FINE STRUCTURE AND CYTOCHEMISTRY OF THE RHEUMATOID SYNOVIAL MEMBRANE, WITH SPECIAL REFERENCE TO LYSOSOMES

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The synovial membrane in active rheumatoid arthritis generally shows characteristic features in hematoxylin and eosin stained sections. Multiple villi contain proliferated lining cells and numerous lymphocytes and plasma cells. Increased vascularity is present and, occasionally, areas of necrosis and fibrinoid degeneration are found at the surface. With more active chronic inflammation, the membrane may show all these changes and areas of dense fibrosis as well. No single feature can be considered diagnostic.

New thoughts about the pathogenesis of rheumatoid arthritis have arisen from recent studies. Mellors, Nowoslawski, Korngold and Sengson,[1] using specific fluorescent reactants, demonstrated the presence of rheumatoid factor in some plasma cells in the synovial membrane. The changes from the normally ordered arrangement of lining cells at the surface of the synovial membrane, and the thickening and blunting of the cell processes were revealed by cytochemical methods.[2] Kulka [3] suggested that focal ischemia due to acute venulitis and capillaritis with fibrin thrombosis contributed to areas of focal necrosis.

Electron microscopic study of normal human synovial membrane [4] has revealed two cell types in the lining: (1) A predominant type A cell contains numerous vacuoles, including pinocytotic vacuoles, and a prominent Golgi apparatus. The cells possess many finger-like extensions, filopodia, which protrude into and surround portions of the extracellular matrix. (2) A less plentiful type B cell contains abundant ergastoplasm, less extensive Golgi apparatus, fewer vacuoles and few filopodia. The lining cells form a layer 1 to 3 cells deep; their long tapered processes extend toward the surface, intertwining and forming a discontinuous layer of cytoplasm at the margin of the synovial membrane facing the joint cavity. These findings form a basis of comparison for the fine structure of lining cells in the rheumatoid synovial membrane.

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Materials and Methods

Specimens of synovial membrane were obtained during surgical arthrotomy from the knee joints of 5 patients with active rheumatoid arthritis. Four of the patients had subcutaneous nodules, 3 had positive latex fixation tests, and roentgenograms in all 5 showed joint changes consistent with rheumatoid arthritis. The synovial membrane in all 5 showed gross and light microscopic features characteristic of rheumatoid arthritis. For comparison, specimens of synovial membrane were obtained from normal, osteoarthritic, or traumatized but otherwise normal knee joints.

At arthrotomy the synovial surface was identified *in situ* by a needle inserted into the capsular side and made to emerge at the free inner surface. A portion of the synovial membrane around the needle was removed and quickly fixed, with the needle in place, in cold 1 per cent osmic acid-veronal buffer (pH 7.2) made isotonic with sucrose (0.22 M), for 2 hours. Following rinsing in veronal buffer, 1 cu. mm. blocks of the synovial surface were dissected out under a low power microscope. This was a simple matter since the needle point marked the synovial surface. The small blocks were dehydrated in ethanol and embedded in a 1:7 mixture of methyl and butyl methacrylate containing 0.75 per cent uranyl nitrate,[5] and, in 2 instances, in Epon 812.[6] Phase contrast examination of sections cut at 1 to 2 μ was used to identify the synovial lining. Thin sections (400 to 600 Å) of areas containing lining cells were cut on an LKB Ultrotome with diamond knives. Methacrylate sections were stained with aqueous potassium permanganate,[7] and sandwiched between two layers of carbon for study in an RCA EMU-3B electron microscope. Epon sections were stained with potassium permanganate and lead citrate [8] and examined on naked grids.

For determination of acid phosphatase activity by light microscopy, specimens were fixed overnight in cold 4 per cent formaldehyde-1 per cent calcium chloride.[9] Frozen sections were cut at 10 μ and were incubated in a freshly prepared Gomori medium,[10] generally for 30 to 90 minutes at 37° C. In this medium, enzymatically-liberated phosphate ions are trapped by lead ions to form insoluble lead phosphate. This is converted to black lead sulfide by brief immersion in dilute ammonium sulfide solution. To examine the intracellular distribution of acid phosphatase reaction product with the electron microscope, one specimen was fixed in cold 5 per cent glutaraldehyde [11] for 90 minutes and sectioned at 40 μ on a freezing microtome. Following incubation in the Gomori medium for 8 minutes at 37° C., the sections were postfixed in osmium tetroxide, embedded in Epon 812 and thin sections prepared. They were examined before as well as after staining by lead citrate.

