CRYSTALLINE EXCREMENTS IN BRONCHITIS AND CHOLECYSTITIS OF MICE

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Haaland[1] described crystals in mouse tissues in 1905 and Tyzzer[2] photographed crystals in mouse lung in 1909. In 1942, Green[3] described extracellular and intracellular crystals in mouse lungs, in the acinic cells of the pancreas, in the lumens of endometrial glands and in a sarcoma of the thigh. In 1952 Horn, Congdon, Eschenbrenner, Andervont and Stewart[4] noted small crystals in mononuclear cells of pneumonitis in two mice with pulmonary adenomatosis. These crystals were usually noted incidentally and their nature has not been established. We present a histologic and histochemical study of similar crystals found in chronic cholecystitis and bronchitis in 6 of 381 mice utilized in experiments concerned with chemical carcinogenesis.[5,6]

MATERIAL AND METHODS

The experimental mice were ICR Swiss or phenotypically normal pituitary dwarf strain virgin females. Two hundred and seven mice had been treated by 20-methylcholanthrene (MC), 0.5 per cent in acetone in bi-weekly intravaginal applications totalling 0.2 to 1.1 ml. of carcinogen solution. Controls consisted of the same strains; 154 were acetone-treated, 10 were saline-treated and 10 were untreated. The 6 mice with crystals lived full life spans or were sacrificed in the course of long-term observations; in each of them complete necropsy was performed (Table I). Formalin-fixed viscera and lymph nodes of various regions were embedded in paraffin, cut at 3 μ and stained with hematoxylin, phloxine, and saffron (HPS). Paraffin sections containing crystals were stained by various techniques (Table II). Frozen sections of lungs were stained with Sudan III and oil red O for lipids and by the Schultz method for cholesterol and cholesterol esters. Sections of fresh and 10 per cent formalin-fixed tissues, stained and unstained, were examined by polarized, ultraviolet and blue light.

X-ray diffraction and electron microanalysis were not employed in this study. The examination of the crystals by these techniques is intended as a future project.

OBSERVATIONS

Experimental and necropsy data in the 6 mice exhibiting crystals are tabulated (Table I). Lungs in 2 mice were firm and had multiple fine nodularities with gray-white centers and yellow peripheries. Microscopically, bronchioles and bronchi had lymphocytic and plasma cell infiltrates in and about their walls. Peribronchial alveoli were filled by

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TABLE I

SUMMARY OF EXPERIMENTAL DATA AND NECROPSY FINDINGS IN 6 MICE WITH CRYSTALS

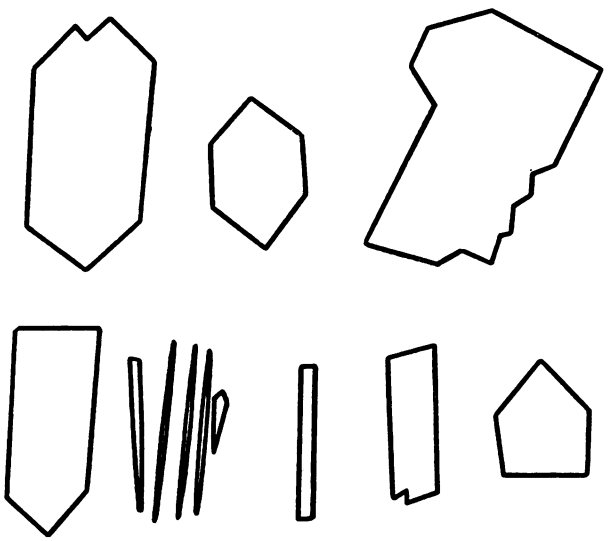
Mouse	Strain	Age (days)	Treatment (vaginal)	Period of observations (days)	Distribution of pulmonary lesions with crystals	Distribution of extrapulmonary lesions with crystals	Accompanying lesions
1	ICR Swiss	340	Acetone 10 appl.	250	Left lung Right lung (mid. and inf. med. lobes)	Gallbladder	None
2	ICR Swiss	310	Acetone 10 appl.	220	Right lung (except inf. lat. lobe) Left lung	None	None
3	Pituitary dwarf	390	Acetone 10 appl.	300	None	Gallbladder	Liver abscess
4	Pituitary dwarf	570	Acetone 10 appl.	480	None	Gallbladder	Bilateral acute bronchopneumonia; suppurative parametritis; pyometria
5	ICR Swiss	490	MC * in acetone 10 appl.	400	None	Gallbladder	Papilloma of vagina; epithelial dysplasia of cervix uteri
6	Pituitary dwarf	767	None	767	None	Gallbladder	Bilateral acute bronchopneumonia; *Klossiella muris* infection of kidneys

