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# ULTRASTRUCTURAL STUDY OF LEUCOCYTES AND URATES IN GOUTY ARTHRITIS

BY

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The demonstration of urate crystals in synovial fluid is pathognomonic for gout. Hollander (1960) first noted the presence of extracellular crystals of sodium biurate in the synovia of patients with acute gout. McCarty (1962) pointed out that urate crystals were also contained within the exudative leucocytes in gouty arthritis, and Zvaifler and Pekin (1963) confirmed these findings. Certain identifying characteristics of these urate crystals have been described by McCarty and Hollander (1961). They were digested by uricase and the shape of the urate crystal found in synovial fluids was reported to be a short rod with parallel sides and rounded ends. In addition, these crystals exhibited a negative birefrigence with polarizing light microscopy.

The morphological features of the emigrated leucocytes and their associated urate crystals in gout have been studied by light, phase, and polarizing microscopy; it is the purpose of this paper to describe the fine structure of the exudative leucocytes and urate crystals.

## Material and Methods

Selected clinical findings in our series of eleven patients (ten men and one woman) with acute gouty arthritis are summarized in the Table. A patient with gout was selected for study if the knee joint was the site of acute arthritis. The ages of the patients studied ranged from 43 to 73 years (mean 51; median 49). Eight male subjects were of the Caucasian race, Case 11 was Negro, and Case 7 was Filipino. The only female (Case 4) was premenopausal and exhibited recurrent, episodic arthritis over a 10-year period. A family history of gout could be documented only in Case 4. Tophaceous gout was evident in only three subjects (Cases 3, 7, and 10). Case 5 was studied during the initial attack of his gouty arthritis, but all the others had experienced their first attack 4 to 17 years before the investigative arthrocentesis. Cases 2, 7, 8, and 10 were hypertensive and had experienced acute attacks of gouty arthritis over a period of 10 to 17 years, two of them (Cases 7 and 10) being in the chronic tophaceous stage. Renal claculi were absent by history and intravenous pyelography in all subjects. Blood urea nitrogen determinations were uniformly within normal limits, but Cases 2 and 9 exhibited albuminuria at the time of investigation.

### Arthrocentesis and Synovianalysis

The synovial fluid was aspirated through an 18-gauge needle and eighteen synovial fluid samples were obtained from the eleven patients studied. Serial samples were collected from the same knee of one patient (Case 1) over a 3-day period during the course of the inflammatory reaction. Both knees were aspirated in five patients, either at short intervals from the onset of exudation or as long as one year after an acute attack of gout. Single samples of synovial fluid were obtained during the acute attack of gouty arthritis in the other five subjects.

Immediately after the aspiration, 9 parts of synovial fluid were mixed thoroughly with one part of 3·8 per cent. sodium citrate to prevent the sample from clotting. The total volume aspirated was recorded and a while blood cell count was performed. A drop of synovial fluid was examined by polarizing microscopy for the identification of intracellular and extracellular urate crystals. The

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TABLE
CLINICAL CHARACTERISTICS OF 11 PATIENTS

| Patient No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | M | M | M | M | M | M | M | M | M | M | F |
| Age (yrs) | 51 | 55 | 49 | 46 | 46 | 46 | 57 | 53 | 74 | 47 | 43 |
| Gout* | I | I | T | I | F | I | T | I | I | T | I |
| Duration of Gout (yrs) | 5 | 17 | 4 | 5 | <1 | 5 | 15 | 16 | 17 | 10 | 10 |
| Hypertension | 0 | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 |
| Albuminuria | − | 1+ | − | − | − | − | − | − | tr | − | − |

* I = Intermittent Arthritis  T = Chronic Tophaceous  F = First Attack

percentage of leucocytes containing crystals was noted. Synovial fluid samples were separated into their fluid and cellular compartments by centrifugation in a table model international Clinical Centrifuge for 10 min. at 1,000 r.p.m. A portion of the concentrated leucocytes was processed immediately for electron microscopy. The remaining concentrated leucocytes were divided and utilized for an *in vitro* viability study (Wintrobe, 1961), observed in a wet mount with phase-fluorescence microscopy and smeared onto glass slides. One of the smears of concentrated leucocytes was processed with Leishman's stain and a differential count of 500 leucocytes was performed.

### Electron Microscopy

The concentrated leucocytes were fixed with either 6·5 per cent. buffered glutaraldehyde (Sabatini, Miller, and Barnett, 1964) for 2 hrs or 1 per cent. buffered osmium tetroxide (Palade, 1952) for 1 hr at 4° C. After fixation the leucocytes were concentrated by centrifugation. Glutaraldehyde-treated leucocytes were stored in 0·2 M. sucrose while leucocytes fixed with osmium were placed in 95 per cent. alcohol. After osmium fixation or after gluteraldehyde fixed leucocytes were stained with osmium, the leucocytes were reconcentrated and mixed with 3 per cent. agar. The agar-leucocyte preparation was next cut into small blocks which were dehydrated with alcohol and propylene oxide. Final embedding was accomplished in Maraglas 655. Thin sections were cut with a diamond knife on either a Porter-Blum MT-1 or MT-2 ultramicrotome. Sections were stained doubly with uranyl acetate (Watson, 1958) and potassium permanganate (Pease, 1964). An RCA EMU 3-H electron microscope operated at 50 kV was used to examine and photograph our material.

## Findings

### Light Microscopy

Leucocyte counts ranged from 500 to 32,950 per mm.$^3$ reflecting the degree of inflammation. In each synovial fluid, the cellular reaction was characterized by a predominance of exudative neutrophils and the number of neutrophils present per 500 leucocytes counted was consistently greater than 66 per cent. Neutral red preparations demonstrated that from 85 to 100 per cent. of the exudative neutrophils were viable. Interestingly, the percentage of neutrophils with pyknotic nuclear lobes counted in Leishman-stained smears of the concentrated leucocytes did not parallel the percentage of nonviable neutrophils as determined by the nuclear exclusion of neutral red. Such findings emphasize the separation which may exist between morphological and physiological criteria of leucocytic function. These findings examplify the value of applying various methods to assess a single parameter when leucocytes are involved in a dynamic process such as the acute inflammatory reaction.

Varying percentages of lymphocytes, hypertrophied lymphocytes, monocytes, and macrophages constituted the mononuclear leucocyte population. Phagocytic macrophages frequently contained single or several whole pyknotic neutrophils as well as cytoplasmic buds which were shed from other exudative neutrophils. The nuclear lobes of the ingested neutrophils were stained dark red in the viability test, which suggested that they were nonviable. No eosinophils or basophils were observed in the differential count from any of the concentrated samples of these exudative leucocytes. A few synovial lining cells were intermixed with the population of leucocytes in three of the eighteen smears examined. Intracellular crystals were observed in from 7 to 100 per cent. of both neutrophils and mononuclear leucocytes. The distribution of the intracellular urate crystals within a leucocyte population was uneven; that is, some leucocytes contained no crystals whereas other adjacent leucocytes exhibited single or several crystals or were gorged with numerous crystals.

