SYNOVIAL GIANT CELLS IN RHEUMATOID ARTHRITIS

PHILIP M. GRIMLEY, M.D., AND LEON SOKOLOFF, M.D.

From the Pathologic Anatomy Branch, National Cancer Institute and Laboratory of Experimental Pathology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

This paper reports the occurrence of a distinctive synovial giant cell in rheumatoid arthritis. Except for passing reference to multinucleated synovial cells in a recent Japanese communication,[1] we have encountered no publications concerning this phenomenon. The synovial giant cells are not mentioned in standard histologic accounts of rheumatoid arthritis.[2-6] These cells at times pose a problem in differential diagnosis, and their pathologic significance requires evaluation.

MATERIAL AND METHODS

Histologic Methods. A majority of the synovial tissues had been fixed in Bouin's solution or 4 per cent acetate-neutralized formaldehyde and embedded in paraffin. From 2 to 10, usually 3 tissue blocks were examined in each case. Sections were stained with hematoxylin and eosin as well as with special procedures as indicated in Table I.

Electron Microscopic Methods. In 3 cases surgically excised tissues were available for electron microscopic examination. In 1 (S65–1341), knee synovium had been fixed in formalin. The other specimens (S66–214, S66–730), from radio-ulnar, carpal or metacarpo-phalangeal joints, were fixed primarily with 4 per cent phosphate-buffered glutaraldehyde.[7] After post-osmication,[8] dehydration through graded ethanol solutions and propylene oxide infiltration, the tissues were embedded in large blocks of Araldite resin.[9,10] Large epoxy sections, 5 to 10 μ thick, were obtained with an automatic sliding microtome.[10,11] These were attached to glass cover slips with 1 per cent gelatin, dried thoroughly and coated with a very thin film of Araldite mixture. The resin film polymerized at 60° C; the sections were then inverted over glycerol and surveyed by phase microscopy. Giant cells and other appropriate areas of synovium were thus selected for re-embedding and ultrathin sectioning.[10] Contiguous sections, 2 to 5 μ thick, were stained for high resolution light microscopy.[11,12] Ultrathin sections (600 to 1,000 Å) for electron microscopy, obtained with an automatic ultratome and diamond knife were treated with uranyl acetate or lead citrate [13] and examined with an RCA EMU-3G microscope at original magnifications of × 1,000 to 12,000.

Clinical Data. The patients had advanced peripheral rheumatoid arthritis and were of both sexes; with one exception, their ages ranged from 40 to 63 years. Thirty-two knee specimens, obtained by anterior synovectomy, had been collected over a period of several years. A total of 3 other specimens were obtained from wrist or hand joints. The joint disease was of 3 to 20 years duration, but was active clinically at the time of operation. Various systemic medications had been employed at different times in the past, principally with broad spectrum antibiotic agents or corticosteroids administered orally.

Serologic tests for rheumatoid arthritis had been carried out in 28 patients. They were positive (sensitized sheep erythrocyte agglutination titer 1:16 or greater, or bentonite flocculation 1:64 or greater) in 19 cases.

Accepted for publication, May 23, 1966.

Control Specimens. In an attempt to assess the specificity of the giant cells, their presence was sought in non-rheumatoid synovial tissues. These included sections from 25 normal human knees; 7 knee synovectomy specimens from cases of severe traumatic or other synovitis; 2 specimens of infectious arthritis of the knee; and 6 of severe chronic villous synovitis in hogs.[14] Several dozen biopsy specimens representing various types of synovitis such as tuberculosis, sarcoidosis and lipoid dermatoarthritis were also examined.

RESULTS

Description of Giant Cells. Well developed giant cells were ovoid and measured approximately 40 μ in greatest dimension. In plastic sections measuring 2 to 5 μ or standard paraffin sections (7 μ), there were usually up to 12 peripherally located nuclei (Figs. 1 to 4). These were uniform and ovoid, averaging 10 and 5 μ in their major and minor axes respectively. They thus resembled the nuclei of the mononuclear lining cells and had a similar marginal disposition of chromatin densities.

The central cytoplasmic zone of the giant cells was abundant and moderately eosinophilic. A conspicuous feature was the presence of numerous cytoplasmic granules. Rarely distinguishable in the hematoxylin and eosin stained sections, the granules were brilliantly differentiated in the PAS preparations (Fig. 4). They were of varying size but reached a maximum diameter of 1.5 μ. Coarse cytoplasmic granules were usually located peripheral to the nuclei; occasionally they were concentrated in protrusions of cytoplasm at the poles of ovoid cells (Figs. 4 and 5). Their histochemical staining reactions[15,16] were those of a neutral mucosubstance with a minor esterified lipid component (Table I). In giant cells adjacent to areas of hemosiderosis; the granules sometimes contained stainable ferric salts. The giant cells and surface lining synovial cells both displayed strong acid phosphatase activity.

The distribution of giant cells was often patchy, but where present, they usually congregated in appreciable numbers. Hyperplasia of the lining cell layers was prominent in these areas. Occasional hyperplastic lining cells possessed several nuclei and in some of the specimens with prominent giant cell formation there appeared to be a spectrum of multinucleated forms culminating in the mature variety described. Conversely, even marked villous hypertrophy was not necessarily accompanied by the giant cell formation.

The location of the giant cells was an identifying characteristic. They were usually disposed within a band of 25 to 100 μ below the synovial surface (Figs. 1 and 2). Less frequently, some directly abutted upon the synovial space (Fig. 3). Tightly knit aggregates or "knots" of superficial lining cells, suggesting a syncytium at the light optical level, were observed in cases of nonspecific synovitis as well as rheumatoid synovitis

TABLE I

STAINING CHARACTERISTICS OF GIANT CELLS

Stain *	Visible granules	Cytoplasm
Paraffin sections †		
Hematoxylin and eosin	Rarely pale golden brown	Moderately eosinophilic
Periodic acid-Schiff (PAS)	Strongly +	Finely granular +
Diastase, PAS	Strongly +	Finely granular +
Direct Schiff	—	—
Periodic acid-phenylhydrazine-Schiff	—	—
Methenamine silver (Grocott-Gomori)	+	—
Aldehyde fuchsin-alcian blue [16]	Purple	—
Periodate-mixed diamine [16]	Grey-violet	Grey
High iron diamine-alcian blue [16]	—	—
Azure A, pH_2	—	—
Alcian blue, pH_2	—	—
Diastase, alcian blue pH 1.0, PAS	Magenta	—
Diastase, alcian blue pH 2.5, PAS	Magenta	Finely granular magenta
Colloidal iron (Rinehart-Abul-Haj)	—	Finely granular +
Hemofuscin (Mallory)	—	—
Ferric-ferricyanide reduction	—	—
Nile blue sulfate	—	—
Sudan black-B	+	—
Carbol-fuchsin	Occasionally dull red	—
Luxol fast blue	+	—
Perls' reaction for ferric salts	Occasionally +	Often weakly +
Brown-Brenn Gram technic	—	—
Feulgen reaction	—	—
Frozen sections ‡		
Oil red O	—	—
Baker's acid hematein test	—	—
Schultz's cholesterol method	—	—
Fluorescence, post-formalin	—	—
Acid phosphatase (Burstone)	+	+
Polariscopy	Isotropic	Isotropic
OsO_4-plastic sections ‡		
Phase optics	Moderately osmiophilic	Weakly osmiophilic
Paraphenylenediamine [9]	Moderate reaction	Weak reaction
$KMnO_4$-oxalic acid [10]	Bleached	Bleached

* Methods quoted in Lillie,[7] unless otherwise specified.

† Many of the specialized tests for lipids and mucosubstances were performed on a single case (s65-1341); the PAS, carbol fuchsin, Brown-Brenn, Perls' reaction and colloidal iron methods were repeated in several cases.

‡ Same cases examined by electron microscopy.

with marked hyperplasia. These "knots" were easily distinguished from the giant cells under consideration by a clumped arrangement of the nuclei and a lack of central cytoplasm. Unless deep giant cells were also identified, cases with superficial multinucleated cells were classified separately for purposes of clinical analysis (Table II).