* Methylcholanthrene

large, prominently striated histiocytes with abundant acidophilic cytoplasm (Fig. 1). Acidophilic crystals, sometimes in histiocytes, were present in bronchial lumens or peribronchial alveoli (Fig. 2). Some bronchial epithelial cells were nearly twice as large as usual, with brightly acidophilic cytoplasm (Fig. 3). In one mouse, a hilar lymph node contained striated histiocytes and intracellular crystals.

Gallbladders in 3 mice had prominent lymphocytic infiltrates. Marked fibrosis was found in 2 mice. Fibrosed gallbladders had eroded mucosa and lumens packed with acidophilic crystals. Gallbladders with prominent lymphocytic infiltrates contained fewer crystals but had enlarged mucosal epithelial cells with markedly acidophilic cytoplasm, occasionally ballooned as though preparatory to secretion (Figs. 4 and 5).

In accord with Green's observations,[3] the crystals were soft enough to cut in paraffin blocks, were insoluble in alcohol, acetone, ether and xylene, but were soluble in water adjusted to pH 1 to 2 or pH 11 to 12. They were needle- or rod-shaped, rectangular or polygonal (Text-fig. 1).



TEXT-FIG. 1. Outline drawings of crystals to illustrate variety of shapes.

They measured from 10 to 127 μ in length and from 1 to 55 μ in width. Birefringence was slight and, in ultraviolet and blue light, fluorescence was faint. Histologic and histochemical findings are tabulated (Table II, Figs. 6 to 8).

Acidophilic epithelial cytoplasm gave the same histologic and histochemical staining reactions as the crystals. The abnormal cytoplasm did not stain for mucins and reacted in negative manner with benzidine, but

did stain with amidoblack and reacted in positive fashion with the Hartig-Zacharias ferrocyanide technique for protein.[7] Normal bronchial and gallbladder epithelial cytoplasm stained for mucins and was negative with amidoblack and the Hartig-Zacharias technique. Except for positive results with the periodic acid-Schiff reaction and with Gomori's technique for iron, the cytoplasm of histiocytes in crystal-bearing lungs stained the same as the abnormally acidophilic epithelial cytoplasm.

TABLE II

STAINING REACTIONS OF CRYSTALS AND ACIDOPHILIC EPITHELIAL CYTOPLASMS

Staining methods	Results
Alcian blue	Negative
Amidoblack for hemoglobin	Light to dark blue
Aniline blue	Red
Benzidine	Negative
Congo red	Light yellow
Gmelin's reaction for hematoidin	Negative
Gomori's iron reaction	Negative
Hartig-Zacharias ferrocyanide reaction for protein	Blue
Hematoxylin and eosin	Eosinophilic
Hematoxylin, phloxine and saffron	Phloxinophilic
Masson's trichrome	Acid fuchsinophilic
Mayer's mucicarmine	Negative
Methylene blue	Negative
Methyl green-pyronine for DNA and RNA	Pyroninophilic
Methyl violet	Blue-violet
Oil red O	Negative
Periodic acid-Schiff (McManus)	Negative
Phosphotungstic acid-hematoxylin (Mallory's)	Blue-purple
Schultz method for cholesterol and cholesterol esters	Negative
Sudan III	Negative
Toluidine blue	Blue
von Kossa stain for calcium	Negative

The staining reactions of crystals and acidophilic epithelial cytoplasm were identical.