Observations

By light microscopy the lining cells of both normal and pathologic synovial membranes contained abundant acid phosphatase activity confined to cytoplasmic granules considered to be lysosomes.[2] In the lining cells of the rheumatoid synovial membrane, the lysosomes were more numerous (Fig. 1) and much larger, attaining a diameter of 1 μ.

Electron microscopy demonstrated numerous differences in the fine structure of type A lining cells in rheumatoid arthritis as compared with those of normal. The most striking feature was the presence of complex cytoplasmic granules, 0.4 to 3.2 μ in diameter. These contained a dense granular material and numerous inclusions in the form of vacuoles and membranous whorls (Figs. 4 to 6). The dense bodies were pres-

ent throughout the cytoplasm but appeared most numerous in the perinuclear region and proximal portions of the cell. Although the number of lining cells containing these bodies varied from one specimen to another, in all rheumatoid membranes examined many were present. The dense bodies were easily differentiated from the large vacuoles commonly noted in normal or osteoarthritic type A synovial lining cells which were less dense and did not contain membranous structures.[4,12,13] Occasionally rheumatoid lining cells showed such large vacuoles.

In the rheumatoid synovial membrane examined for distribution of acid phosphatase by electron microscopy, the reaction product was confined to large cytoplasmic granules in some of the type A cells. These granules corresponded in size and location to the complex dense bodies (Figs. 2 and 3). Their identification was more firmly established by the observation of membranous whorls and vacuolar structures within some of the granules exhibiting acid phosphatase activity (Fig. 3).

Other differences between type A cells of rheumatoid arthritic and those in normal or osteoarthritic membranes[4] may be briefly summarized. The Golgi apparatus was smaller as judged by its infrequent presence in most of the rheumatoid type A cells studied. There were fewer of the large moderately dense vacuoles adjacent to the Golgi apparatus. Fewer filopodia were present. Some of the mitochondria appeared swollen and contained fewer cristae, and the cristae occasionally appeared disrupted and shortened. Large clear vacuoles were present in some of the lining cells. On occasion these vacuoles occupied a major portion of the cytoplasm. The type B cells appeared to have the same structure in the rheumatoid as in the normal and osteoarthritic membranes.

The lining cells in the synovia of rheumatoid arthritis were packed together more closely than in the normal membrane and extended to a depth of 2 to 6 cells. Collagen fibers were present in the intercellular matrix in the deeper layers only.

Discussion

The prominent departures from normal in the fine structure of the lining cells in rheumatoid synovitis may reflect phases of the evolution and progression of this disease. However, the interpretation of these changes is made particularly difficult because there is no comparable description of the lining cells in other pathologic states of the synovial membrane. Moreover, concepts of the function of the lining cells are based on indirect evidence obtained from histologic studies of the synovial membrane[14–16] and on interpretations from the electron microscopic findings.

Light microscopic studies revealed increased lysosomal activity in rheumatoid synovial lining cells.[2] The lysosomes were more numerous and larger than those in normal or osteoarthritic lining cells. Chemical assays for acid phosphatase activity revealed increased levels in the rheumatoid synovial membrane,[17] and in rheumatoid synovial fluids[18] when compared with normal controls. Electron microscopy showed intracytoplasmic dense bodies with acid phosphatase activity and numerous inclusions. They thus bore a striking resemblance to the structures called cytolysomes[19] (or autophagic vacuoles[20]) and residual bodies. Such structures have been described in cells of many organs including liver,[21,22] pancreas,[23] kidney,[24] endometrium,[25] intestine[26] and brown fat.[27] They are presumed to represent, in the case of the cytolysome, focal areas of cytoplasmic degeneration in response to noxious or physiologic stimuli, and in the case of the residual bodies, the indigestible residues of intracellular digestion.[19,20,28]

There is a possibility that these bodies in the type A synovial cells represent breakdown products of phagocytized material. These cells have been shown to take up material from the synovial fluid.[12] Muirden[13] recently reported that ferritin injected into the rabbit knee joint is localized within these cells. We have made similar studies with the human knee joint, confirming these findings. In addition, we have observed that the filopodia of the type A cells often give the appearance of engulfing portions of extracellular material. One can only speculate, at this time, on the nature of the substances that might be taken up by the lining cells in rheumatoid arthritis. Bodies resembling those seen in rheumatoid type A lining cells appear in the liver cells of mice inoculated with hepatitis virus.[29] Moreover, viral infection of monkey kidney and mouse liver cells causes increased concentration of hydrolytic enzymes and apparent solubilization from lysosomes.[30] Although cytolysomes and residual bodies may arise from widely different processes, their presence in type A synovial cells may stimulate renewed efforts to isolate a specific virus as the etiologic agent in rheumatoid arthritis.