Giant cells were often situated amidst numerous large mononuclear cells laden with prominent oil red O staining droplets. These droplets

TABLE II

OCCURRENCE OF GIANT CELLS IN KNEE SYNOVIUM

Type of synovium	Number studied	Type of multinucleate cells *			
		Giant cells	Unclassified multinucleated	Superficial multinucleated cells or "knots" †	Foreign-body or Touton cells
Rheumatoid arthritis, sero-positive	19	9	2	1	3
Rheumatoid arthritis, sero-negative ‡	9	0	2	1	3
Rheumatoid arthritis, serologic unknown	4	0	1	1	2
Rheumatoid arthritis Total	32	9	5	3	8
Normal	25	0	0	0	0
Severe traumatic or idiopathic synovitis	7	0	0	1	0
Infectious synovitis	2	0	0	1	0
Porcine polyarthritis	6	0	0	1	0

* Excluding multinucleated plasma cells.

† Exclusive of the cases having distinctive giant cells.

‡ One of these patients may have had Marie-Strümpell disease.

measured up to 2 μ in diameter. Many were birefringent and their histochemical properties, including positive Schultz reaction, indicated a neutral triglyceride and cholesterol content.[15] The general deep distribution of the lipid-laden cells was most clearly demonstrated in plastic-embedded tissue sections. Here, the osmium-fixed lipid reacted intensely with paraphenylenediamine (Fig. 13) and resisted bleaching by potassium permanganate and oxalic acid.[11,12] Such lipid droplets were almost never observed in giant cells.

The deep lipid-laden cells were more loosely arranged than the superficial lining cells. Many possessed elongated or "fibroglial" processes which made contact with adjacent deep and giant cells or insinuated between surface cells. They thus corresponded to the deep group of lining cells present in normal synovium and designated type B by Barland and co-workers [17] or type F by Hirohata and co-workers.[1,18] The nuclei of the deep, lipid-laden cells averaged slightly larger than the surface or giant cell nuclei.

In addition to features detailed, the affected tissues had other usual characteristics of villous synovitis. These included chronic inflammatory cell infiltration and proliferation of supportive vascular connective tissue.

Electron Microscopy. The nuclei of the giant cells were located close to the plasma membrane, often separated from it by less than 1μ of cytoplasm. Their contours generally were smooth, but occasionally were irregularly angular or saw-toothed (Fig. 5). The nuclei contained unobtrusive nucleoli and minimal, peripherally located, slightly clumped chromatin.

The plasma membrane was elaborately ruffled into intertwining slender filopodia (Fig. 6). No plasmolemmal partitions of the type sometimes observed in the cytoplasm of foreign body or Langhans giant cells were found.[19,20] The cytoplasm of synovial giant cells contained a variable quantity of granular endoplasmic reticulum in the form of simply developed, irregularly arranged short tubular profiles. There were a moderate number of smooth surfaced vesicles dispersed throughout. Cytoplasmic filaments measuring about 60 to 100 Å in diameter and of indeterminate length were randomly distributed in the central areas. Microtubules measuring about 200 Å diameter were sparse. Golgi elements were frequently encountered and multiple diplosomes were occasionally noted at the border between nuclei and the central cytoplasm.

The cytoplasmic granules appeared as complex dense bodies or cytosomes.[21] The granule contents were enveloped by a single limiting membrane, and were often separated from it by a clear space, 20 to 50 mμ wide. Large granules, located in the paranuclear regions, corresponded

to the brilliantly staining PAS reactive forms observed in conventional sections. These displayed ovoid, rounded, polygonal or irregularly protuberant profiles in various planes of sections. They were occasionally arranged in short stacks with flattened apposing margins (Fig. 6), suggesting a low order of structural rigidity. Finer granules (average diameter, 0.3 μ), were most numerous in the central portions of giant cells and generally not detected by light microscopy. They were more frequently rounded, ovoid or dumbbell shaped than the large granules, and possessed fewer, less voluminous inclusion structures (Figs. 7 and 8). In these respects, they more closely resembled the cytoplasmic dense bodies illustrated in normal synovial lining cells[22–24] and in certain active macrophages.[25–27] Some small granules were located in proximity to dilated cisternae of granular endoplasmic reticulum which were filled with amorphous material of similar density to the granule matrix. No consistent spatial relationship of the granules to Golgi elements or diplosomes was apparent[26]; rare horseshoe shaped or elongate dense bodies, however, suggested an origin in membranous saccules.

Within the matrix of large granules, eccentric or bulging globular inclusions of homogeneous density occasionally suggested incorporation of lipid or mucin droplets. Other forms of irregular dense inclusions probably derived from a variety of ingested components. Compact opaque inclusions, 30 to 100 mμ in greatest dimension were often concentrated at the margin of granules. Particularly in the case of smaller granules, this arrangement imparted a beaded appearance to the edges (Fig. 7). High magnification of the opacities rarely revealed a substructure consisting of closely packed dense particles in the size range of ferritin. Similarly sized dense particles were also dispersed individually within the matrix of granules or the surrounding cytoplasm.[1]

The fine structural characteristics of the giant cells were basically similar to those of macrophage-like cells which formed the predominant component of the synovial surface (Fig. 9). Both lacked the prominent osmiophilic lipid droplets and abundance of better developed, regularly organized ergastoplasm seen in the deeper, fibroblast-like cells (Figs. 5 and 14).[1,17,18] The granules in the surface macrophage-like cells were as numerous, but generally smaller than those in the giant cells (Figs. 9 and 10). The exuberance of filopodial processes at the surface (Fig. 10) suggested considerable phagocytic activity[19,28] and masses of engulfed amorphous material were sometimes observed. Smooth endoplasmic reticulum of tubular form was best developed at the periphery of surface macrophages and pinocytotic vesicles were also numerous in these regions (Fig. 10). Coated "pits"[24] occasionally indented the surface of macrophage-like cells as well as giant cells (Fig. 8).

Macrophage-like, surface cells were often closely juxtaposed, and two types of intimate cell contact were noted: adjacent filopodia enmeshed forming interlocking pairs or clusters of cells (Fig. 10), or straight portions of contiguous plasma membranes were directly attached at specialized junctions. In the latter circumstance, parallel segments of plasma membrane, 0.5 to 0.75 μ long, were thickened by accretion of dense material on the inner aspect; and separated by a 60 mμ gap filled with less dense, amorphous material (Fig. 11). These junctions were sometimes serially arranged in "belts" along the membranous interface of two adjacent cells (Fig. 12). They thus exhibited morphologic features of desmosomes or maculae adherentae[29,30]; radiating tonofilaments, however, were not identified. The intercalated cement substance in some of the junctional zones appeared to be contiguous with other amorphous material, probably fibrin or mucin, precipitated upon the synovial surface. Connection of synovial lining cells by desmosomes has not hitherto been reported[1,17,18,22-24,28,31,32] and might represent a pathologic modification, perhaps extraneously induced by the adhesive properties of abnormal coating materials. The specialized surface cell connections described might well resist shrinkage separation better than adjacent unattached cells,[30] and thus offer an explanation for the superficial cell "knots" occasionally observed in conventional sections of hyperplastic synovium.

Abundant rod-shaped bodies were identified within endothelial cells of many small caliber synovial vessels. These were usually linear and measured up to 0.12 μ in thickness and 0.6 μ in length in any plane of section. Some rods were angulated or twisted and could well have measured a greater length if serially reconstructed. The internal structure of the rods, including 150 Å diameter tubules arranged parallel to the long axis, appeared identical to the components described by Weibel and Palade in other types of capillary endothelium.[33] Another feature of interest was the presence of lipid droplets within some synovial mast cells or plasma cells participating in the chronic inflammatory reaction.

Occurrence and Clinicopathologic Correlations. Multinucleated superficial synovial lining cells or surface cell "knots" were occasionally seen as an isolated phenomenon in several types of synovitis (Table II); the deep giant cells, however, were not found in any conditions other than rheumatoid arthritis. Acceptable giant cells were present in 9 of the 19 seropositive cases. They were readily distinguished from multinucleated plasma cells and incidental lipophagic or foreign-body giant cells also frequently present in rheumatoid synovium.

No direct correlation of the giant cell reaction with titers was evident. In one seropositive case, a knee specimen containing giant cells was followed 2 weeks later by a histologically negative contralateral synovec-

tomy. In another patient, repeated synovectomy from the same knee at an interval of 1 year yielded differing histologic results.

Rarely, a few deeply placed multinucleated cells were observed in seronegative as well as seropositive types of synovitis. They often possessed pyknotic or irregularly arranged nuclei; and sometimes occurred in areas of ulceration. Because of their small numbers, atypism and possible confusion with the foreign body type of giant cells, they have been listed as "unclassified" (Table II). In one seronegative case, a few deeply placed giant cells were detected only after search through several large blocks of tissue. Because most of these cells were atypical, this case has been included in the "unclassified" group. An additional specimen, from a patient with seronegative Still's disease, contained no giant cells, but was excluded from tabulation because the synovial surface was extensively ulcerated.