DISCUSSION

The crystals appeared to contain protein,[7] and their pyroninophilia was equated with RNA content.[8] Despite affinity for amidoblack stain,[9] the crystals appeared not to contain hemoglobin, for reaction to benzidine was negative and unstained crystals were colorless.[10] Gmelin's reaction for hematoidin and methylene blue staining for bilirubin were also negative.[11,12] Exposure to methylcholanthrene was evidently unrelated to crystal formation. As a haptene, acetone might lead to production of abnormal crystallizing antibodies, but one of the mice reported here was an untreated control. The crystals were unlike Charcot-Leyden crystals.[3]

The crystals may be products of the abnormal epithelium in the company of which they occur, because the acidophilic cytoplasm of the ab-

normal epithelial cells stains and reacts the same as the crystals with a variety of techniques (Table II). RNA-containing "differentiated secretion" of cultivated chick embryo tissue described by Rose [13] may be an analogous tissue product, and Griffin [14] has demonstrated that similar crystals occur as nitrogenous excretory products in *Chaos chaos* and other large amebas. We postulate that, in chronic inflammations in bronchi or gallbladders in our 6 mice, RNA and protein-containing products of bronchial and gallbladder epithelium crystallized interstitially and intraluminally, and was to some extent phagocytosed by histiocytes. Intra-epithelial inclusion bodies were absent, but the mononuclear inflammatory infiltrates and the positive staining for RNA in abnormal epithelial cells and crystals hint at the occult participation of a virus.

Summary

Crystals apparently containing protein and RNA occurred in chronic bronchitis and cholecystitis of mice. We postulate that the crystals were products of abnormal epithelium.

References

1. Haaland, M. Les tumeurs de la souris. *Ann. Inst. Pasteur,* 1905, 19, 165–201.
2. Tyzzer, E. E. A series of spontaneous tumors in mice with observations on the influence of heredity on the frequency of their occurrence. *J. Med. Res.,* 1909, 21, 479–518.
3. Green, E. U. On the occurrence of crystalline material in the lungs of normal and cancerous Swiss mice. *Cancer Res.,* 1942, 2, 210–217.
4. Horn, H. A.; Congdon, C. C.; Eschenbrenner, A. B.; Andervont, H. B., and Stewart, H. L. Pulmonary adenomatosis in mice. *J. Nat. Cancer Inst.,* 1952, 12, 1297–1315.
5. Yang, Y. H. Dysplasia and carcinoma of the uterine cervix and vagina in mice and rats. A thesis presented to the University of Ottawa in partial fulfilment of the requirements of the degree of Doctor of Philosophy, April, 1963.
6. Yang, Y. H., and Campbell, J. S. Induced dysplasia and carcinoma of the uterine cervix and vagina in mice. (In preparation)
7. Gomori, G. Microscopic Histochemistry, Principles and Practice. University of Chicago Press, Chicago, 1952, p. 112.
8. Culling, C. F. A. Handbook of Histopathological Technique. Butterworth & Co., London, 1957, pp. 197–199.
9. Puchtler, H., and Sweat, F. Amidoblack as a stain for hemoglobin. *Arch. Path.,* 1962, 73, 245–249.
10. Karsner, H. T. Human Pathology. J. B. Lippincott Co., Philadelphia, 1955, ed. 8, p. 29.
11. Culling, C. F. A. Handbook of Histopathological Technique. Butterworth & Co., London, 1957, pp. 267–268.
12. Pearse, A. G. E. Histochemistry, Theoretical and Applied. J. & A. Churchill, Ltd., London, 1953, pp. 358–359.
13. Rose, G. G. Biologic crystals and particles produced in tissue culture. *Cancer Res.,* 1963, 23, 279–284.