Conceivably the lysosomes in the lining cells might contain parenterally administered gold previously ingested by the cells. Gold was used in the treatment of all 5 patients studied. Tonna, Brecher, Cronkite and Schwartz,[31] using radioautography, showed that systemically administered Au^{198} was concentrated, among other sites, in the rabbit synovial lining cells.

Some observations suggested that sequestration of damaged or altered elements within the cytoplasm of the lining cells produced the cytolysome or residual bodies. Fewer mitochondria were seen in those cells contain-

ing numerous dense bodies (Fig. 4). These mitochondria, in some instances, appeared abnormal. Their cristae seemed shortened and disrupted, and, in at least one mitochondrion, a dense inclusion was observed.

Changes in mitochondria and areas of cytoplasmic degeneration conceivably leading to loss of normal cellular compartmentation[32] may partly account for the altered pattern of metabolism observed when synovial membrane from active rheumatoid arthritis is studied *in vitro*, viz., high aerobic glycolysis and lactate production, and failure of air to suppress glycolysis (Pasteur effect).[33,34] Cytochemical studies have also shown increased activity of glyceraldehyde-3-phosphate dehydrogenase in rheumatoid synovial cells, while, with similar methods, there were little or no demonstrable succinic dehydrogenase and isocitric dehydrogenase activities.[2]

We have previously suggested that normal type A cells are structurally adapted for active secretory and absorptive interchange with components of the extracellular matrix and synovial fluid. Similar activities of lining cells in normal synovial membrane were suggested by Langer and Huth[35] and Chapman and Muirden.[12] The observations reported here suggest altered activities of type A lining cells in rheumatoid arthritis. One function of the synovial cells is thought to be the synthesis of hyaluronate, and thus their abnormal structure may be related in some manner to the secretion of an abnormal product. From rheumatoid synovial fluid there has recently been isolated a compound of hyaluronate and protein, containing more protein than normal, and showing unusual physical properties.[36]

Summary

Electron microscopic and cytochemical studies of the synovial lining cells in rheumatoid arthritis revealed prominent changes in the type A cells. There were fewer filopodia, a smaller Golgi apparatus and altered mitochondria. Many cells exhibited large numbers of cytoplasmic granules not present in normal lining cells. These granules were characterized by variable electron-opaque content, often membranous in appearance. The granules contained high levels of acid phosphatase activity and may tentatively be considered "residual bodies."

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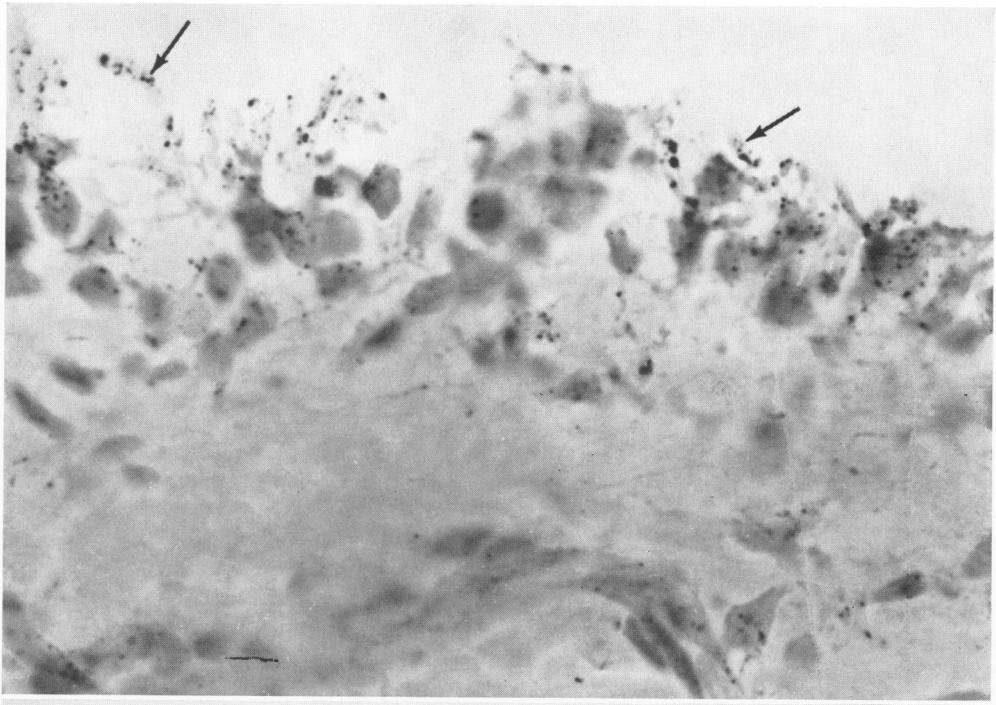
[*Illustrations follow*]

