GIANT CELL FOREIGN BODY REACTION TO NON-CHOLESTEROL LIPID PLATE CRYSTALS

A Case Report with Chemical Studies *

Ralph L. Engle, Jr., M.D.

(From the Department of Pathology, the New York Hospital—Cornell Medical Center, New York, N.Y.)

There have been numerous reports of foreign body reaction in which plate crystal slits have been a prominent feature of the granulomas and the reaction was assumed to be due to cholesterol crystallized in the tissue. This is the report of a case in which giant cell granulomatous lesions with plate crystal slits were found in the lungs and the crystals were shown to be lipid but not cholesterol or an ester of cholesterol.

Report of Case

The patient was a white female, 68 years old, who, 1 year before death, developed asthma with "wheezing," slight dyspnea, and a cough productive of small amounts of gray sputum. There was a weight loss of 10 lbs. When examined at the New York Hospital, her temperature was 38° C.; respirations, 20 per minute; blood pressure, 163/100 mm. of Hg. She appeared chronically ill. The trachea was deviated markedly to the right. Physical examination and roentgenograms of the chest suggested a differential diagnosis between tuberculosis and carcinoma of the upper lobe of the right lung. Shortly after a bronchoscopic examination under local cocaine anesthesia, the patient died suddenly in respiratory distress.

At necropsy, the lungs together weighed 1350 gm. There were dense fibrous adhesions from the upper and middle lobes of the right lung to the chest wall and between the lobes, completely obliterating the upper portion of the pleural cavity and pulling the trachea to the right. The right upper and middle lobes of the lungs were small, purple, rubbery hard, and, on section, gray-green. No alveoli could be identified. The other lobes of the lungs appeared normal. There were a few small areas of pleural thickening at the apex of the left lung. The hilar and tracheobronchial lymph nodes were not enlarged.

On microscopic examination the lesion in the upper and middle lobes of the right lung resembled a foreign body reaction. The alveolar structure was almost completely obliterated by moderately dense fibrous tissue containing foci of lymphocytes, monocytes, and foamy macrophages (lipophages), as well as numerous large giant cells of foreign body type without any surrounding epithelioid cells. A number of the giant cells contained stellate asteroid bodies (Fig. 7), a few cells containing as many as three or four. Many of the giant cells were arranged

* Read by title at the Forty-seventh Annual Meeting of The American Association of Pathologists and Bacteriologists, Madison, April 13 to 15, 1950.

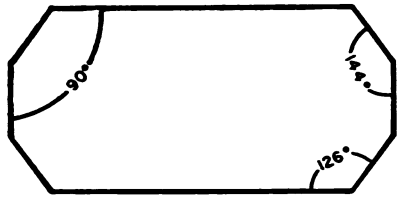
Received for publication, June 8, 1950.

in groups around several elongated, spindle-shaped slits. Slits were located also within some of the giant cells (Figs. 1, 2, and 3). A frozen section stained with sudan IV revealed plate crystals in place of the slits seen in paraffin sections. Some of the macrophages contained numerous tiny globules of fat, stainable with sudan IV. Acid-fast, Giemsa, and Gram stains revealed no organisms. Sections of the remaining lobes of the lungs revealed moderate hemorrhage and edema. There was a slight bronchopneumonia of the lower lobe of the right lung (beta hemolytic streptococcus cultured post mortem). A few small foreign body granulomas, as described above, were seen also in the lower lobe of the right lung and in the thickened visceral pleura of the upper lobe of the left lung. A section through a hilar lymph node in the upper lobe of the right lung revealed moderate numbers of macrophages and red blood cells in the sinuses as well as considerable black and golden-brown pigment. No granulomas were seen.

Other significant lesions included obliterative fibrous pericarditis (no granulomas demonstrable), and moderate congestion of the liver and spleen. Death was attributed to hypersensitivity to cocaine.

Chemical Studies

Sufficient material was available for tests on the nature of the plate crystals. When unstained frozen sections of the lung were made without running the sections through the usual fat solvents, a crystalline material was present in place of the previously seen slits (Fig. 4). Under a dissecting microscope at 72 ×, it was possible to dissect out the clumps of crystals from the formalin-fixed tissue blocks and place them on a slide (Fig. 5).



Text-figure 1. Plate crystal showing measured angles.