A retrospective review of the clinical records disclosed that many of the patients had received intra-articular injections of hydrocortisone acetate or prednisolone at some time during their illness. Information was available in 17 cases; 12 patients had been treated in this manner. There was no obvious association between the presence of the giant cells and the intra-articular therapy. Giant cells occurred in several joints that had never received injections. The synovium in 2 patients, who had received the injections a few days prior to operation, contained no giant cells. By contrast, these cells were abundant in a patient who had received her last intra-articular treatment over a year prior to synovectomy. Giant cells were also abundant in at least one patient who was known never to have been treated with gold compounds.

Discussion

Histogenesis of the Giant Cells. The synovial lining normally comprises a heterogeneous population of connective tissue cells, in which two principal types have been distinguished morphologically. One, type A[17] or type M,[18] possesses many cytologic features in common with macrophages, particularly elaborate surface filopodia and abundant lysosomal bodies.[19,20,22–28,34,35] The other cell type (type B or type F) has fibroblast-like features, including elongated cytoplasmic processes and prominent regularly arranged ergastoplasm.[1,17,18,35] The macrophage-like cells are known to accumulate preferentially at the surface of both normal and rheumatoid synovium.[1,17,18,32] In rheumatoid arthritis they form a thicker mantle than normally, so that layering of the surface macrophage-type and deeper fibroblast-type cells is exaggerated. It appears likely that, as in other granulation tissues, the cell population of rheumatoid synovium actually constitutes a functional spectrum of macrophages and fibro-

blasts which transform from one to another under appropriate environmental conditions [19,20,35]; indeed, the macrophages may originally derive from circulating monocytes.[36,37]

Although characteristically deep in location, the giant cells of rheumatoid arthritis clearly possess cytologic features in common with synovial surface cells of macrophage type. While their precise precursor and mode of formation cannot be established in a static morphologic study such as the present one, a histologic hypothesis for their situation can be offered: In rheumatoid arthritis, the synovial lesions are quite labile, ulceration and proliferation of granulation tissue seemingly alternating with each other. The synovial lining layers might thus be conceived as an advancing front of granulation tissue with macrophage-like cells selectively migrating to, or proliferating at the surface. The giant cells because of greater size would be retarded in emigration toward the joint cavity and remain embedded in proliferating granulation tissue. This proposal finds some support in the observation that multinucleated cells, developing in tissue cultures or rheumatoid synovium [38,39] lack the mobility characteristic of single macrophages.[38] An alternative possibility that giant cells develop in the surface layers and migrate to a deeper level of the synovium cannot presently be excluded.

Multinucleated giant cells of various sorts are generally believed to arise through confluence of pre-existing mononuclear cells,[19,36,40,41] but amitotic division may also participate in their development.[20,36,37] Residual plicated segments of paired plasma membranes sometimes seen in foreign-body or Langhans giant cells [19,20] have been considered evidence for the confluence mechanism,[19] but they were not present in the synovial giant cells.

If the type A lining and synovial giant cells do indeed originate from macrophages, it is particularly interesting that the type B fibroblast-like cells, rather than they, contained prominent lipid droplets. This was also observed by Hirohata and Kobayashi.[1] Cytoplasmic lipid inclusions are not a prominent feature of inactive fibroblasts or fibroblasts proliferating during normal wound healing [35]; they do, however, develop abundantly in the fibroblasts of scorbutic guinea pig wounds.[42] In rheumatoid arthritis, the synovial fluid contains elevated amounts of lipids and rarely assumes a chylous character.[43] Copious quantities of lipid may also occur in the synovium and subcutaneous nodules of this disorder. At times, xanthoma-like foam cells or Touton giant cells are also found in the synovial lesions and such cells are presumably derived from macrophages. It may be surmised, that lipids accumulating in the fibroblast-like synovial cells result from an intrinsic disturbance of cellular metabolism, while the lipophage lipid is acquired by endocytosis.

Significance of the Multinucleation. Multinucleation of itself conveys no specific information about the nature of a tissue reaction. It may be experimentally induced by diverse types of stimuli both *in vivo* and *in vitro*. From the standpoint of pathologic diagnosis, the occurrence of giant cells in response to certain infectious agents is most immediately relevant. No virus particles, or other formed micro-organisms were identified in the synovial giant cells by electron microscopy or conventional staining methods. Synovial fluid cultures were negative in several cases.

It is noteworthy that acidification of the nutrient medium supporting macrophages in tissue cultures encourages the proliferation of multinucleated forms.[36,41] The pH of synovial fluid in rheumatoid arthritis is low,[44] presumably because of the concomitant accelerated glycolysis and lactate production.[45]

Significance of the Giant Cell Granules. The PAS reactive granules in the macrophage-like lining and synovial giant cells possessed ultrastructural characteristics generally associated with lysosomes or lysosomal derivatives: They consisted of a dense matrix material with heterogeneous inclusions and limited by a single membrane.[21,46] They were present in cells demonstrating a high acid-phosphatase reactivity.[46]

Barland and colleagues earlier demonstrated an increased number of lysosomal granules in macrophage-like rheumatoid synovial lining cells [32]; possible roles of these granules in rheumatoid arthritis have been amply speculated upon.[47] Analagous granules sometimes associated with PAS or hydrolytic enzyme reactivity increase during the maturation of macrophages or giant cells in a number of experimental situations [26,27,36–38,40,48] and alterations of lysosome size or content have been specifically related to endocytic activity under experimentally manipulated nutritional conditions in tissue culture.[26,49,50] Analysis of our micrographs suggests participation of the synovial giant cell granules in the digestive activities characteristic of morphologically similar "solid" lysosomes.[26,27,46,50] The well documented phagocytic function of cytologically comparable synovial surface cells [28,51] supports this concept. Small granules, apparently formed as cellular secretory products,[46] seemed to fuse into larger masses through stages of dumbbell or stacked formations.[27] During this process they evidently became associated with a larger complement of inclusion structures, probably representing material in the process of digestion.[21,26,27,34,50] The internal structure of cytoplasmic granules observed in our rheumatoid cases differed in minor respects from comparable dense bodies described by others.[32] In particular, distinctly organized membrane configurations were not presently identified.

The biochemical composition of lysosomal granule residues in rheumatoid arthritis is unknown. The roles of various phagocytic cells in

rheumatoid synovium, however, are a subject of considerable current interest.[47,52] The production of rheumatoid serologic factors in the synovial tissue as well as phagocytosis of this factor and its aggregate with gamma globulin in synovial exudate have recently been investigated.[39,52,53] Rheumatoid factor has been detected by several investigators, using fluorescein-labeled gamma globulin, in the cytoplasm primarily of synovial plasma cells, but not in the multinucleated giant cells formed *in vitro*.[39] Application of these techniques to the synovial giant cells in question remains to be performed.

Among other explanations for the prominent dense bodies in rheumatoid synovial lining cells, Barland and colleagues[32] considered residual deposition of therapeutically administered gold compounds. These have been shown to concentrate in the synovium after injection at distant sites.[54] This mechanism *per se* can be excluded in the tissues in at least 1 case currently examined by electron microscopy, since chrysotherapy had never been employed in this carefully supervised patient.

Correlation of the granule sizes, histochemical staining reactions (Table I), and ultrastructural appearances in the synovial giant cells suggests that the esterified lipid component represents some of the residual products in the large granules, and that ferric salts are also frequently incorporated. Neutral mucosubstance, presumably sequestering hydrolytic enzymes,[55] evidently comprises a major portion of the granule matrix, particularly in the smaller forms. Neither histochemical nor fine structural features resembled those of mast cell granules.[56]

Diagnostic Significance. Multinucleated giant cells of several sorts may occur in synovial tissue. Aside from Langhans cells of specific infections and sarcoidosis, the foreign-body cells of gout, Touton cells of pigmented villonodular synovitis, multinucleated sarcoma cells and multinucleated plasma cells, the commonest giant cells in chronic synovitis are formed as a foreign body reaction to joint detritus. Shards of necrotic cartilage and bone are frequently recognized in such cells with the polarizing microscope. A rare type of synovitis, in which numerous multinucleated giant cells occur, is that of lipoid dermato-arthritis; the giant cells are of Touton type[57] and lack the characteristic marginal orientation of nuclei observed in the synovial giant cells of rheumatoid arthritis.