14. GRIFFIN, J. L. Quoted by ALLEN, R. D. Amoeboid Movement. In: The Cell; Biochemistry, Physiology, Morphology. BRACHET, J., and MIRSKY, A. E. (eds.). Academic Press, Inc., New York and London, 1961, Vol. 2, Chapt. 3, pp. 135–216.

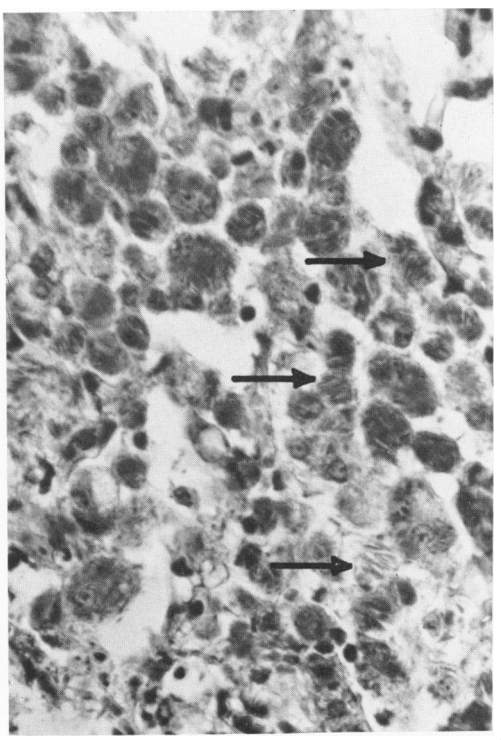
LEGENDS FOR FIGURES

FIG. 1. Aggregations of histiocytes in pulmonary alveoli. The needle-shaped striations in the cytoplasm of histiocytes are demonstrated. Mallory's phosphotungstic acid hematoxylin stain. × 620.

FIG. 2. Crystals and histiocytes in the lung. Both crystals and histiocytes are deeply stained with phloxine. Hematoxylin, phloxine and saffron stain. × 225.

FIG. 3. Acidophilic bronchial epithelium and crystals in a bronchial lumen. Some bronchial epithelial cells are filled with acidophilic material which gives staining reactions identical to those of crystals. Arrows indicate crystals and bronchial epithelium with abnormal cytoplasm. The bronchial wall is infiltrated by lymphocytes and plasma cells. Aniline blue stain. × 180.

FIG. 4. Crystals in the lumen of a gallbladder. Many crystals are stained intensely (red). Mallory's aniline blue stain. × 65.



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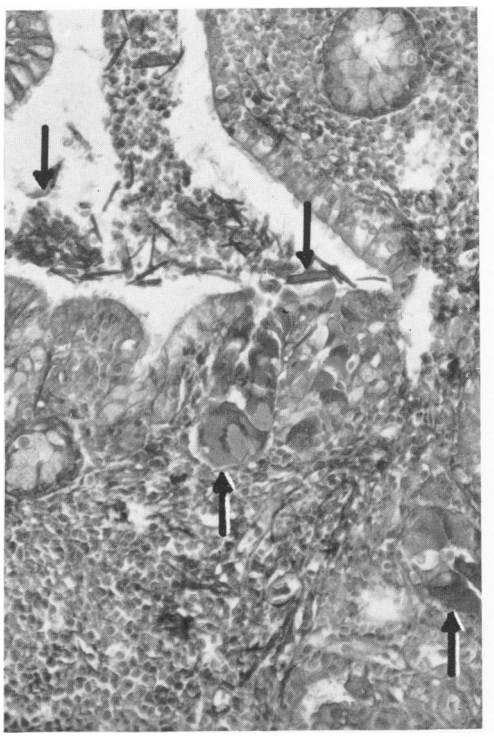