The angles of one of the perfectly formed plate crystals were measured. Text-figure 1 is a diagram of the crystals. The crystals were anisotropic, melted at 77° to 78°C. (microscopic technic), and gave a strongly positive Liebermann-Burchard reaction. It was possible to test the solubility of the crystals by applying a drop of solvent to them on a slide. Using this technic, they were found to be soluble at room temperature in ether, chloroform, carbon tetrachloride, benzene, hexane, xylol, toluol, and hot ethanol; and insoluble in water, cold ethanol, acetone, methanol,

N/10 hydrochloric acid, N/10 sodium hydroxide, sodium bicarbonate solution, dilute and glacial acetic acid, and ethylacetate. In relatively much larger quantities of acetone the crystals were soluble.

On the basis of solubility, extraction of the crystals from the fibrosed lung tissue that had been fixed in formalin was attempted. The lung tissue was minced and dried on a paper towel. Twenty grams of tissue were added to 20 cc. of cold ethanol and the mixture placed in the refrigerator for 4 days, at which time the ethanol was poured off and discarded. Twenty cc. of ether were added to the lung tissue and kept at room temperature for 4 days, the evaporating ether being replaced. The ether solution was washed with water in a separatory funnel, filtered, and then placed at room temperature to evaporate. When half of the ether had evaporated, a drop of solution was placed on a slide, the ether quickly evaporating and leaving behind small, round droplets of an oily substance. The plate crystals expected were not seen so the slide was placed aside. Two hours later the slide was again examined under the microscope. In each droplet of oil there was a small, almost perfectly shaped, crystalline asteroid body, some of the larger areas of oil containing several bodies (Fig. 6). In some there was a round, central body from which radiated thin, needle-like spicules, some of the spicules being gracefully curved as in the asteroid bodies seen in the tissue. The melting point of the crystals was found to be 76° to 77° C. (microscopic technic). The crystals were doubly refractile, soluble in ether and xylol, insoluble in cold ethanol, and relatively insoluble in acetone.

Repeated attempts to get similar crystals using similar technics sometimes were successful and sometimes not. Most often needle crystals appeared but did not have the shape of a typical asteroid, many of the crystals branching out unevenly in all directions, forming a tree-like structure. On several occasions, however, typical asteroids were formed. Since these crystals looked so strikingly like the asteroid bodies seen in the giant cells, the asteroid bodies in unstained frozen tissue sections were examined. Some of the same giant cells that contained the plate crystals contained as many as three or four asteroid bodies. The asteroid bodies, however, did not seem to have a crystalline structure, but rather had the appearance of condensed cytoplasm in stained and unstained preparations (Fig. 7). These bodies were not doubly refractile, in contrast to the doubly refractile plate crystals.

Further attempts were made to obtain plate crystals from the ether extract. When the ether had completely evaporated, a light yellow, amorphous material remained. Enough ether was then added to dissolve all of the material completely. Five times that amount of acetone was added and the solution kept at room temperature for evaporation. The

total volume was 20 cc. After 1 week all but 4 cc. of liquid had evaporated, leaving behind small clumps of white crystals which, under the microscope, had the same appearance as the plate crystals dissected from the lung. The crystals were washed with acetone and recrystallized several times in this manner. The crystals had a melting point of 77°C. (microscopic technic) and gave a strongly positive Liebermann-Burchard reaction. The ammonium molybdate-amidol spot test for phosphorus was negative after prolonged hydrolysis of the crystals with hot, concentrated sulfuric acid.

The material was dissolved in carbon disulfide, and the infra-red absorption spectrum was obtained.* The absorption spectrum in the region from 1180 to 750 cm.$^{-1}$ exhibited absorption bands which were not characteristic of those given by cholesterol or cholesterol acetate. The material was recovered and submitted to hydrolysis with a mixture of alcohol, acetone, and normal potassium hydroxide at 50° C. This method would lead to the recovery of free cholesterol, if present as one of the cholesterol esters obtained from blood or tissue. Infra-red analysis of this material gave a spectrum which was different from that given by the original material and also was different from that given by cholesterol. This strongly suggests that the material submitted was neither cholesterol nor a known cholesterol ester.

A drop of the supernatant fluid was placed on a slide. The liquid quickly evaporated, leaving behind small oil droplets. After 1 hour, numerous very small plate crystals having the appearance of the crystals dissected out of the lungs had formed (Fig. 8). A few asteroid crystals were seen also. In one or two instances a single crystal seemed to be forming into both a plate and an asteroid.