### Phase-Fluorescence Microscopy

Fresh, wet preparations of some of the concentrated leucocyte populations were examined. The intracellular and extracellular crystals of sodium urate were well delineated. The crystal was characterized by a dark rim which outlined a brightly fluorescent interior (Fig. 1, opposite). The colour of the crystal edge varied depending upon the combinations of exciter and barrier filters employed.

Some exudative neutrophils were distinctive in that their cytoplasm contained numerous defined areas of light transmission which gave the cytoplasm a mottled or "honeycomb" appearance (Fig. 2, opposite). These cytoplasmic areas did not convey the impression that they were bounded by a membrane.

### Fine Structure of the Exudative Neutrophil

#### Surface Features

The surface of the exudative neutrophil presented several forms. The contour of some neutrophils was smooth, whereas the outline of other neutrophils was irregular. By examining large numbers of serial sections, we observed morphological features which suggested that these exudative neutrophils were engaged in the processes of pinocytosis and micropinocytosis. Short single pseudopods jutted from the neutrophil and formed hooks whose tips eventually fused with the plasma membrane. Also parallel pairs of pseudopodia were extended by the neutrophil and joined by membrane fusion at their

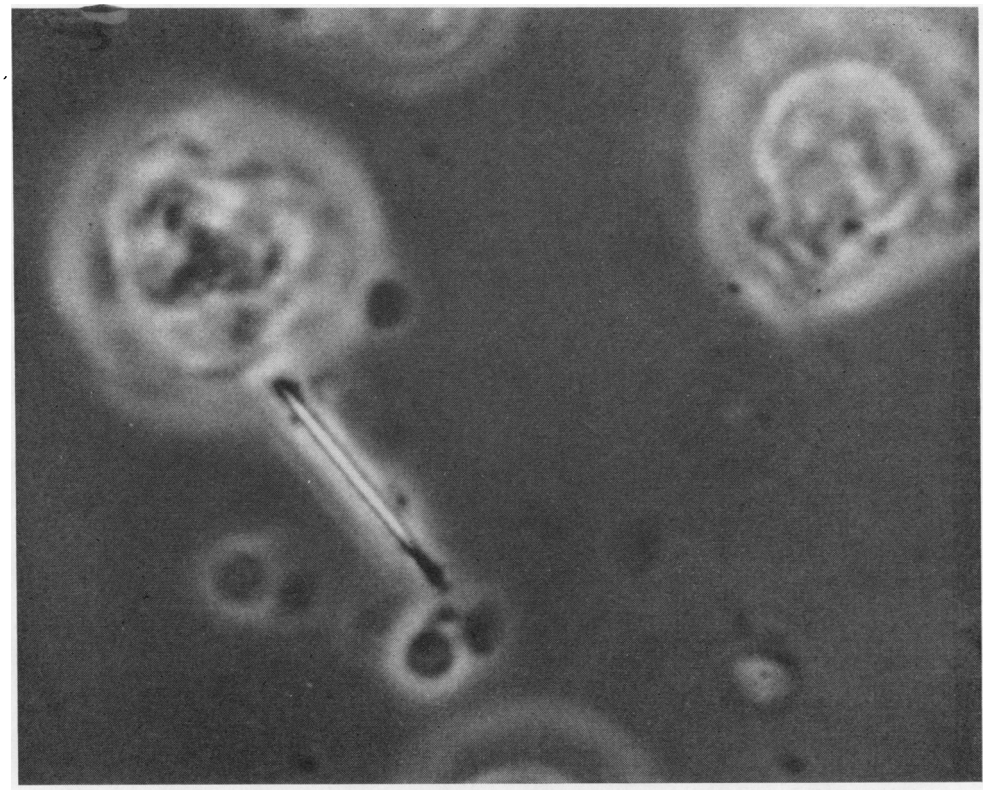

Fig. 1.—Urate crystals in gouty synovial fluid revealed a bright interior and were outlined by a darker edge when viewed by phasefluorescence microscopy. ×2,500

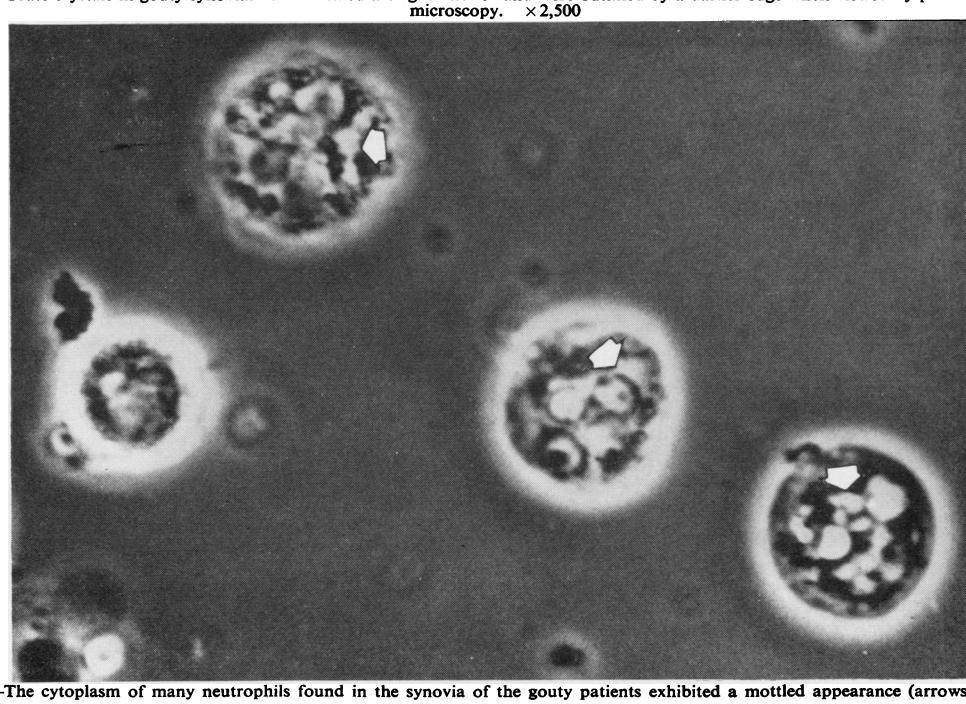

Fig. 2.—The cytoplasm of many neutrophils found in the synovia of the gouty patients exhibited a mottled appearance (arrows). ×2,000

tips. Rarely, minute invaginations of the plasma membrane were observed and formed smooth-contoured vesicular inpocketings. Numerous vesicles of a similar size were distributed throughout the cytoplasm of many of the exudative neutrophils and in some instances were concentrated centrally. Although it was not possible to trace sequentially the formation and detachment of these vesicles, the cytoplasm of exudative neutrophils from our patients with gouty arthritis contained many more vesicular elements than their counterparts in the peripheral blood. It appeared therefore, that the membranes of these exudative neutrophils were stimulated and participated in the uptake of small amounts of the synovial fluid with its dissolved solutes.