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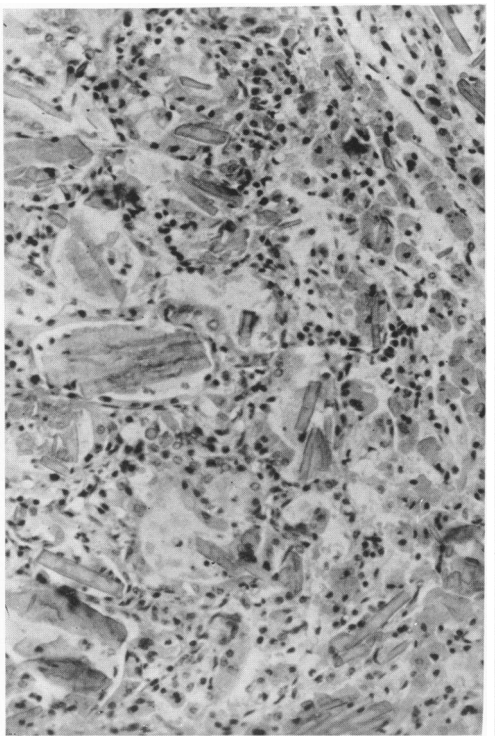

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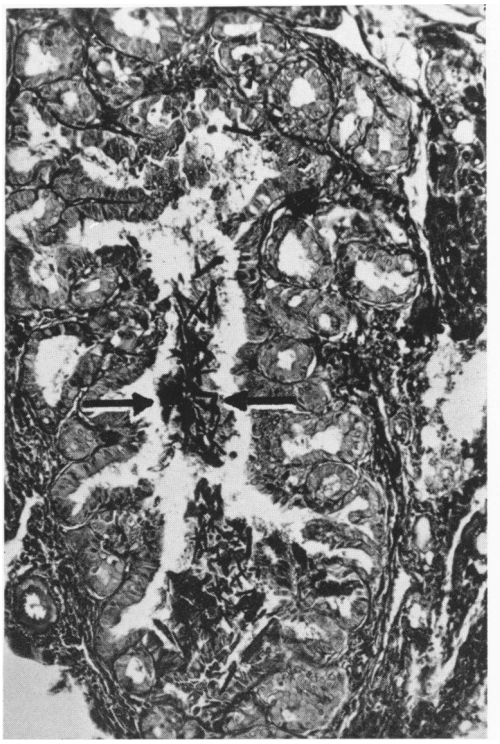

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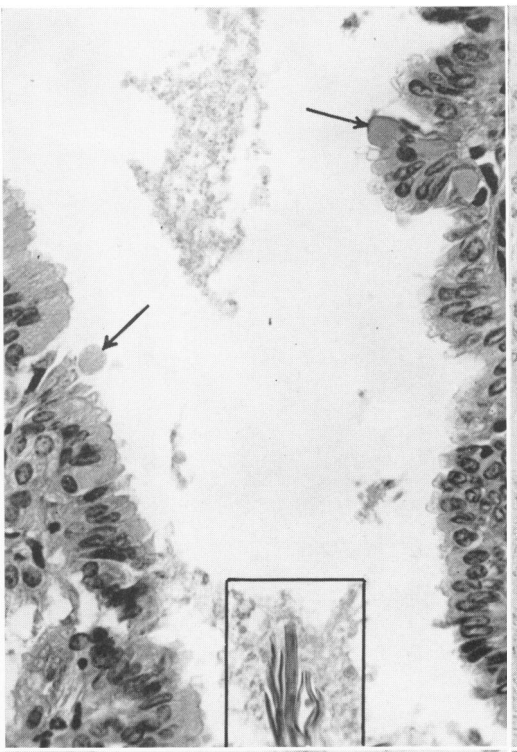
FIG. 5. Ballooning of gallbladder epithelium (arrows). Crystals in the lumen (enclosed in square) are seen on the bottom. Hematoxylin, phloxine and saffron stain. × 370.

FIG. 6. Crystals are stained with pyronine, a finding equated with RNA content. Methyl green-pyronine stain. × 180.

FIG. 7. Crystals and histiocytes in the lung. A positive iron reaction in histiocytes is shown at the upper right corner while crystals in the lower left corner of the field do not react. Gomori's technique for iron. × 225.

FIG. 8. Crystals and bronchial epithelium give a positive reaction for protein by the ferrocyanide method of Hartig-Zacharias.[7] × 180.