Discussion

From the chemical studies it has been impossible to identify the plate crystals. The solubilities seem to indicate that the substance is a lipid, and the strongly positive Liebermann-Burchard reaction, if not due to impurities, would indicate a steroid. The infra-red absorption spectrum before and after hydrolysis was not that of cholesterol or an ester of cholesterol. The best possibility would seem to be that the substance is a steroid but not cholesterol or an ester of cholesterol.

That there is a possible relationship between the plate crystals and the produced asteroid crystals can be seen from the similarities in their properties. They have similar melting points and solubilities. In addition, the two crystal forms were seen to originate in a few instances

* I am indebted to Dr. K. Dobriner and Mrs. P. Humphries for data on the infra-red absorption spectra and their interpretation. The value in the following sentence expresses frequency and not wave length.

from the same nucleus. It is probable that the two types represent two crystal forms of the same substance. Whether these asteroid crystals are related to the asteroid bodies seen in the tissue can only be surmised. A number of substances can crystallize in needle form, producing structures similar to asteroids.[1] However, in this instance it seems highly probable that the same substance that caused the foreign body reaction could also be responsible for the production of the co-existing asteroid bodies, especially since it has been demonstrated that it can crystallize out in such a characteristic manner. It should be remembered, however, that such doubly refractile asteroid crystals are not seen in the asteroid bodies of giant cells when they are viewed in unstained frozen sections. Rather, the asteroid bodies have the appearance of condensed cytoplasm.

Stahel-Stehli [2] reported a case of cholesterol foreign body granuloma of the lung in diabetes mellitus. The lesions pictured were identical with those described here. On the basis of extracting excess cholesterol from the tissues, the author concluded that the crystals in the giant cells were cholesterol. No attempts were made to identify the foreign crystals directly.

Hirsch,[3] in 1935, gave an excellent review of the literature. He was the first to work on the chemistry of the asteroid bodies. He concluded that asteroid bodies are crystalline forms of fats which are solid at body temperature, such as palmitin and stearin, separated from an oil system containing cholesterol or a similar substance. The conclusions were based on the known cholesterol-fat relationship in tissues; on chemical analysis of the spleen containing asteroids and finding excess stearin and palmitin; and on the production in lungs of rabbits of a foreign body reaction to rosette-shaped crystals following intravenous injections of cholesterol-palmitin mixtures. Furthermore, he concluded that the formation of the asteroid crystals is according to the usual rules of crystallization. Chemical changes occur in the tissues so that the crystals are no longer soluble in fat solvents and stain with elastin stains. Friedman,[4] in 1944, concluded that asteroid bodies give no specific staining reaction and have the same staining properties as cytoplasm of endothelial or giant cells. They are not disease-specific since they have been seen in cases of talcum powder granuloma,[5] leprosy,[6] and in the tophi of gout.[6]

Summary

In the material from a patient with pulmonary foreign body giant cell granulomas, plate crystal slits and asteroid bodies were found. The plate crystals have been shown to be a lipid, possibly a steroid, but

even though they gave a positive Liebermann-Burchard reaction, were not cholesterol or an ester of cholesterol. A possible relation between the plate crystals and the asteroid bodies is suggested by observations made in the course of this study.

REFERENCES

1. Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical Physiological Chemistry. The Blakiston Co., Philadelphia, 1947, ed. 12, pp. 90; 125; 253.
2. Stahel-Stehli, J. Cholesterin-Fremdkörper-Granulomatose der Lunge bei Diabetes mellitus. *Virchows Arch. f. path. Anat.*, 1939, 304, 352–370.
3. Hirsch, E. F. Radial inclusions of giant cells. *Arch. Path.*, 1935, 20, 665–682.
4. Friedman, M. Sarcoidosis of the spleen. Report of a case with autopsy and a study of intracellular "asteroid bodies." *Am. J. Path.*, 1944, 20, 621–635.
5. Skavlem, J. H., and Ritterhoff, R. J. Coexistent pulmonary asbestosis and sarcoidosis. *Am. J. Path.*, 1946, 22, 493–517.
6. Personal observations.