**Nucleus**

The nucleus in the majority of the exudative neutrophils was typical and exhibited a single lobe or several lobes depending on the plane of section. Each nuclear lobe exhibited peripheral as well as central chromatin condensation. The nuclear characteristics were compatible with a functional neutrophil and these observations correlated well with our viability studies. However, an occasional neutrophil contained a single lobe or several nuclear lobes which were filled with a homogeneous dense material, a pattern characteristic of the pyknotic alteration. This ultrastructural finding parallels our light microscopic finding of a few pyknotic neutrophils in the stained smears of the concentrated leukocytes. Nuclear pores and a nuclear membrane were visible.

**Cytoplasm**

The cytoplasm of the exudative neutrophils contained a number of components and demonstrated certain interrelationships between structural elements.

*Electron-Lucent Spaces.*—As mentioned previously, the cytoplasm of some of these exudative neutrophils appeared "honeycombed". In ultrathin sections we observed that this mottled effect was created when the moderately dense cytoplasmic matrix contained numerous spaces which varied in size and shape (Fig. 3).

These spaces were electron-lucent, contained widely dispersed particles of low electron density and were not limited by a membrane. Neutrophil granules were associated frequently with the outer margins of the electron-lucent areas. This striking cytoplasmic architecture was easily discernible in leucocytes fixed with glutaraldehyde. However in the same population of leucocytes fixed with buffered

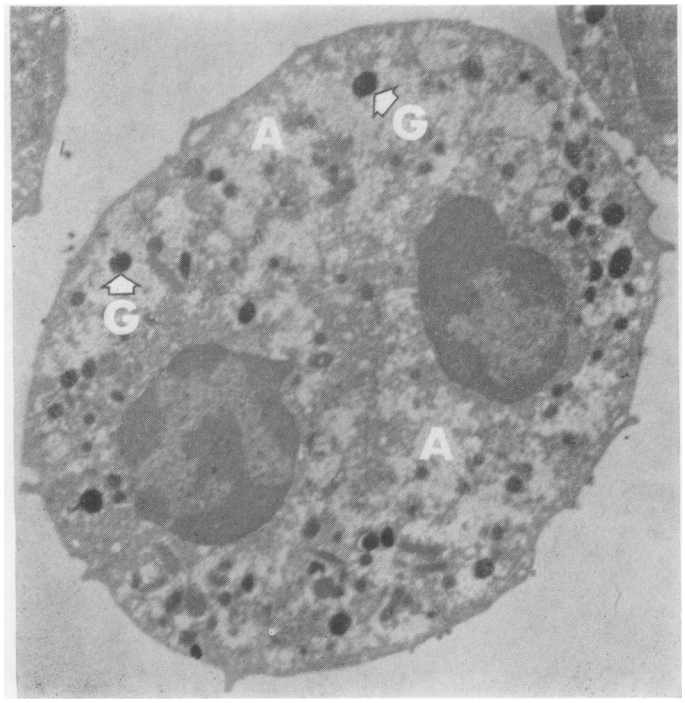


Fig. 3.—In ultrathin sections, the mottled, cytoplasmic architecture was related to electron-lucent areas (A) which were distributed throughout the more dense cytoplasmic matrix. Neutrophil granules (G) were associated with these areas. ×7,360

osmium, the "honeycombed" appearance although present was much more subtle (Fig. 4). This finding may relate to a basic difference between the properties of osmium and glutaraldehyde fixatives, since it is recognized that many substances are extracted from the cytoplasmic matrix of cells after osmium fixation. If this extraction of the neutrophils' cytoplasm occurred, it would be more difficult to delimit the electron-lucent spaces in a cytoplasmic matrix with a decreased density.

*Urate Crystals.*—The outlines of urate crystals contained within neutrophils and mononuclear leucocytes or observed extracellularly were essentially of three shapes. Some crystals were long with parallel sides and ends which tapered to a fine point. Other crystals were rod-shaped with two blunt ends. A third type of crystal had parallel sides with one blunt end while the other end was needle-like. The interior of the crystal was either electron-transparent, showed a substructure, or exhibited a density which was identical with that of the surrounding embedding media. Since the intracellular urate crystals did not usually appear electron dense, we surmised that the crystal was not present in the ultrathin section when it was viewed in the electron microscope. It seemed possible that the crystal might have been fragmented and dislodged during the sectioning step; however, the cytoplasmic detail immediately adjacent to the intracellular urate crystal was never observed to be damaged. In addition, the crystal occasionally contained substructure. Moreover, the contents of neutrophil granules frequently aligned the outer edge of crystals which lay free in the cytoplasm or were contained within membrane-bound vacuoles. The preservation of these minute details seemed to minimize the possibility of mechanical displacement of the crystal. Next we considered that perhaps the urate crystals were dissolved as the ultrathin sections were floated on the water bath before being mounted on the grid. This was a partial explanation for a few electron dense urate crystals were present in ultrathin sections floated on a water bath saturated with uric acid. However, the majority of the urate crystal images still remained electron transparent. Finally, we investigated the possibility that these biological crystals were electron-lucent. When we viewed whole urate crystals supported on a carbon film, we found that the urate crystals did vary in their degree of electron density (Fig. 5A, overleaf).

Furthermore, as the intensity of the electron beam was increased, the internal structure of the crystal was altered (Fig. 5B, overleaf).