Langhans cells typically are larger than the synovial giant cells in question. They may have fine, granular PAS reactive material in the central portions of their cytoplasm, but lack the conspicuous peripheral granules of the sort observed here.[20,27] Furthermore, they usually are associated with tuberculoid granuloma formation not seen in rheumatoid arthritis.

Although the synovial giant cells have been observed only in our

seropositive cases of rheumatoid arthritis, some conservatism about their diagnostic specificity appears justified. The clinical analysis of the older case material was, by its retrospective nature, not ideal; serologic and historical data were incomplete in some cases. The control specimens were roughly comparable to the rheumatoid ones in terms of the location, amount of tissue studied, intensity of inflammatory infiltration and lining cell hyperplasia. The chronicity of the rheumatoid cases was, however, not present in any of them and the number of cases studied was limited. It is important to recognize the occurrence of giant cells in rheumatoid arthritis and not let their presence militate against the correct diagnosis. Giant cells develop in cultures of normal and rheumatoid synovial tissues, but more frequently in the latter.[38,39]

Summary

A distinctive multinucleated synovial giant cell is described in rheumatoid arthritis. The cells were present in synovium from the knee in 9 of 19 patients with active, seropositive disease. None were found in 9 seronegative rheumatoid or in a series of control cases. The giant cells have also been observed in joints other than the knee. They were ordinarily readily distinguished from multinucleated plasma cells, foreign-body cells, and Touton cells that also occur with some frequency in rheumatoid arthritis. There was no association with the intra-articular administration of therapeutic agents.

The cells exhibited peripherally arranged nuclei and conspicuous paranuclear, PAS reactive granules. They were characteristically located deep to the synovial lining. In 3 cases studied by electron microscopy, the giant cells had fine structural features comparable to those of macrophage-like synovial lining cells. The granules appeared as membrane-limited heterogeneous dense bodies. They were interpreted as lysosomal derivatives. No micro-organisms were identified in the giant cells. An incidental new finding was the presence of desmosomes between some synovial surface cells. Rod-shaped bodies were abundant in the endothelial lining of small blood vessels.

Addendum

Since submitting this manuscript, rod-shaped bodies in the capillary endothelium have been described elsewhere (Highton, T. C.; Caughey, Z. E., and Rayns, D. G. A new inclusion body in rheumatoid synovia. *Ann.Rheum. Dis.*, 1966, **25**, 149–155). Desmosome-like structures have also been reported recently in other types of connective tissue (Ross, R., and Greenlee, T. K., jr. Electron microscopy: attachment sites between connective tissue cells. *Science,* 1966, **153**, 997–999).

References

1. Hirohata, K., and Kobayashi, I. Fine structures of the synovial tissues in rheumatoid arthritis. *Kobe J. Med. Sci.*, 1964, 10, 195–225.
2. Allison, N., and Ghormley, R. K. Diagnosis in Joint Disease. A Clinical and Pathological Study of Arthritis. Wm. Wood, New York, 1931.
3. Nichols, E. H., and Richardson, F. L. Arthritis deformans. *J. Med. Research*, 1909, 21, 149–222.
4. Collins, D. H. The Pathology of Articular and Spinal Diseases. E. Arnold, London, 1949.
5. Klinge, F. Die rheumatischen Erkrankungen der Knochen und Gelenke und der Rheumatismus. In: Handbuch der speziellen pathologischen Anatomie und Histologie. Lubarsch, O., and Henke, F. (eds.). Julius Springer, Berlin, 1934, Vol. 9, Part. 2, pp. 107–251.
6. Gardner, D. L. Pathology of the Connective Tissue Diseases. E. Arnold, London, 1965.
7. Sabatini, D. D.; Bensch, K. G., and Barrnett, R. J. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.*, 1963, 17, 19–58.
8. Millonig, G. Further Observations on a Phosphate Buffer for Osmium Solutions. In: Electron Microscopy. Fifth International Congress. Breese, S. S., jr. (ed.). Academic Press, New York, 1962, Vol. 2,
9. Luft, J. H. Improvements in epoxy embedding methods. *J. Biophys. & Biochem. Cytol.*, 1961, 9, 409–414.
10. Grimley, P. M. Selection for electron microscopy of specific areas in large epoxy tissue sections. *Stain Techn.*, 1965, 40, 259–263.
11. Grimley, P. M.; Albrecht, J. M., and Michelitch, H. E. Preparation of large epoxy sections for light microscopy as an adjunct to fine structure studies. *Stain Techn.*, 1965, 40, 357–366.
12. Estable-Puig, J. F.; Bauer, W. C., and Blumberg, J. M. Technical note. Paraphenylenediamine staining of osmium-fixed, plastic-embedded tissue for light and phase microscopy. *J. Neuropath. Exp. Neurol.*, 1965, 24, 531–535.
13. Venable, J. H., and Coggeshall, R. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.*, 1965, 25, 407–408.
14. Sikes, D.; Neher, G. M., and Doyle, L. P. The pathology of chronic arthritis following natural and experimental erysipelothrix infection of swine. *Amer. J. Path.*, 1956, 32, 1241–1251.
15. Lillie, R. D. Histopathologic Technic and Practical Histochemistry. The Blakston Division, McGraw-Hill Book Co., New York, Toronto, Sydney, London, 1965, ed. 3, 715 pp.
16. Spicer, S. S. Diamine methods for differentiating mucosubstances histochemically. *J. Histochem. Cytochem.*, 1965, 13, 211–234.
17. Barland, P.; Novikoff, A. B., and Hamerman, D. Electron microscopy of the human synovial membrane. *J. Cell Biol.*, 1962, 14, 207–220.
18. Hirohata, K.; Mizuhara, K.; Fujiwara, A.; Sato, T.; Imura, S., and Kobayashi, I. Electron microscopic studies on the joint tissues under the normal and pathologic conditions. I. Normal joint tissues (1st report), *J. Jap. Orthop. Ass.*, 1963, 36, 871–883.
19. Davis, J.M.G. The ultrastructural changes that occur during the transformation of lung macrophages to giant cells and fibroblasts in experimental asbestosis. *Brit. J. Exp. Path.*, 1963, 44, 568–575.

20. GUSEK, W. Submikroskopische Untersuchungen zur Feinstruktur aktiver Bindegewebszellen. *Veroff. Morph. Path.*, 1962, 64, 1–115.

21. ERICSSON, J.L.E.; TRUMP, B. F., and WEIBEL, J. Electron microscopic studies of the proximal tubule of the rat kidney. II. Cytosegresomes and cytosomes: their relationship to each other and to the lysosome concept. *Lab. Invest.*, 1965, 14, 1341–1365.

22. LEVER, J. D., and FORD, E.H.R. Histological, histochemical and electron microscopic observations on synovial membrane. *Anat. Rec.*, 1958, 132, 525–534.

23. LANGER, E., and HUTH, F. Untersuchungen über den submikroskopischen Bau der Synovialmembran. *Z. Zellforsch.*, 1960, 51, 545–559.

24. WYLLIE, J. C.; MORE, R. H., and HAUST, M. D. The fine structure of normal guinea pig synovium. *Lab. Invest.*, 1964, 13, 1254–1263.

25. KARRER, H. E. The ultrastructure of mouse lung: the alveolar macrophage. *J. Biophys. & Biochem. Cytol.*, 1958, 4, 693–700.

26. COHN, Z. A., and WIENER, E. The particulate hydrolases of macrophages. *J. Exp. Med.*, 1963, 118, 991–1020.

27. DUMONT, A., and SHELDON, H. Changes in the fine structure of macrophages in experimentally produced tuberculous granulomas in hamsters. *Lab. Invest.*, 1965, 14, 2034–2055.

28. BALL, J.; CHAPMAN, J. A., and MUIRDEN, K. D. The uptake of iron in rabbit synovial tissue following intra-articular injection of iron dextran. A light and electron microscope study. *J. Cell Biol.*, 1964, 22, 351–364.

29. FARQUHAR, M. G., and PALADE, G. E. Junctional complexes in various epithelia. *J. Cell Biol.*, 1963, 17, 375–412.

30. FAWCETT, D. W. Intercellular bridges. *Exp. Cell Res.*, 1961, 8, suppl. 174–187.

31. LUSE, S. A. A synovial sarcoma studied by electron microscopy. *Cancer*, 1960, 13, 312–322.

32. BARLAND, P.; NOVIKOFF, A. B., and HAMERMAN, D. Fine structure and cytochemistry of the rheumatoid synovial membrane with special reference to lysosomes. *Amer. J. Path.*, 1964, 44, 853–866.