DESCRIPTION OF PLATES

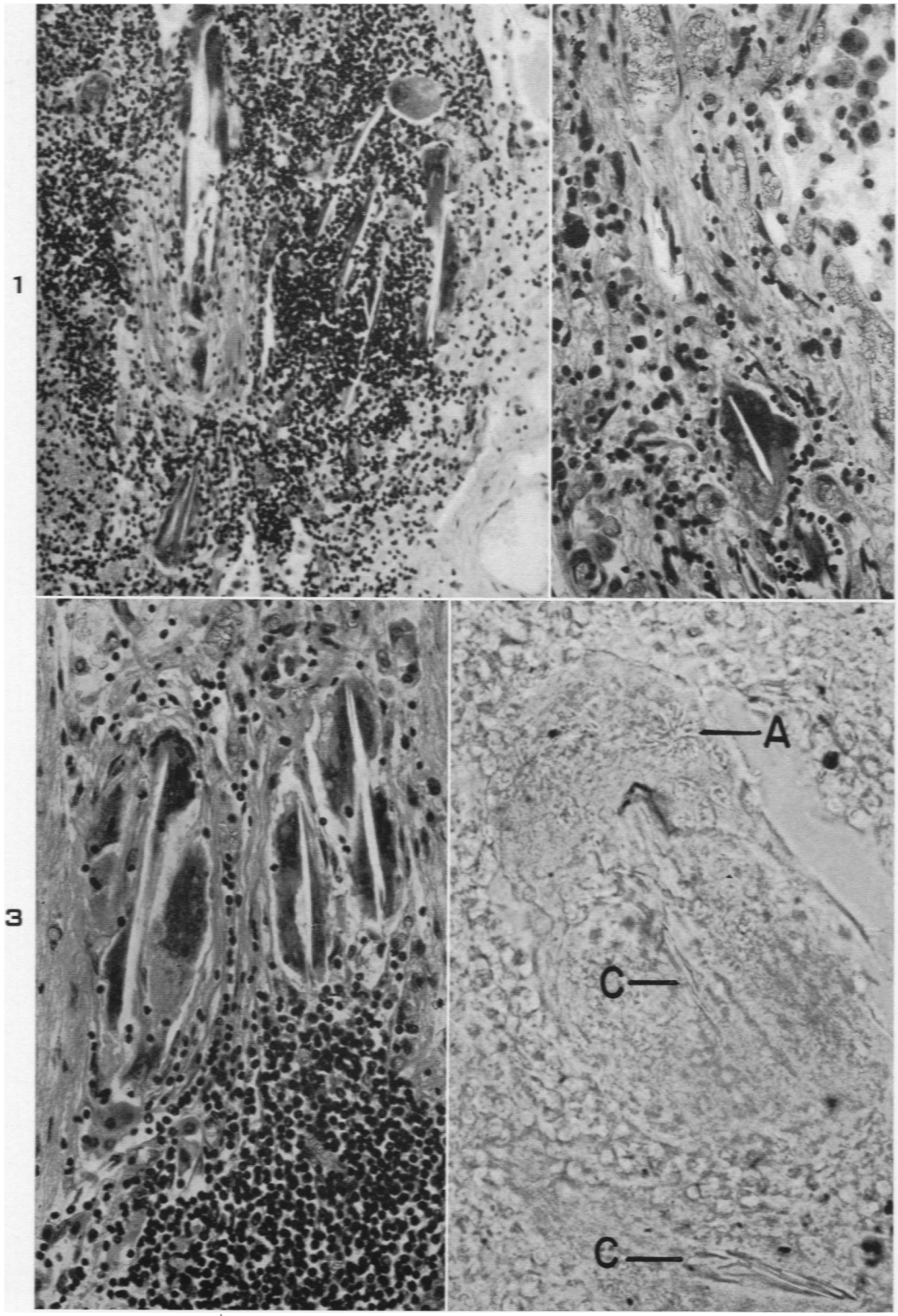
PLATE 54

FIG. 1. Lung, demonstrating foreign body giant cell granuloma with plate crystal slits. Hematoxylin and eosin stain. × 145. (Armed Forces Institute of Pathology Neg. 104567.)

FIG. 2. Lung, demonstrating foreign body giant cell granuloma with plate crystal slit and asteroid body in giant cell. Hematoxylin and eosin stain. × 285. (A.F.I.P. Neg. 103535.)

FIG. 3. Lung, demonstrating foreign body giant cell granuloma with plate crystal slits. Hematoxylin and eosin stain. × 285. (A.F.I.P. Neg. 103536.)

FIG. 4. Lung. Unstained frozen section fixed in formalin, demonstrating crystalline material (c), and asteroid bodies (a) in giant cells. × 450. (A.F.I.P. Neg. 103356.)