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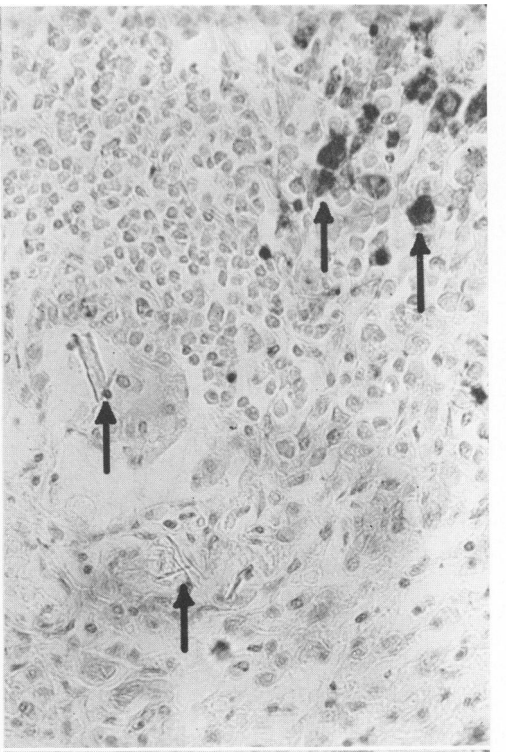

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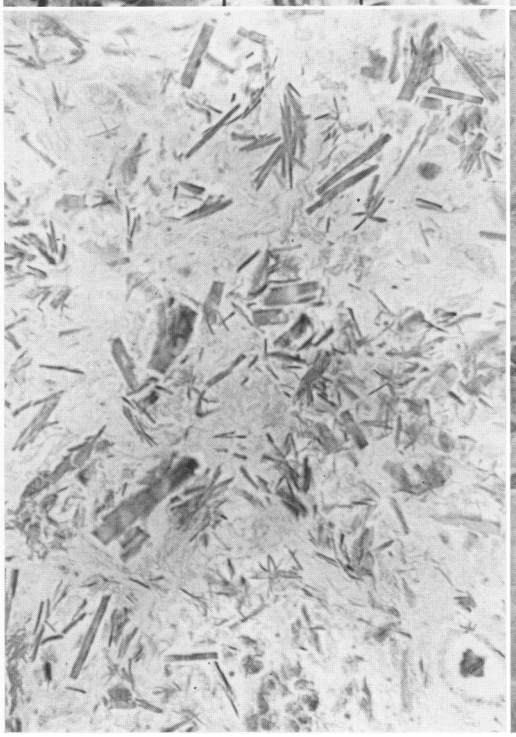

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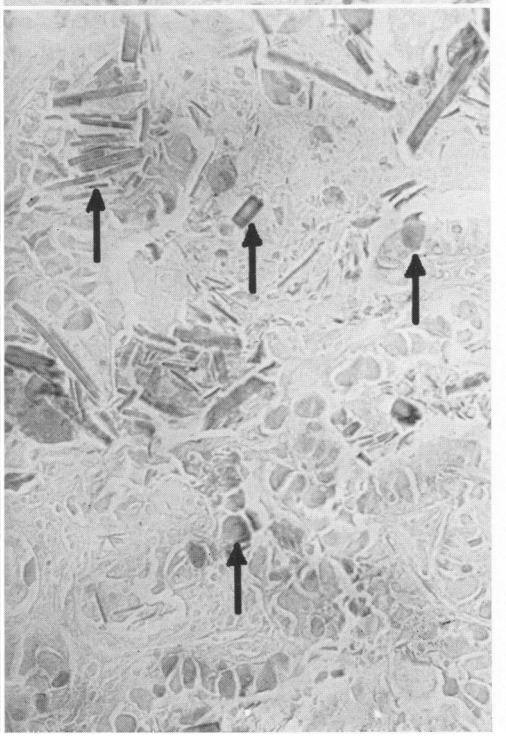

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