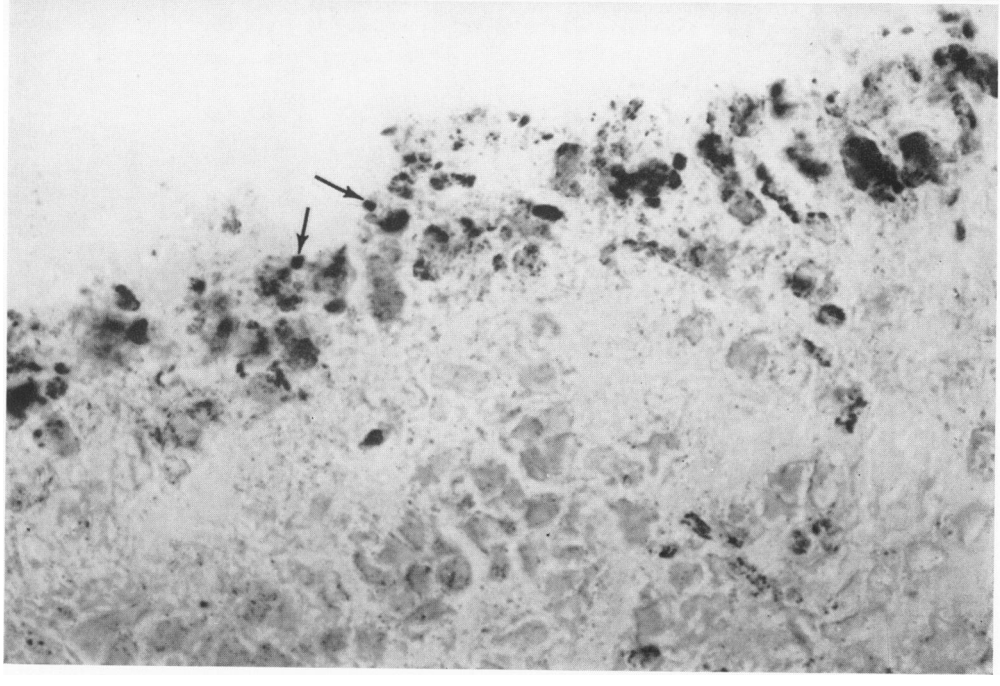
LEGENDS FOR FIGURES

Unless stated otherwise, all electron micrographs are of sections from tissue embedded in 7:1 mixture of butyl and methyl methacrylate containing 0.075 per cent uranyl nitrate [5] and stained with aqueous potassium permanganate.[7]

FIG. 1. Two human synovial membranes fixed in cold formol-calcium and incubated for acid phosphatase activity. A. Normal membrane. Most of the enzyme activity is confined to small granules (lysosomes) within the cytoplasmic processes of the lining cells (arrows). × 490. B. Rheumatoid membrane. Lysosomes are in greater abundance and are larger in size (arrows) in the lining cells. The underlying chronic inflammatory cells exhibit little acid phosphatase activity. × 490.