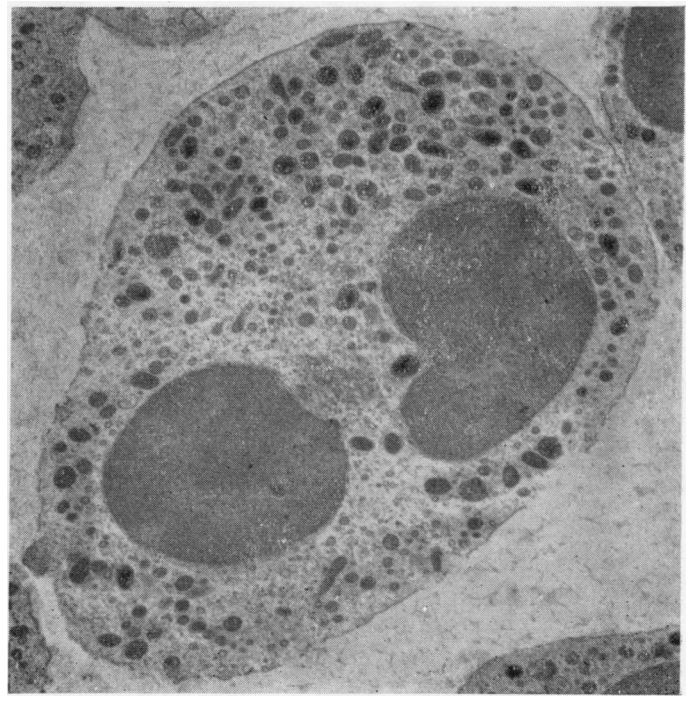

Fig. 4.—The electron-lucent areas were less well delimitated in the same neutrophil population fixed with buffered osmium tetroxide. ×6,550

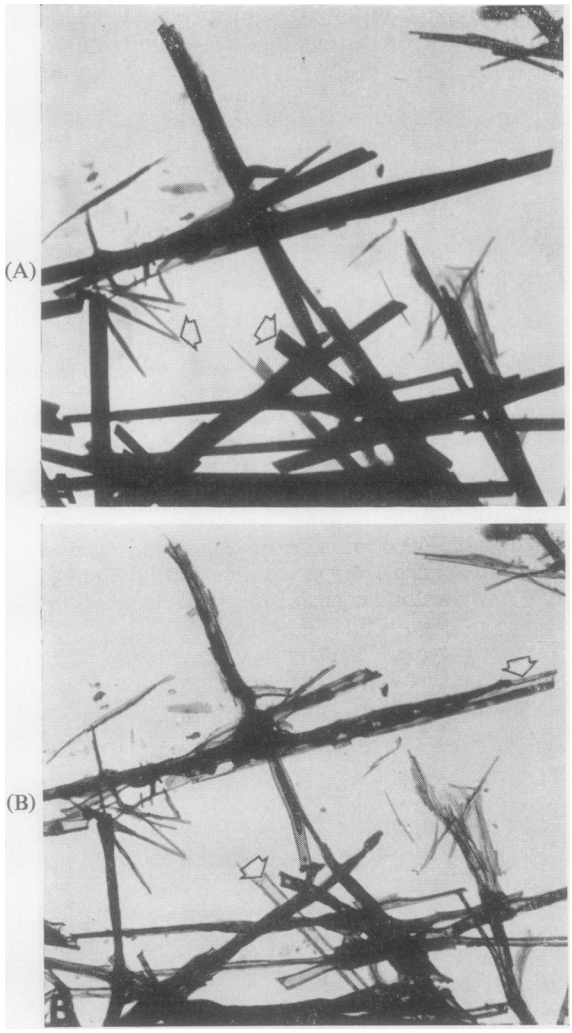


Fig. 5.—Whole urate crystals exhibited variable densities (arrows) when they were viewed in the electron microscope (A). These urate crystals were altered (arrows) after intens.ve bombardment with the electron beam (B). ×7,660

The degree of density was decreased and certain urate crystals showed a substructure within their interior. The crystal outline was unchanged and remained stable during the bombardment with electrons. The appearance of the altered whole crystal was similar to the appearance of the crystals observed in our ultrathin sections.

Since the outline of the urate crystals was not modified by either dissolution or alteration by the electron beam, the linear and width dimensions of 108 crystals were measured to gather additional information about the morphology of these crystals associated with gouty arthritis. The crystal at its greatest breadth averaged 0·17$\mu$ and ranged from 0·03 to 1·98$\mu$. The length of the urate crystal was variable and averaged 1·37$\mu$ with a range from 0·12

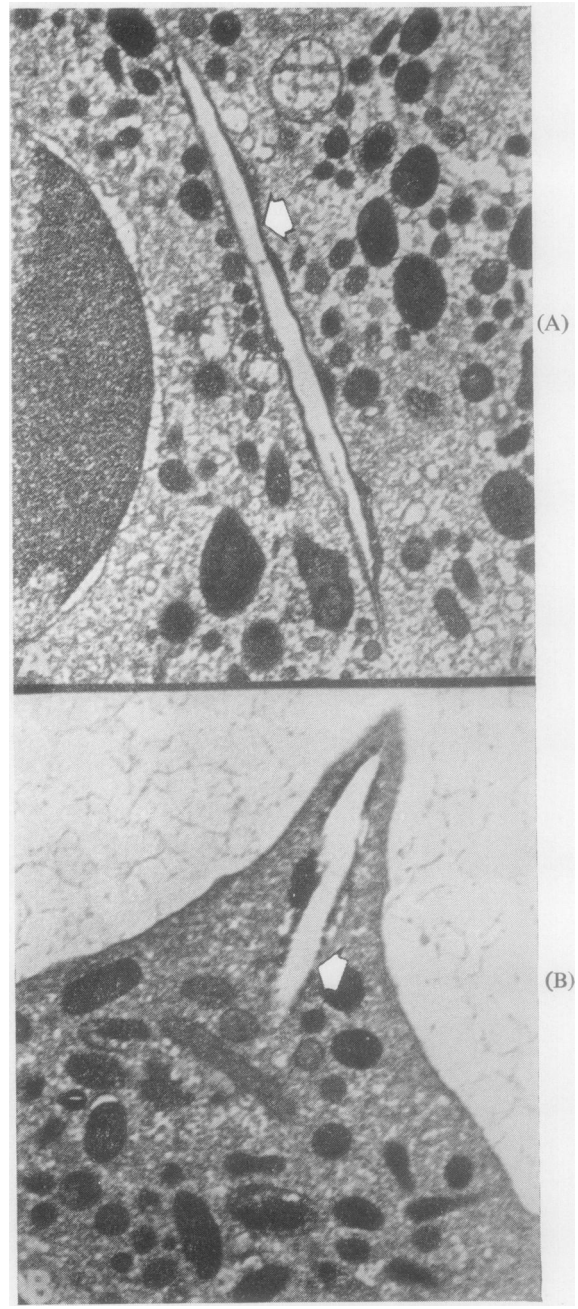


Fig. 6.—Other urate crystals within neutrophils were lined with a dense linear structure which was continuous (A) along the crystalline surface (arrow) or was interrupted (arrow) at intervals (B). ×12,000 and 22,500

to 34·44$\mu$. The surface of intracellular and extracellular urate crystals was outlined frequently by a dense line which had an average width of 0·026$\mu$. This structure varied in width from 0·045 to 0·010$\mu$ and was either continuous (Fig. 6A) or interrupted (Fig. 6B). In the latter case, the surface of the crystal had a beaded appearance. The most striking feature, particularly of the crystals contained within the exudative neutrophil, was that the crystal was not membrane-bounded but appeared to lie in contact with the cytoplasm (Fig. 7). This observation was valid even when serial sections of the neutrophil were examined. Urate crystals in neutrophils were found rarely within a phagosome (Fig. 8, overleaf).

