. There is a notable absence of inflammatory cells other than macrophages. × 95.

FIG. 2. Frozen section stained with Sudan IV. The alveolar spaces are filled with sudanophilic débris and colorless crystals. The crystals are disposed in sheaves or parallel masses, less often in single plates. × 130.

FIG. 3. Same field as in Figure 2, but with crossed polaroid films. × 130.

FIG. 4. Lymph node with deposits of antimony trioxide in cortex. There is no demonstrable tissue reaction to the pigment, which is entirely intracellular. × 130.



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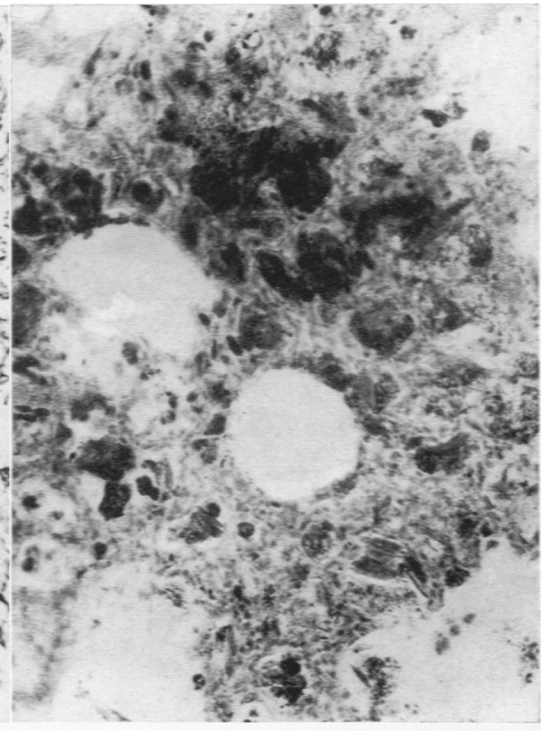

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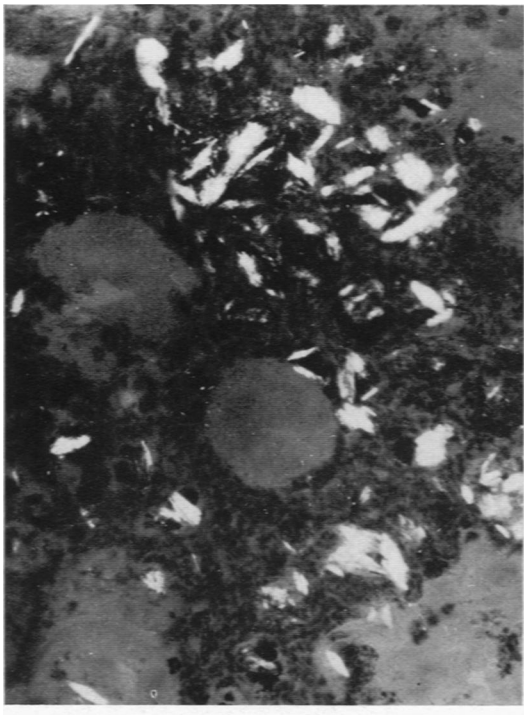

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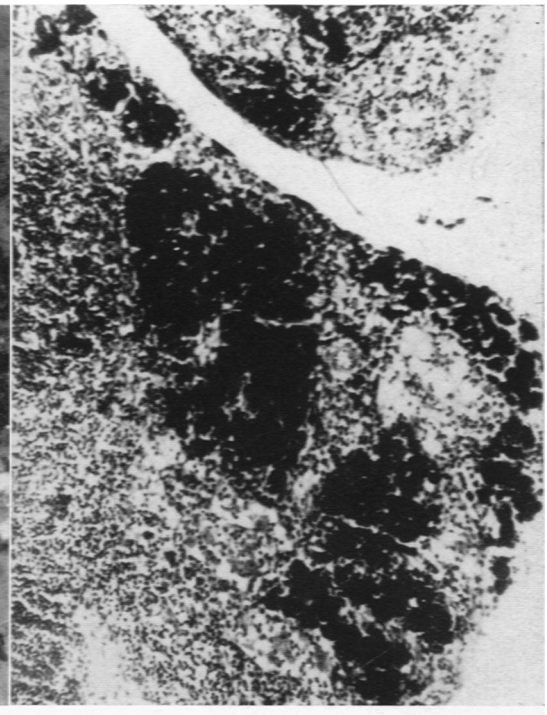

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Endogenous Lipoid Pneumonia