33. WEIBEL, E. R., and PALADE, G. E. New cytoplasmic components in arterial endothelia. *J. Cell Biol.*, 1964, 23, 101–112.

34. NOVIKOFF, A. B. Lysosomes and Possible Roles in the Reticuloendothelial System. In: Colloques Internationaux du Centre National de la Recherche Scientifique, Paris, 1963, Vol. 115, pp. 67–88.

35. ROSS, R., and BENDITT, E. P. Wound healing and collagen formation. I. Sequential changes in components of guinea pig skin wounds observed in the electron microscope. *J. Biophys. & Biochem. Cytol.*, 1961, 11, 677–700.

36. WEISS, L. P., and FAWCETT, D. W. Cytochemical observations on chicken monocytes, macrophages and giant cells in tissue culture. *J. Histochem. Cytochem.*, 1953, 1, 47–65.

37. LEDER, L. D., and NICOLAS, R. Untersuchungen zur Genese der Fremdkorperriesenzellen mittels der Hautfenstermethode. *Frankfurt. Z. Path.*, 1965, 74, 620–639.

38. STANFIELD, A. B., and STEPHENS, C.A.L., JR. Studies of cells cultured from 188 rheumatoid and non-rheumatoid synovial tissues. *Texas Rep. Biol. Med.*, 1963, 21, 400–411.

39. BARTFELD, H. Rheumatoid arthritic and non-rheumatoid synovium in cell culture. Morphological observations, acridine orange, and fluorescent fraction II studies. *Ann. Rheum.Dis.*, 1965, 24, 31–39.

40. KRACHT, J., and GUSEK, W. Autoradiographische und histochemische Untersuchungen am Mycolsäuregranulom. *Verh.Deutsch. Ges. Path.*, 1964, **48**, 300–305.

41. FRANKLIN, R. M. Some observations on the formation of giant cells in tissue culture of chicken macrophages. *Z. Naturforsch.*, 1958, **13b**, 213–214.

42. ROSS, R., and BENDITT, E. P. Wound healing and collagen formation. II. Fine structure in experimental scurvy. *J. Cell Biol.*, 1962, **12**, 533–552.

43. NEWCOMBE, D. S., and COHEN, A. S. Chylous synovial effusion in rheumatoid arthritis. Clinical and pathogenetic significance. *Amer. J. Med.*, 1965, **38**, 156–164.

44. CUMMINGS, N. A., and NORDBY, G. L. Measurement of synovial fluid pH in normal and arthritic knees. *Arthritis Rheum.*, 1966, **9**, 47–56.

45. DINGLE, J.T.M., and THOMAS, D.P.P. *In vitro* studies on human synovial membrane. A metabolic comparison of normal and rheumatoid tissue. *Brit. J. Exp. Path.*, 1956, **37**, 318–323.

46. NOVIKOFF, A. B. Lysosomes in the Physiology and Pathology of Cells: Contributions of Staining Methods. In: Lysosomes. Ciba Foundation Symposium. DE RUECK, A.V.S., and CAMERON, M. P. (eds.). Little, Brown & Co., Boston, 1963, pp. 36–73.

47. HAMERMAN, D. New thoughts on the pathogenesis of rheumatoid arthritis. (Editorial) *Amer. J. Med.*, 1966, **40**, 1–9.

48. COHN, Z. A., and BENSON, B. The differentiation of mononuclear phagocytes. Morphology, cytochemistry and biochemistry. *J. Exp. Med.*, 1965, **121**, 153–170.

49. COHN, Z. A., and BENSON, B. The *in vitro* differentiation of mononuclear phagocytes. II. The influence of serum on granule formation, hydrolase production and pinocytosis. *J. Exp. Med.*, 1965, **121**, 835–848.

50. GORDON, B. B.; MILLER, L. R., and BENSCH, K. G. Studies on the intracellular digestive process in mammalian tissue culture cells. *J. Cell Biol.*, 1965, **25**, No. 2, Pt. 2, 41–56.

51. COCHRANE, W.; DAVIES, D. V., and PALFREY, A. J. Absorptive functions of the synovial membrane. *Ann. Rheum. Dis.*, 1965, **24**, 2–15.

52. RAWSON, A. J.; ABELSON, N. M., and HOLLANDER, J. L. Studies on the pathogenesis of rheumatoid joint inflammation. II. Intracytoplasmic particulate complexes in rheumatoid synovial fluids. *Ann. Intern. Med.*, 1965, **62**, 281–284.

53. MELLORS, R. C.; NOWOSLAWSKI, A.; KORNGOLD, L., and SENGSON, B. L. Rheumatoid factor and the pathogenesis of rheumatoid arthritis. *J. Exp. Med.*, 1961, **113**, 475–484.

54. TONNA, E. A.; BRECHER, G.; CRONKITE, E. P., and SCHWARTZ, I. L. The autoradiographic localization and distribution of neutron activated gold (Au^{198}) in skeletal tissue and synovia of mice. *Arthritis Rheum.*, 1963, **6**, 1–10.

55. KOENIG, H. Histological distribution of brain gangliosides: lysosomes as glycolipoprotein granules. *Nature (London)*, 1962, **195**, 782–784.

56. THIÉRY, J. P. Étude au microscope électronique de la maturation et de l'excrétion des granules des mastocytes. *J. Microscopie*, 1963, **2**, 549–556.

57. BORTZ, A. I., and VINCENT, M. Lipoid dermato-arthritis and arthritis mutilans. *Amer. J. Med.*, 1961, **30**, 951–960.

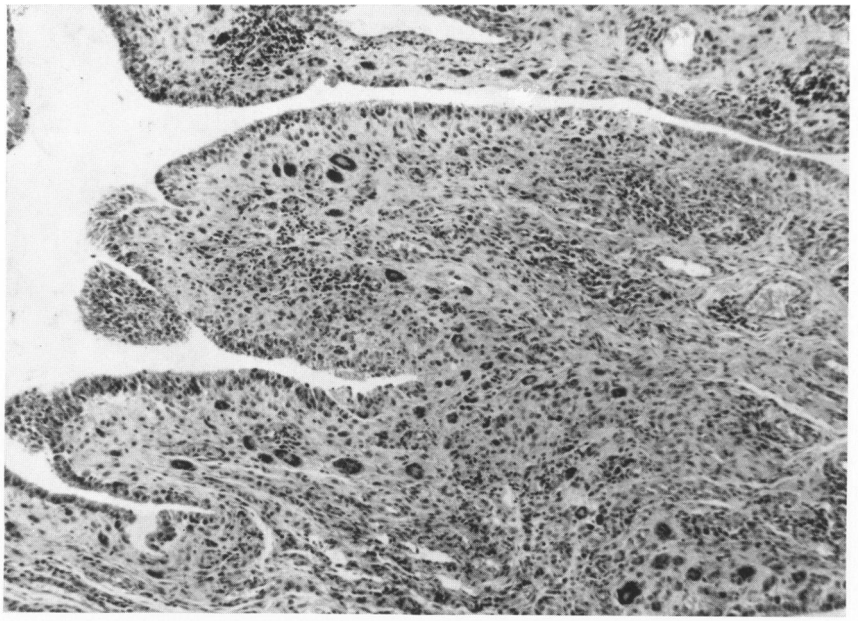
We are indebted to Drs. Stewart Bush, William R. Felts and John L. Decker for many of the clinical data, and to Drs. Dennis Sikes, Lent C. Johnson and William Newman for control synovial tissues. Assistance in a number of the histochemical procedures and other special techniques was graciously provided by Drs. Samuel S. Spicer, Norman S. Cooper and Bruce K. Wetzel and Miss Mary C. Bowling.