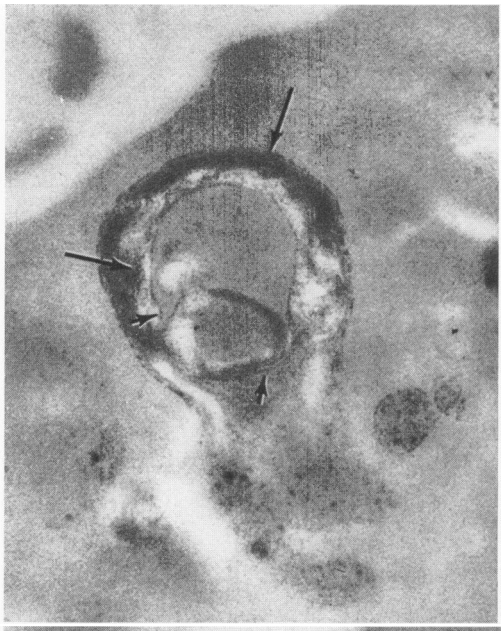
1A



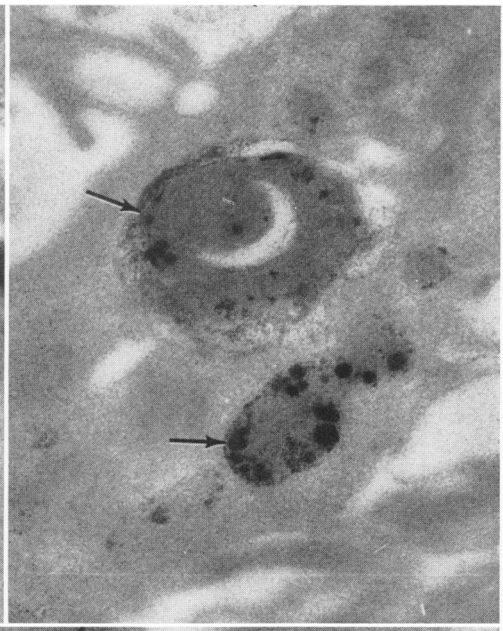
1B

FIG. 2. A portion of a type A lining cell in rheumatoid arthritis following incubation for acid phosphatase activity. Though there is considerable loss of ultrastructural detail, dense precipitates of lead phosphate may be seen in cytoplasmic bodies similar in size and density to those seen in unincubated tissue. A. A large cytoplasmic granule contains membranous structures (short arrows) and acid phosphatase reaction (long arrows). B. Two cytoplasmic granules contain reaction product (arrows). × 35,000.

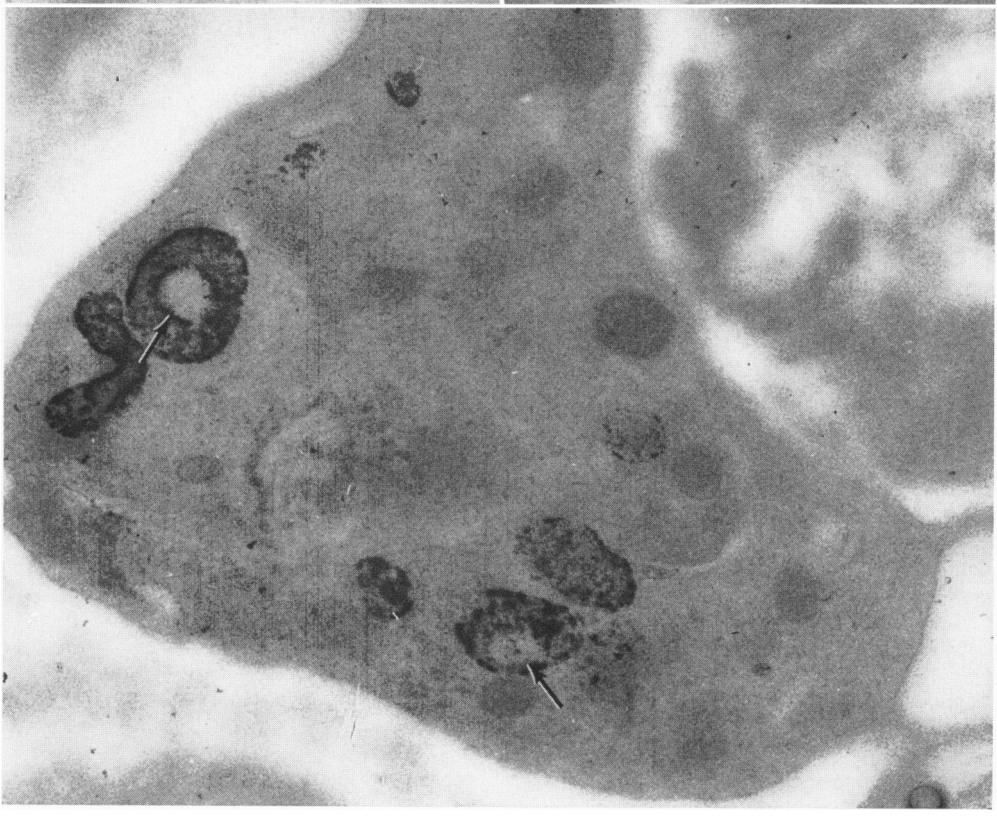
FIG. 3. Part of a type A cell incubated for acid phosphatase activity. Reaction product is confined to cytoplasmic granules. In two of the granules (arrows) vacuoles are seen which do not contain any precipitate. × 20,000.