Engle

Giant Cell Reaction to Non-Cholesterol Crystals

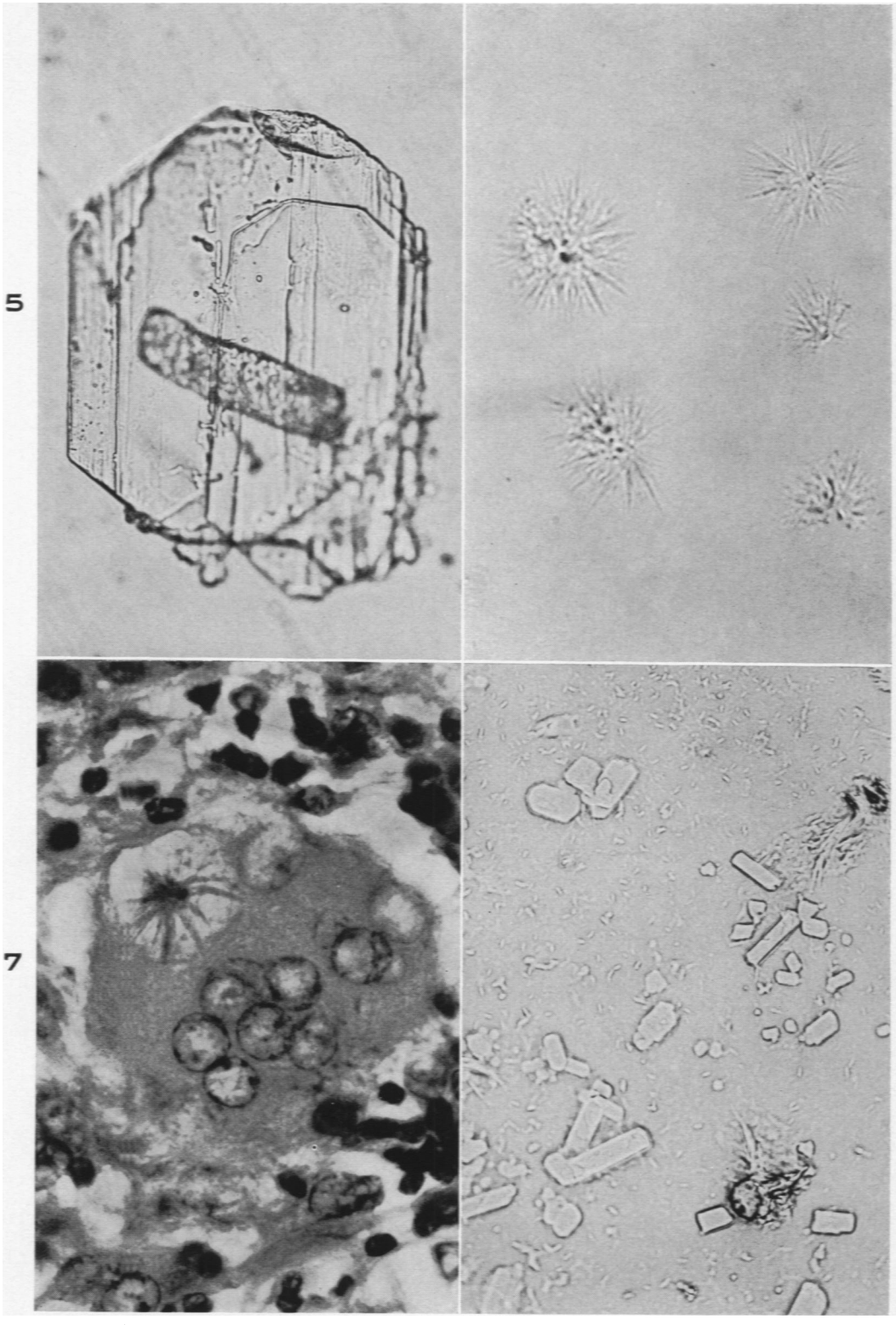
PLATE 55

FIG. 5. Plate crystals dissected from the granulomas in the lung. Unstained. × 315. (A.F.I.P. Neg. 103362.)

FIG. 6. Asteroid crystals obtained by extraction from the tissues of the lung. Unstained. × 450. (A.F.I.P. Neg. 103360.)

FIG. 7. Asteroid body in a giant cell. Hematoxylin and eosin stain. × 900. (A.F.I.P. Neg. 103347.)

FIG. 8. Plate crystals obtained by extraction from the tissues of the lung are similar in shape to the crystals dissected from the lung and pictured in Figure 5. Unstained. × 450. (A.F.I.P. Neg. 103359.)



Engle

Giant Cell Reaction to Non-Cholesterol Crystals