Legends for Figures

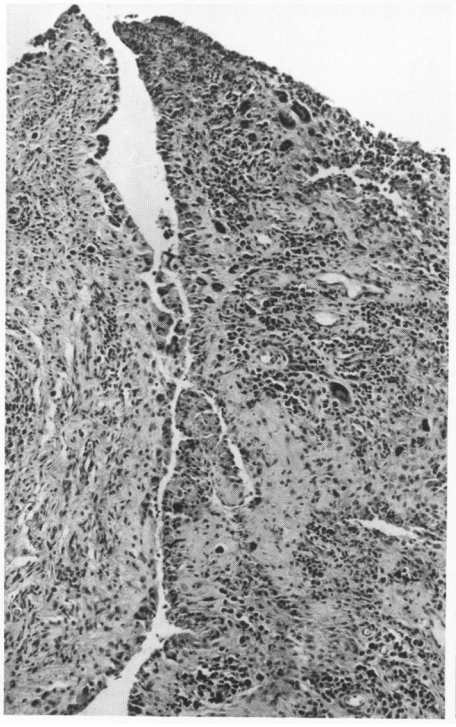
FIG. 1. Knee synovectomy specimen (s65-1341), 25-year-old white woman with clinically active rheumatoid arthritis (bentonite flocculation titer 1:1024). Each hyperplastic villus contains up to a dozen giant cells with peripherally arranged nuclei. They are characteristically located deep to the lining surface. Hematoxylin and eosin stain. × 73.

FIG. 2. Metacarpal synovium (s66-214), 53-year-old white man with clinically active rheumatoid arthritis (bentonite flocculation titer 1:4096). Giant cells are similar to those shown in Figure 1. Hematoxylin and eosin stain. × 75.

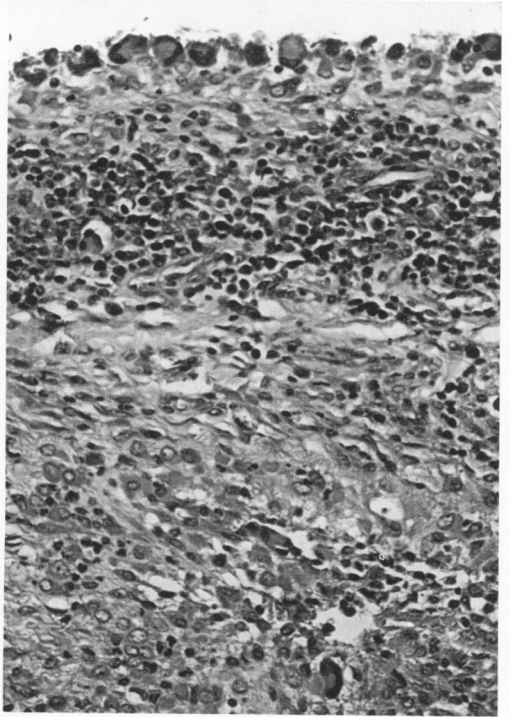
FIG. 3. Wrist tendon sheath (s66-730), 35-year-old white man with active rheumatoid arthritis (bentonite flocculation titer 1:1024). Prominent multinucleated cells aligned at the synovial surface are striking, but unusual. Elsewhere in the specimen, deep giant cells were numerous. Hematoxylin and eosin stain. × 195.



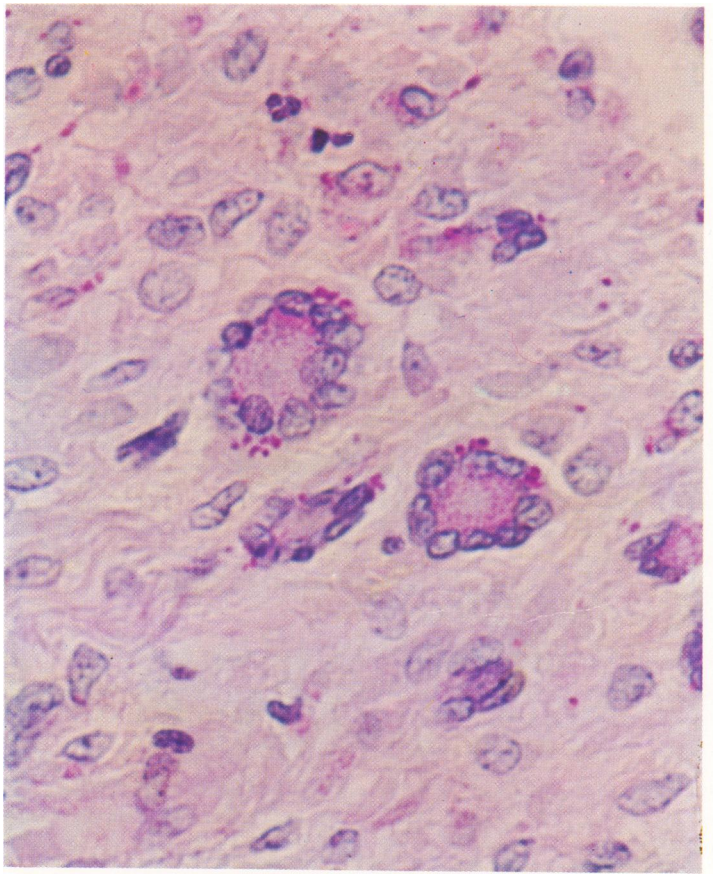
1



2



3



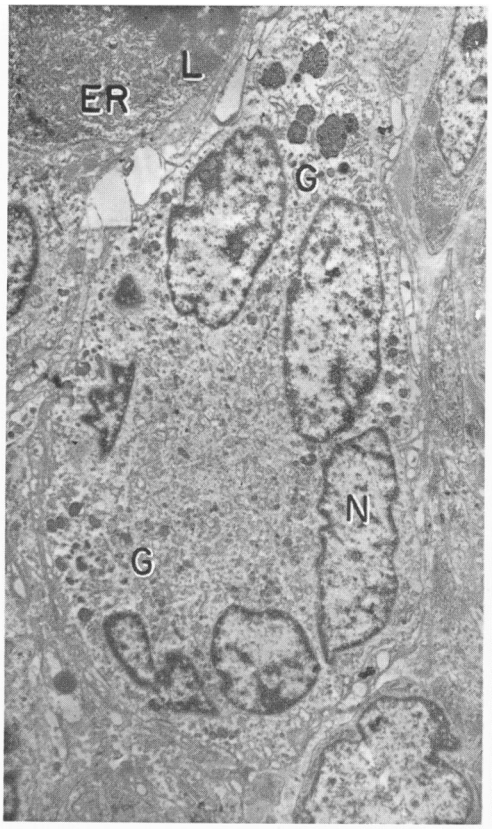
4

FIG. 4. Cytoplasmic granules in synovial giant cells; this is the same case shown in Figure 1. Diastase, periodic acid-Schiff stain. × 690.

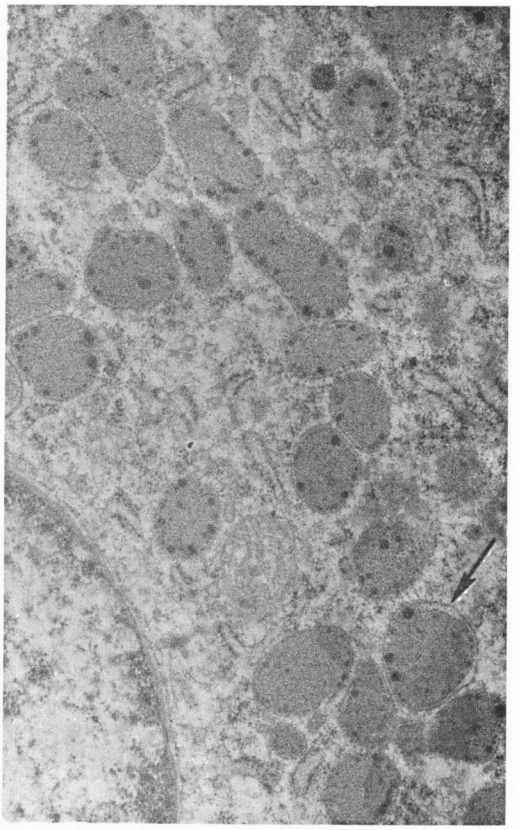
FIG. 5. A deep giant cell. This is the formalin-fixed specimen illustrated in Figures 1 and 4. Nuclei (N) are arranged in a peripheral ring. A number of large irregular granules (G) are clustered in a cytoplasmic process at the upper pole. Smaller granules (G) are sprinkled between nuclei and the central cytoplasm. An adjacent lipid-laden cell (L) contains prominent granular endoplasmic reticulum (ER). Uranyl acetate and lead citrate stain. × 3,400.

FIG. 6. The periphery of a giant cell illustrates the larger, more complex form of granules. Irregular granule profiles and an occasional stacked arrangement (*) are evident. Surface filopodia (F) are numerous. Portions of 2 nuclei (N) intrude at the lower margin of the micrograph. Lead citrate stain. × 17,900.