*Neutrophil Granules.*—These were positioned adjacent to crystals lying free within the neutrophils' cytoplasm (Fig. 6*b*) and sometimes their contents were distributed along the outer surface of the urate crystal (Fig. 8). Intact granules were also adjacent to the phagosomal membrane. The numbers of granules present per neutrophil were decreased, although the amount of degranulation varied with different neutrophil populations.

*Vacuoles.*—Large membrane-limited vacuoles containing a homogenous material of varying density from light to medium grey were observed in the cytoplasm of these exudative neutrophils (Fig. 8). In addition, a rare autophagic vacuole was found. These membrane-limited structures probably explain our light microscopic finding of a single or several spherical refractile, cytoplasmic inclusions in 4 to 46 per cent. of the exudative neutrophils when a wet preparation was viewed with phase microscopy.

**Mononuclear Leucocytes**

The mononuclear leucocytes in these synovial fluids from gouty arthritis were either lymphocytes, hypertrophied lymphocytes, or macrophages. Crystals with the three shapes described above were contained within the cytoplasm of the phagocytic mononuclear leucocytes (Fig. 9, overleaf). In addition, nonviable whole neutrophils, partially degraded neutrophils, and numerous crystal-containing shed cytoplasmic buds from exudative neutrophils were also phagocytosed by the hypertrophied lymphocytes and macrophages (Fig. 10, overleaf).

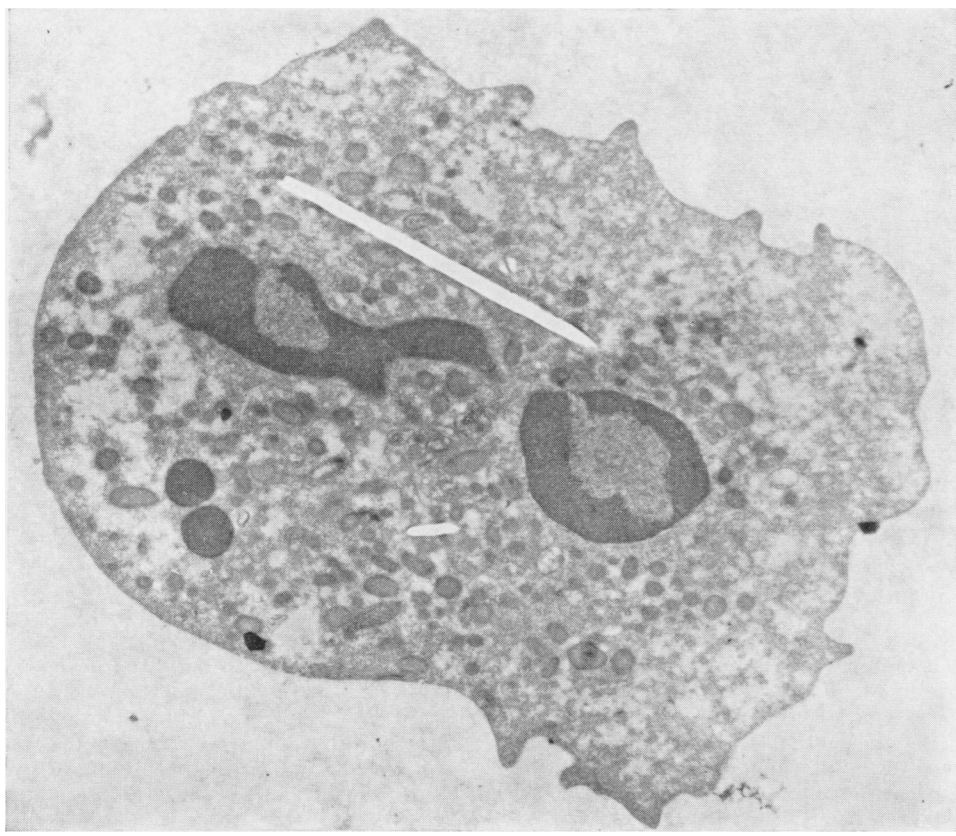

Fig. 7.—Numerous urate crystals in neutrophils were devoid of a limiting membrane and appeared to lie in direct contact with the cytoplasm. Note that other membrane systems such as the golgicomplex are observed easily in this micrograph. ×11,800.

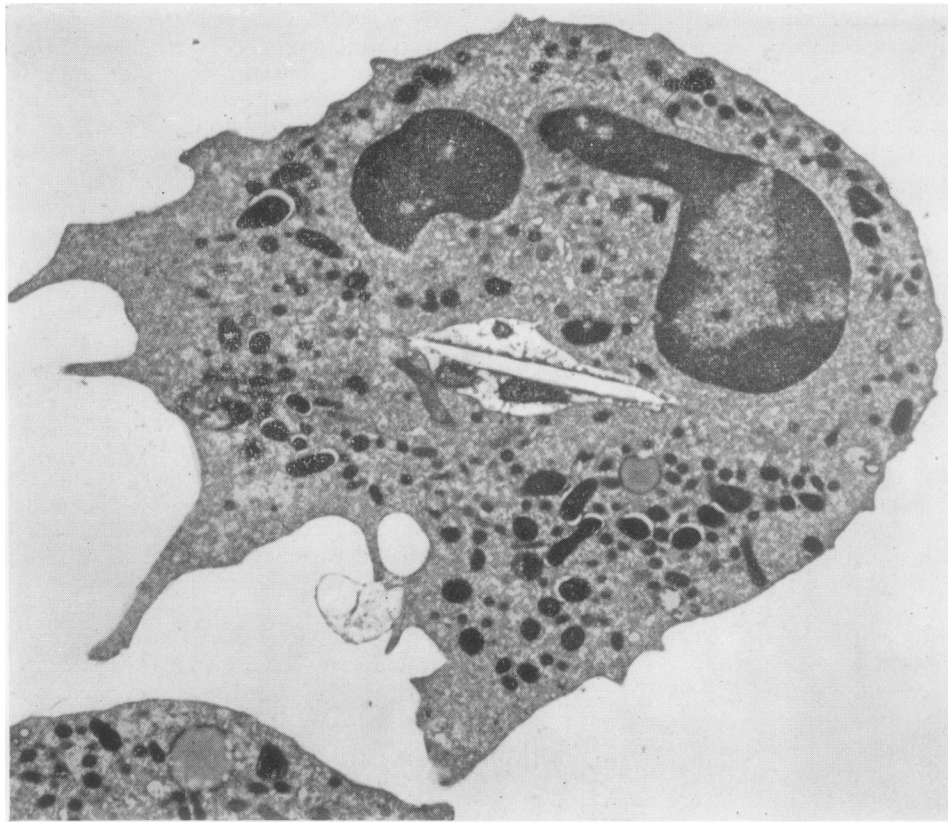

Fig. 8.—A few urate crystals within exudative neutrophils were contained within a typical phagosome. ×12,000.