2A



2B



3

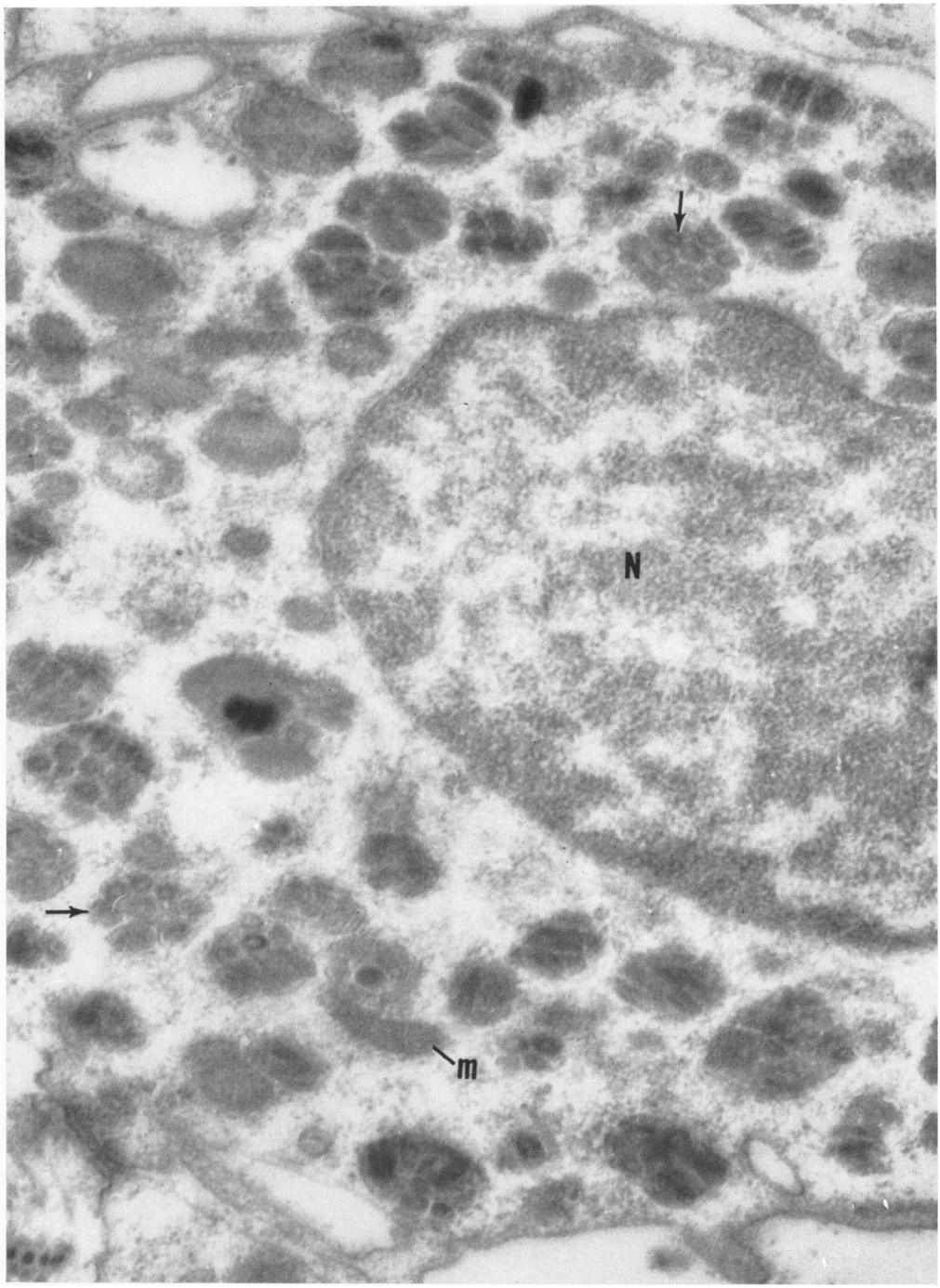


FIG. 4. A rheumatoid type A cell contains numerous cytoplasmic dense bodies. Within these bodies inclusions appear. They generally take the form of whorls of parallel membranes (arrows). A portion of the nucleus is seen at N. Fewer recognizable mitochondria (m) are present in this cell. × 42,000.