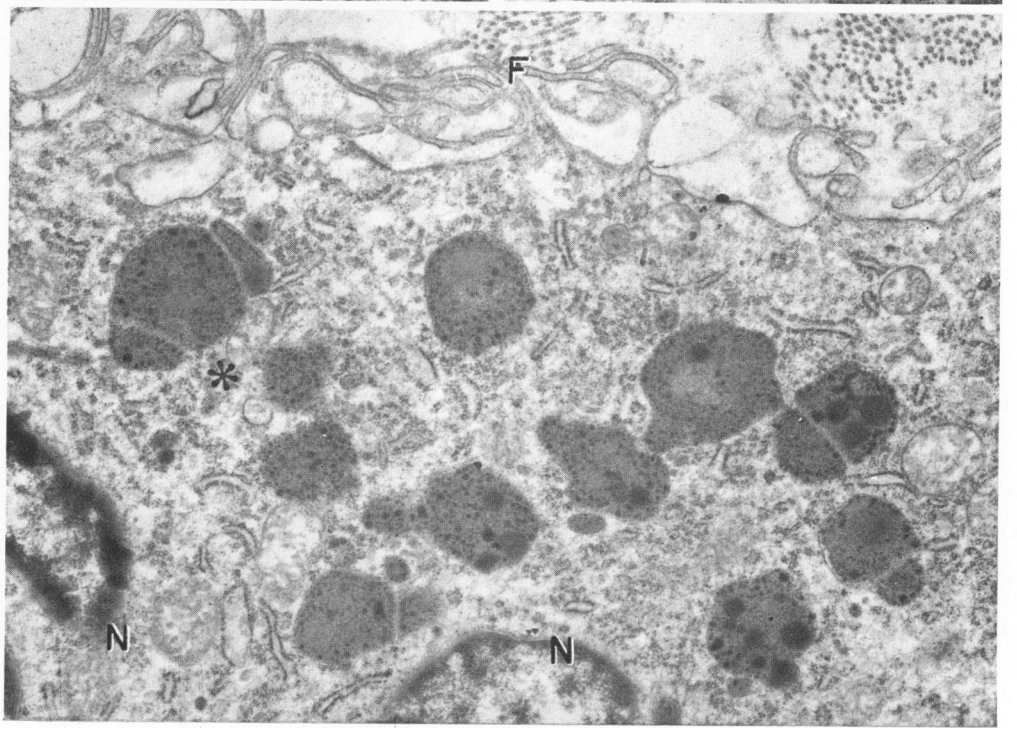
FIG. 7. Detail of small granule forms. The matrix is moderately electron dense and stippled at the edge by compact, electron opaque inclusions. The limiting membrane of these granules is well demonstrated in a zone of artifactual separation from the matrix (arrow). Lead citrate stain. × 28,400.



5



7



6

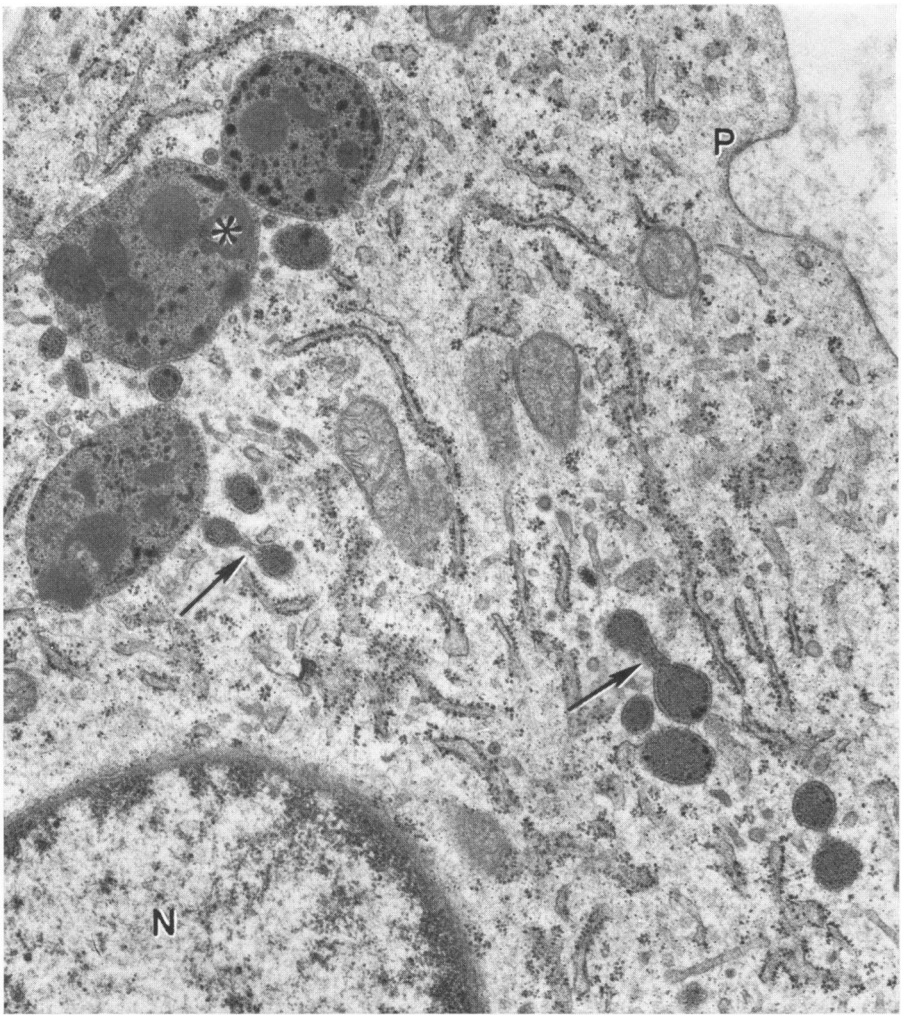


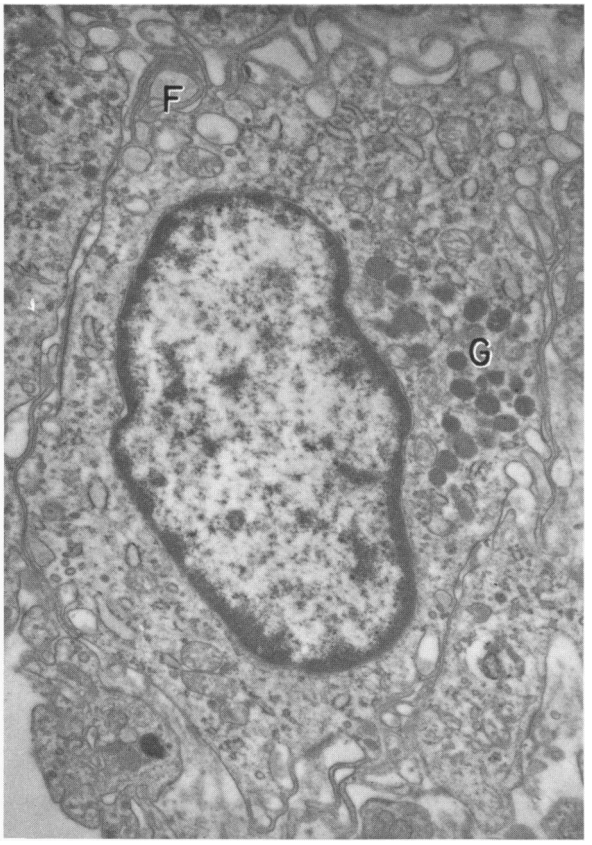
FIG. 8. Granules in a giant cell fixed with glutaraldehyde, the same case shown in Figure 2. Large granule forms (*) contain globular inclusions which are often less dense than the surrounding matrix. Cisternae of granular endoplasmic reticulum are filled with dense substance. Arrangement of small granules suggests budding or fusion of contents (arrows). A "pit" (P) in the surface membrane is associated with fluffy extracellular material. Uranyl acetate and lead citrate stain. × 26,800.

FIG. 9. A typical macrophage-like surface cell in rheumatoid synovium. Joint space is at the upper right. Distinguishing features include numerous filopodia (F) and small granules (G). The cytoplasm contains relatively sparse ergastoplasm. Uranyl acetate and lead citrate stain. × 9,700.