## Discussion

Urate crystals and emigrated leucocytes are recognized as associated components in synovial fluid during episodic attacks of gout. The capacity of synthetic crystalline urates to incite an inflammatory response when introduced by either subcutaneous (Freudweiler, 1899) or intra-articular routes (Faires and McCarty, 1961; Seegmiller, Howell and Malawista, 1962) has also been documented. The magnitude of the inflammatory response correlated not only with the numbers of crystals injected but also with their size and the degree of aggregation. Synthetic microcrystalline sodium urate prepared by the method of Seegmiller, and others (1962) induced inflammation. These crystals were needle-shaped and varied in length from 0·5 to 8·0$\mu$ (Seegmiller, 1965). Conversely, amorphous sodium urate was a smaller particle with a length of less than 1$\mu$ (Kellermeyer and Breckenridge, 1965) and was not capable of provoking recognizable signs of inflammation. Comparable information regarding urate crystals in synovial fluids from patients with gout has been reported by McCarty, Gatter, Brill, and Hogan (1965). These authors stated that the crystals were composed of monosodium urate monohydrate. They were found either in a monoclinic or triclinic form, appeared as rods or needle-shaped crystals, and ranged in size from 0·5 to 20$\mu$ (approximate mean 8 to 10$\mu$). We studied the various crystal planes in ultrathin sections and derived additional information about the forms of uric acid which crystallized in gouty arthritis. Our studies yielded the average width dimension of the crystals, further defined their shapes, established a calculated linear measurement, and demonstrated that no specific crystalline shape or size was associated more frequently with either neutrophils or mononuclear leucocytes. We observed also that the needle-shaped urate crystals were the longest intracellular forms.

The general morphology of the urate crystals found in the synovia and within the leucocytes during attacks of gout is therefore appreciated; however, no definitive information outlining the origin of these crystals is known. The hypothesis suggested by Seegmiller, Laster, and Howell (1963) proposes that sodium urate crystals are precipitated from

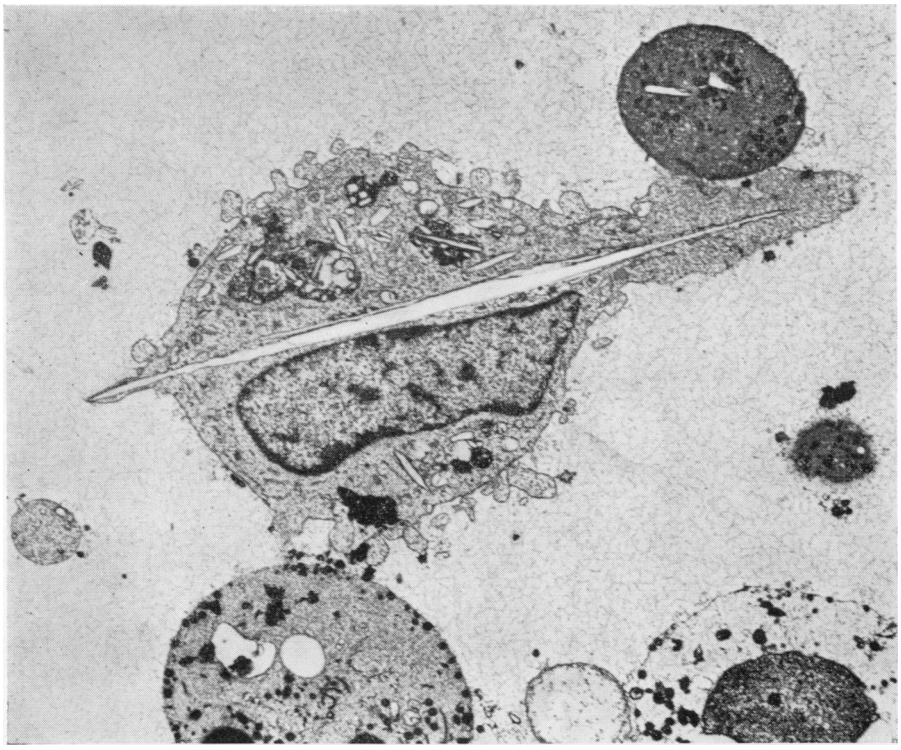

Fig. 9.—Phagocytic macrophages present in the synovia of the gouty patients contained urate crystals and crystal-containing cytoplasmic buds which were shed from the associated exudative neutrophils. ×3,100.

hyperuricaemic body fluids and are phagocytosed by exudative leucocytes. These authors presented a cyclic scheme in which phagocytosis promoted increased lactic acid production, which in turn lowered the local extracellular pH and caused further crystallization of sodium urate from the surrounding fluids. This hypothesis, although attractive, leaves several basic questions unanswered. Where is the first urate crystal formed? Why do some exudative leucocytes contain a single crystal or several urate crystals, while a neighbouring leucocyte is literally stuffed with crystals of various sizes? One possible answer may be that the urate crystal is assembled at an intracellular site.

Information regarding the morphologic characteristics of crystals formed within the cytoplasm of cells is scanty since there are few examples of this process in humans. However, the transformation of eosinophil granula into Charcot-Leyden crystals does provide one example of intracellular crystal formation (Welsh, 1959) within a type of leucocyte. The Charcot-Leyden crystals formed as the denser portions of the eosinophil granules fused. As crystallization proceeded, the surrounding membranes of the granules ruptured and the Charcot-Leyden crystals formed were devoid of a limiting membrane. We observed that many of the urate crystals found within the cytoplasm, particularly of the exudative neutrophils, were also devoid of a limiting membrane.