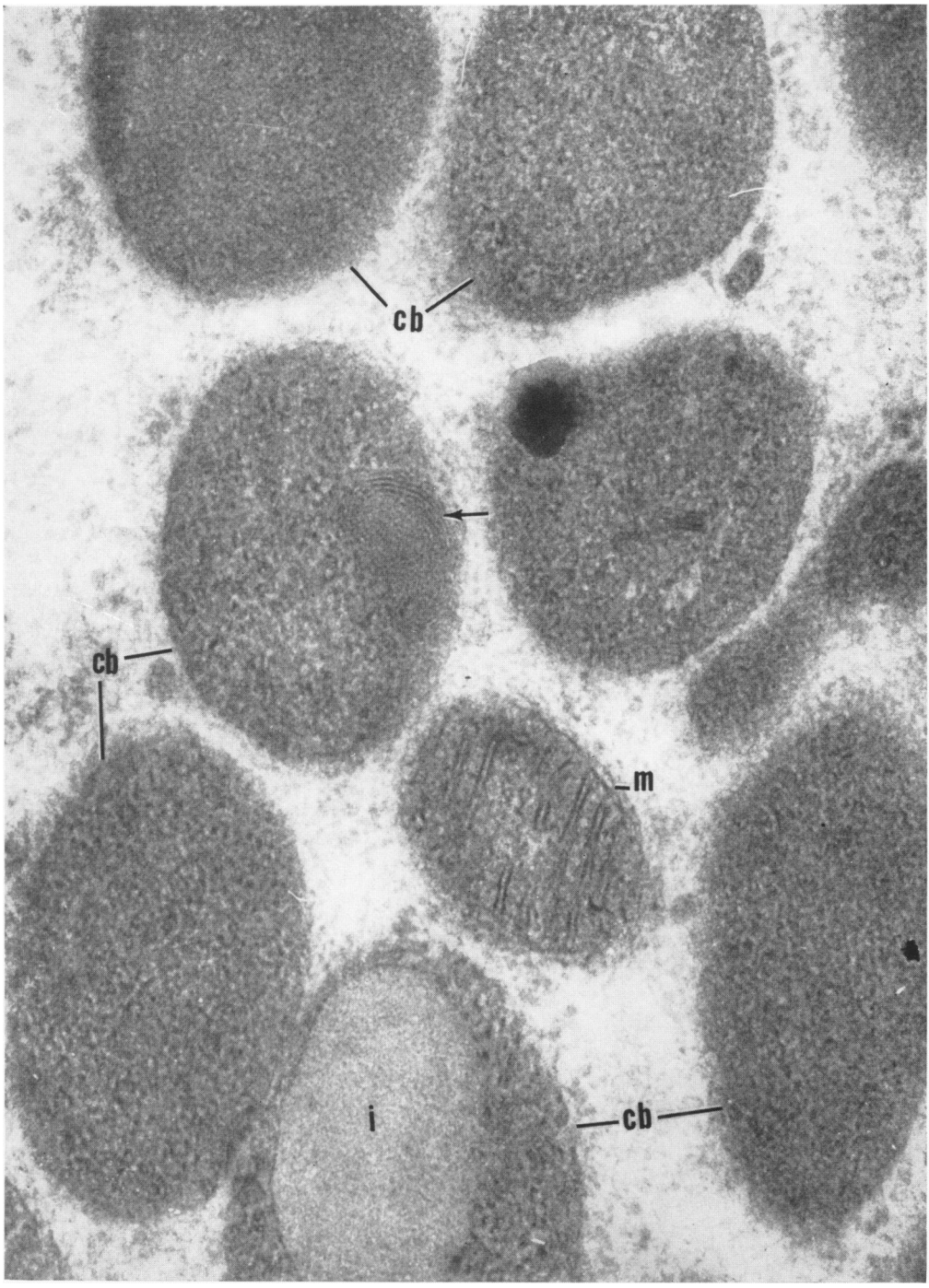


FIG. 5. Area in a type A lining cell with numerous dense cytoplasmic bodies (cb). One contains a membranous array (arrow); another, a membrane-bound ovoid inclusion (i). Normal mitochondrion, m. The matrix of the dense bodies and the mitochondrion are similar in opacity and granularity. × 88,000.