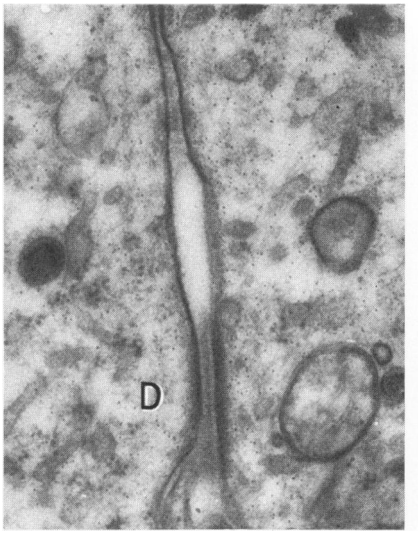
FIG. 10. Filopodial processes (F) from several adjacent macrophage-like surface cells interlock in an elaborate manner. Small granules are abundant in these cells. The limiting membrane and minute opaque inclusions are clearly visible features of the granule in the cell at the upper left. Uranyl acetate and lead citrate stain. × 20,300.

FIG. 11. Detail of a desmosome (D). Cell membranes are thickened in the region of attachment. An equatorial condensation subdivides the intercalated cement substance. Uranyl acetate and lead citrate stain. × 40,700.

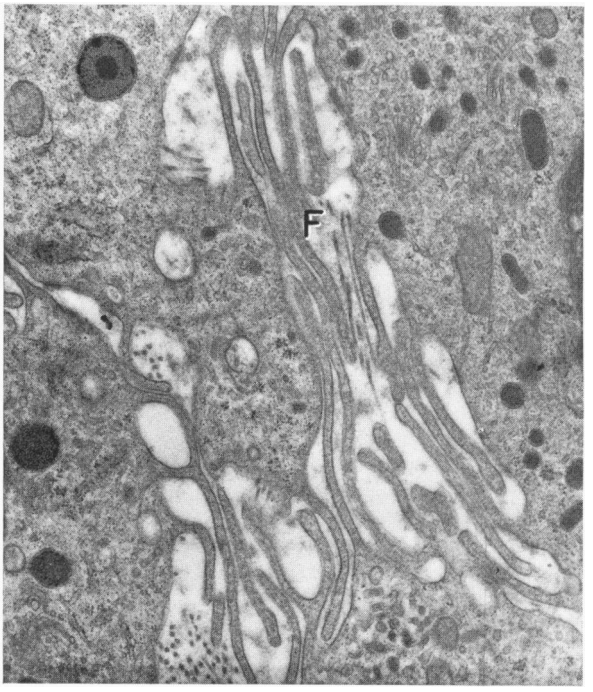
FIG. 12. A series of desmosomes between two surface cells of the macrophage type. Smooth vesicles (V) are numerous just below the plasmolemma. The cell at the left contains small granules (G). Uranyl acetate and lead citrate stain. × 29,500.



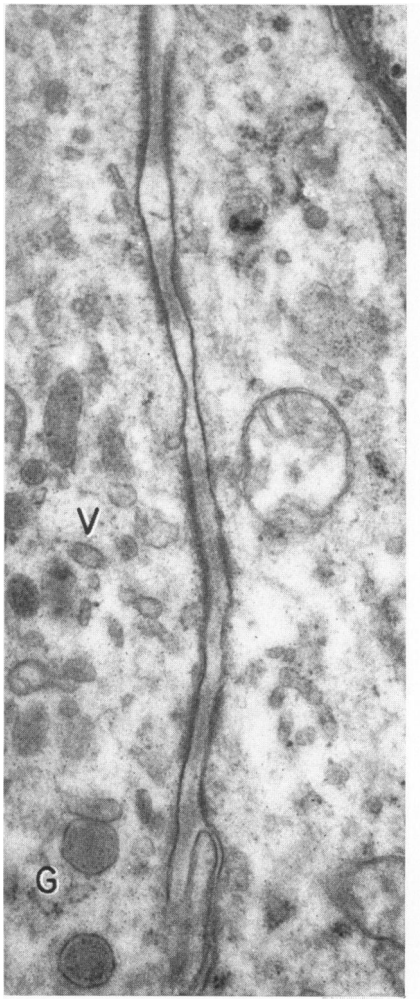
9



11

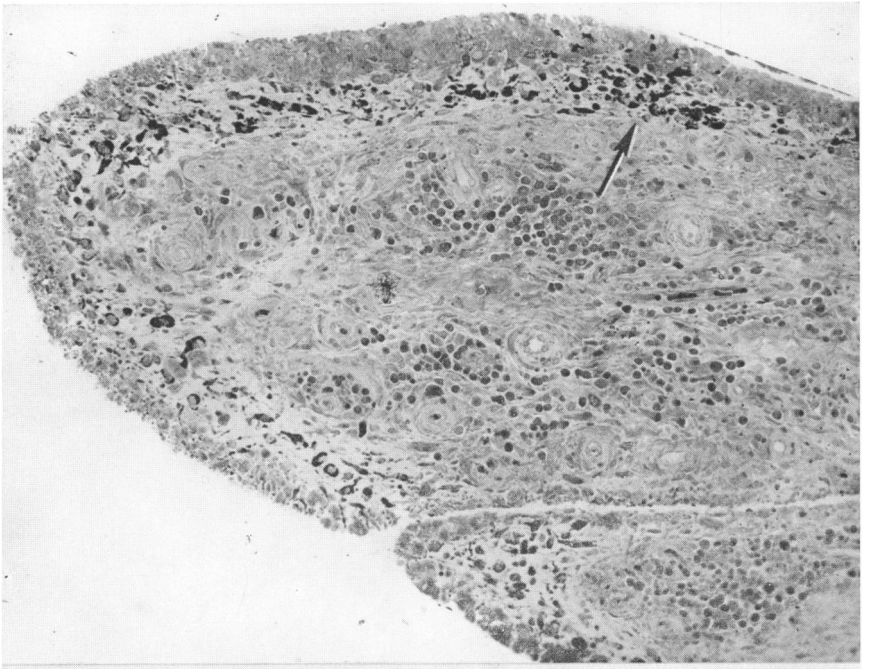


10



12

13



14

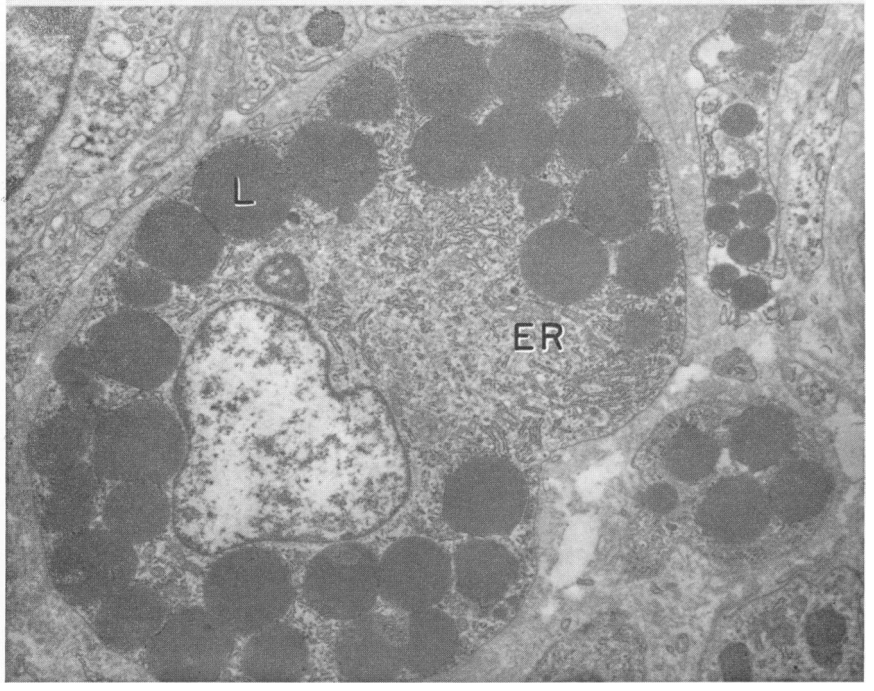


FIG. 13. Rheumatoid synovium in a thin Araldite section, formalin-fixed, post-osmicated. Lipid-laden cells (arrow) in the deep portion of the lining of a hyperplastic villus are highlighted by reaction with paraphenylenediamine. × 108.

FIG. 14. Electron micrograph of a deep cell. Lipid droplets (L) are abundant in the outer rim of cytoplasm. The granular endoplasmic reticulum (ER) is well developed. (See also Figure 5). × 6,200.