Perhaps then we should examine more carefully the accepted idea that urate crystals are formed extracellularly from a synovial fluid primed with an increased amount of uric acid. It may be timely to consider the possibility that the cytoplasm of the exudative neutrophil is the locus of crystal formation. It is possible that leucocytes exposed to a uric acid-rich synovial fluid may sequester the available urate *via* endocytosis (pinocytosis and/or phagocytosis) and thus accumulate a hyperuricaemic internal environment. Increased metabolic activity, prolonged exposure to the synovial fluid environment, and ageing of the neutrophil may promote a decreased intracellular pH, thereby favouring aggregation and crystallization of the accumulated urate. Electolyte shifts which accompany the inflammatory process may also be important, since it is known that the solubility of uric acid is inversely related to the concentration of sodium ion (Allen, Milosovich, and Mattocks, 1965). A urate crystal formed in this manner, like

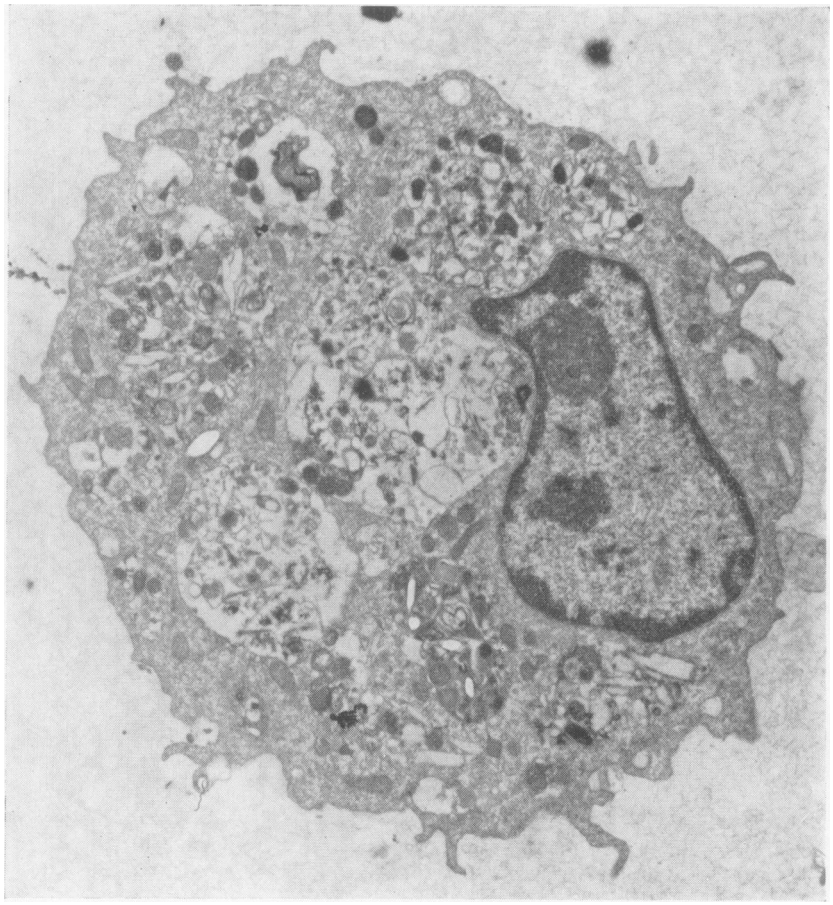

Fig. 10.—Other macrophages contained disintegrating, nonviable neutrophils and cytoplasmic buds from neutrophils. ×9,400.

the Charcot-Leyden crystal, would be devoid of any surrounding membrane. Disintegration of the crystal-containing neutrophil or extrusion of the crystal from the neutrophil by the process of cytoplasmic shedding (Rebuck and Crowley, 1955), both established functions of exudative neutrophils, would provide the first crystal which could subsequently act as the nidus for further crystallization in the synovial fluid.

A second feature of certain exudative neutrophils, namely the mottled appearance of their cytoplasm, may represent an early phase of the accumulation of either polymeric urate (Wolfson and Levine, 1948) or urate complexed with substances such as albumin (Alvsaker, 1965) or $\gamma$1-$\gamma$2-globulin (Alvsaker, 1966). Mordhorst (1897) reported that he had observed globular urates within the cytoplasm of cells in articular cartilage, connective tissue, and fibrocartilage. He noted that these globules were in various states of coalescence in their intracellular location. As coalescence continued, the globular form was transformed into needle urates which penetrated the limiting membrane and killed the cell. The solubility of the globular urates was found to be related to their sodium ion content. From this early observation and our recent ultrastructural findings, we might speculate and introduce the following sequence of leucocyte-urate dynamics as an explanation for the development of gouty arthritis.

Either amorphous or globular urate might accumulate in the synovial fluid and provoke the emigration of the exudative neutrophils and also institute the exudation of fluid. Once contact is established between the particulate urate and the neutrophil, the urate might be transferred to a cytoplasmic location. The cytoplasm of these urate-laden neutrophils might appear "honeycombed" as sequestration and fusion of the globular urates proceeded. Shifts in fluid volume and electrolytes between the extracellular and intracellular environments, which occur during the inflammatory process, may

favour transformation of the intracellular globular urates to needle urates. Urate crystals formed in this manner would not be surrounded by a membrane. A single neutrophil in the synovial fluid of a patient with gout might then contain either no urate, different concentrations of globular urates in various states of coalescence, or single and multiple urate crystals, as well as combinations of these states. The morphological characteristics of the leucocyte population in any one gouty patient would then depend upon the state and concentration of urate present within the synovial fluid, the functional qualities of the emigrated leucocytes, and their length of exposure to the accumulated synovia.

Mordhorst (1897) also stated that in gout signs of inflammation such as pain, redness, and oedema owed their appearance to the accumulation of the globular urates in the cells and intercellular substance as well as blockage of the lymph lacunoe and lymphatics by this form of urate. Synovial fluid aspirated during this phase of the gouty attack then should not contain urate crystals. Recently we have observed a patient who developed an acute arthritis of the left knee 3 weeks after Allopurinol therapy was instituted. A 70 ml. sample of synovial fluid was aspirated. The leucocyte count was 6,700 per $mm^3$. and exudative neutrophils dominated the cytopopulation. No intracellular or extracellular urate crystals were observed when an unstained wet mount was surveyed immediately with polarizing microscopy. This specimen was sealed to prevent drying and chilled for 48 hours. When the preparation was surveyed after the 48-hour period, numerous needle-shaped urate crystals measuring as much as $5\mu$ in length were present. These crystals were found only within the cytoplasm of the exudative neutrophils. No extracellular urate crystals were observed. These data provide indirect support for our concept of the intraleucocytic formation of urate crystals. Further experimentation designed to detail the conditions necessary to induce intracellular crystal formation is in progress.

Some urate crystals are unquestionably acquired by phagocytosis since occasionally these crystals were observed within a typical phagosome. In these instances, the urate crystal was separated from the phagosomal membrane by a large vacuole. More frequently, urate crystals within the cytoplasm of the exudative leucocytes were coated with a continuous or interrupted dense line. The exact composition of this structure is unknown. One might postulate that it represents the plasma membrane which is firmly adherent to the crystalline surface. Or, the dense line may be organized structure other than the plasma membrane. Kellermeyer and Breckenridge (1965) have reported that microcrystals of monosodium urate are procoagulants which promote fibrin formation. It might therefore be possible that a monomolecular layer of fibrinogen or fibrin layers onto the surface of the crystal in its extracellular environment of a fibrinogen-rich synovial fluid. The coating of the crystals by either fibrinogen or fibrin-related molecules might serve to attract neutrophils and augment the phagocytosis of the crystal. The intermittent interruptions of this dense line might be related to unknown toxic properties of crystalline urate or might represent partial digestion of this organized structure by the enzymes released from neutrophil granules. Experiments in progress are designed to elucidate the nature of this linear structure.