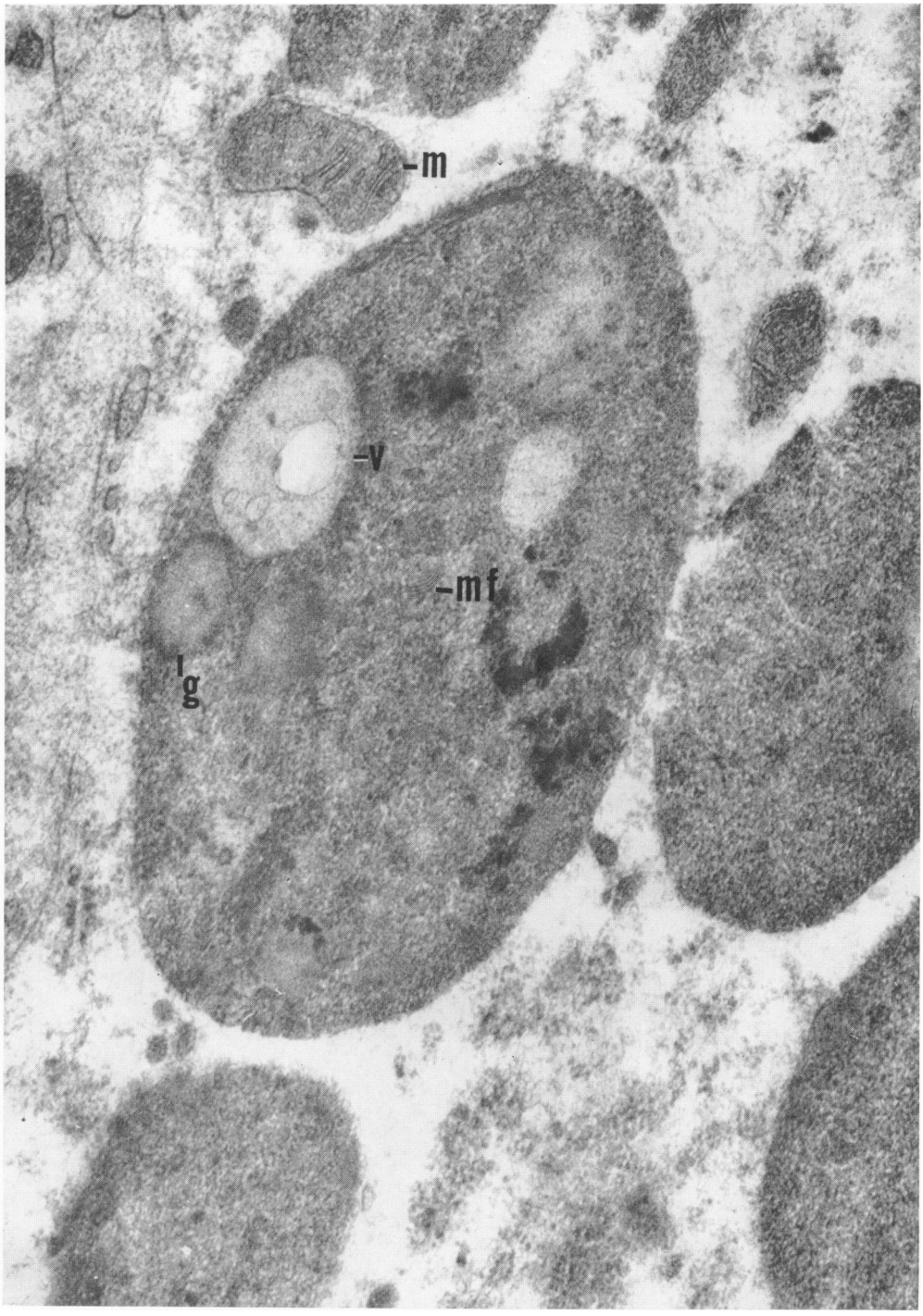


FIG. 6. Portion of a type A cell. The large size and complexity of some cytoplasmic inclusions are evident. Within a single inclusion are a membranous array (mf), and membrane-bound vacuolated structure (v) and densely granular ovoid structures (g). Normal mitochondrion, m. × 50,000.