The ultimate fate of the intracellular crystal is also unknown. Using human leucocytes and labelled uric acid, Howell and Seegmiller (1962) have demonstrated that leucocytes can degrade sodium urate either as microcrystals or in supersaturated solution. This uricolysis is reportedly mediated by the release of enzymes bound normally within the neutrophil granules. We noted in our ultrathin sections that neutrophil granules frequently formed a line on the crystalline surface and that the granule contents seemed to be distributed along the surfaces of the crystals. We were unable, however, to find any obvious morphological evidence of crystal dissolution in these areas of crystal-granule contact. However, since the morphological features which characterize crystal breakdown are unknown, it is possible these features were present but were not recognized. A single urate crystal may be subjected to digestion *via* the enzyme systems not only of exudative neutrophils but also of the mononuclear phagocytes as well. We have observed that crystal-containing cytoplasmic buds shed from exudative neutrophils as well as pyknotic neutrophils with their entrapped crystals were phagocytosed by macrophages. Therefore, after the neutrophil constituents were digested, the acquired urate crystals would be subjected to any additional or different enzymes contained within the macrophage.

The persistence of urate crystals in tophi and their presence in quiescent joints between attacks of gouty arthritis seems to indicate that neither the fluid system nor the cellular mechanism for uricolysis is entirely effective. Further study of these problems is needed to determine the fate of this biological crystal. Many unanswered questions continue to cloud our understanding of the various interrelationships between the cellular components and the fluid compartment in gouty arthritis. It seems that the fundamental knowledge required for

complete understanding of gouty arthritis will be obtained only by correlating clinical, biochemical, and morphologic information.

**Summary**

Exudative leucocytes and associated urate crystals found in eighteen synovia from eleven patients with gouty arthritis were studied by light, polarizing, and electron microscopy. The cytopopulation consisted mainly of emigrated neutrophils. Varying numbers of mononuclear leucocytes were present and the phagocytic macrophages contained pyknotic neutrophils, shed cytoplasmic buds of neutrophils, and urate crystals. The morphological appearance of urate crystals in ultrathin sections and their characteristics during electron bombardment were investigated.

In ultrathin sections the majority of urate crystals contained within the cytoplasm particularly of neutrophils, were devoid of a limiting membrane and appeared to lie free within the cytoplasmic matrix. Other intraleucocyte urate crystals were outlined by a dense line which was either continuous or interrupted at intervals. Rarely was a urate crystal found in a typical phagosome.

The cytoplasm of certain other neutrophils in synovia from each of our patients with gout revealed a striking "honeycombed" appearance. This mottled effect was evident because the more dense cytoplasmic matrix was separated by electron-lucent spaces. These spaces varied in size and shape, were not membrane-bounded and contained widely dispersed particles of low electron density. Our findings that neutrophils in gouty arthritis contain accumulations of a low-density material and urate crystals which are devoid of a membrane may suggest that the urate crystals are formed at an intracellular site.

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## Études de l'ultrastructure des leucocytes et des urates dans l'arthrite goutteuse

RÉSUMÉ

Les leucocytes exsudatifs et les cristaux d'urate y associés, trouvés dans 18 synoviales provenant de 11 malades atteints d'arthrite goutteuse, furent étudiés par la microscopie lumineuse, polarisante et électronique. La cytopopulation consistait surtout en neutrophiles émigrés. Un nombre variable de leucocytes mononucléaires y fut présent et les macrophages phagocytaires continrent des neutrophiles pycnotiques, des bourgeons cytoplasmiques détachés des neutrophiles et des cristaux d'urate. On étudia l'apparence morphologique des cristaux d'urate sur des coupes ultra minces ainsi que leurs caractéristiques sous bombardement électronique.

Dans ces sections très minces la plupart des cristaux d'urate contenus dans le cytoplasme, surtout celui des neutrophiles, était dépourvue de toute membrane limitrophe et semblait être libre dans la matrice cytoplasmique. D'autres cristaux d'urate intraleucocytaire s'ébauchaient par une ligne dense, continue ou interrompue. On ne trouva que rarement un cristal d'urate dans un phagosome typique.

Le cytoplasme de certains autres neutrophiles dans la synoviale de chacun de nos malades atteints de goutte révéla une apparence alvéolée frappante. Cet effet marbré était dû aux parties plus denses de la matrice cytoplasmique séparées par des espaces translucides électroniquement. La forme et l'extension de ces espaces étaient variables, il n'y avait pas de membrane et on y trouvait des particules très dispersées de basse densité électronique.

Nos résultats indiquant que les neutrophiles dans l'arthrite goutteuse contiennent des accumulations d'un matériel de basse densité et des cristaux d'urate sans membrane suggèrent que les cristaux d'urate se forment à un niveau intracellulaire.

## Investigación de la ultrastructura de los leucocitos y de los uratos en la artritis gotosa

SUMARIO

Los leucocitos exudativos y los cristales de urato asociados con ellos, encontrados en 18 sinovias de 11 enfermos con artritis gotosa, fueron estudiados por microscopía luminosa, polarizante y electrónica. En su mayoría, la población citológica se composaba de neutrófilos emigrados. Un número variable de leucocitos fué observado y los macrófagos fagocitarios contuvievon neutrófilos picnóticos, fragmentados brotes citoplásmicos de neutròfilos y cristales de urato. El aspecto morfológico de los cristales de urato en cortes delgadísimos así como sus rasgos bajo el bombardeo electrónico fueron investigados.

En cortes delgadísimos la mayoría de los cristales de urato citoplásmico, en particular en los neutrófilos, no tenían membrana limítrofe y parecían libres en la matriz citoplásmica. Otros cristales de urato leucocitarios se veían delimitados por una línea densa, contínua o interrumpida. Cristales de urato en un fagosomo típico eran raros.

El citoplasma de ciertos otros neutrófilos en la sinovia de todos nuestros enfermos con gota reveló una apariencia alveolar asombrosa. Este efecto abigarrado se debió a partes más densas de la matriz citoplásmica separadas por espacios translúcidos electrónicamente. Estos espacios fueron de forma y tamaño variables, no tuvieron membrana limítrofe y contuvieron muy dispersas partículas de baja densidad electrónica.

Nuestros resultados, indicando que los neutrófilos en la artritis gotosa contienen una acumulación de materias de densidad baja y de cristales de urato sin membrana, sugieren que los cristales de urato se forman en un sitio intracelular.