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Synovial plasma cells in rheumatoid arthritis

Electron microscope and immunofluorescence studies

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Electron microscopic analyses of the rheumatoid synovial membrane have been carried out by several investigators (Hirohata and Kobayashi, 1964; Barland, Novikoff, and Hamerman, 1964; Norton and Ziff, 1966; Wyllie, Haust, and More, 1966; Highton, Caughey, and Rayns, 1966; Ghadially and Roy, 1967), who have given minute description of the ultrastructural changes in the synovial lining layer. The fine structural details of the subsynovial tissue, in particular in the plasma cells have not been so carefully described. Norton and Ziff (1966) demonstrated a group of plasma cells but did not comment on their submicroscopical features. It is the purpose of this paper to describe the ultrastructural changes in rheumatoid synovial plasmocytes which are known to be the cellular sites of rheumatoid factor formation (Mellors, Heimer, Corcos, and Korngold, 1959; Mellors, Nowoslawski, Korngold, and Sengson, 1961; Kazakova, Orlovskaya, and Pavlov, 1967). Preliminary reports on this subject have already been published (Orlovskaya, 1969; Orlovskaya, Muldiyarov, and Kazakova, 1969).

Material and methods

Twenty synovial membrane biopsies were obtained at synovectomy from the knee joints of patients with seropositive rheumatoid arthritis. Specimens were removed from the regions where the synovial membrane showed marked villous proliferation, which have been found by previous immunofluorescent studies to contain rheumatoid factor (Kazakova and others, 1967).

The tissue samples were divided into two parts for immunofluorescent study and electron microscopy.

IMMUNOHISTOCHEMISTRY The fresh tissue blocks were cooled by dry ice and sectioned in the cryostat at − 18°C.; sections were mounted on slides, fixed in absolute acetone at room temperature, placed in the thermostat at 37°C. for 1 hour, stored at 4°C., treated by fluorescein-labelled aggregated human γ-globulin, and examined under the light microscope ML-2 (USSR). In order to compare the same areas in light microscope parallel sections were stained with haematoxylin and eosin, methyl green-pyronin, toluidine blue, and sudan black.

ELECTRON MICROSCOPY Pieces of villous synovial membrane were fixed in 3 per cent. phosphate buffered glutaraldehyde (Sabatini, Bensch, and Barnett, 1963) for 1 hour at room temperature, followed by 1 per cent. ice-cold osmium tetroxide (Millonig, 1962) for 1½ to 2 hours. After dehydration in ethanol, the tissue blocks were embedded in methacrylate or araldite mixture, and 0·5 to 2 μ sections were cut from the methacrylate blocks and stained with methyl green-pyronin stain and/or toluidine blue to locate the plasmocyte infiltrates in the subsynovial tissue. Ultrathin sections were cut on a LKB 4801 microtome from the selected areas, stained with lead citrate (Reynolds, 1963), and examined with a JEM-7 electron microscope at 80 kV.

Results

Light microscopy and immunohistochemistry

The subsynovial tissue under the hyperplastic lining layer of villous synovial membrane contained many plasma cells, lymphocytes, and macrophages. The plasma cells were usually found in dense accumulations along the blood vessels. In these infiltrates immature and mature plasma cells with the pyroninophilic clumps in cytoplasm often fitted to each other so closely that angular (polygonal) outlines appeared. In some areas diffusely pyroninophilic cytoplasms of the plasma cells did not have clear profiles, and nuclei did not show the characteristic spoke-like distribution of chromatin; there were also many plasmocytes with obvious disintegrated cytoplasm, pyroninophilic clumps being found in the intercellular space. Some of the plasma cells in such infiltrates contained typical Russell bodies, and these were sometimes seen in the extracellular spaces among the clumps of pyroninophilic material. Both

intracellular and extracellular Russell bodies were periodic acid-Schiff-positive, weakly pyroninophilic globules. These globules showed marked variation in size, some of extracellular ones being occasionally larger than the young plasmocytes.

Macrophages usually accumulated in areas where the plasmocytes showed marked disintegration of cytoplasm. These were large cells with oval to round clear nuclei. Their cytoplasm was broad, without obvious outlines, and sometimes contained fine inclusions which stain pale with pyronin and periodic acid-Schiff.

Rheumatoid factor was mainly found in the cytoplasm of mature plasma cells (Fig. 1), in small flocculates around degenerating plasma cells that corresponded to the clumps of extracellular material, and in both intracellular and extracellular Russell bodies. The Russell bodies usually appeared as bright green globules with clear-cut borders.

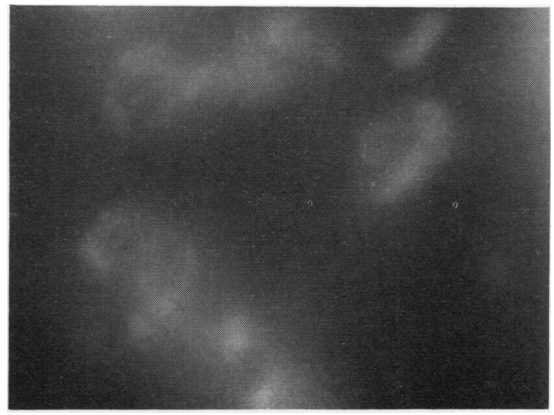

FIG. 1 *Bright fluorescent plasma cell of rheumatoid synovial membrane. Stained with fluorescein-labelled aggregated human γ-globulin.* × 720.

Electron microscopy

The plasma cells of mononuclear cell infiltrates along the blood vessels were usually adjacent to each other. Many were characterized by a tight arrangement of parallel rough endoplasmic reticulum lamellae. Oval to spheroid mitochondria and free ribosomes were situated between the lamellae. An extensive Golgi zone adjacent to the eccentric nucleus comprised numerous fine vesicles containing a moderately osmiophilic substance (Fig. 2). Some plasma cells had two nuclei.

Endoplasmic reticulum cisternae were dilated in many plasma cells. These cisternae contained material of moderate electron density (Fig. 3). Such cells often had numerous cytoplasmic processes with narrowed stems.

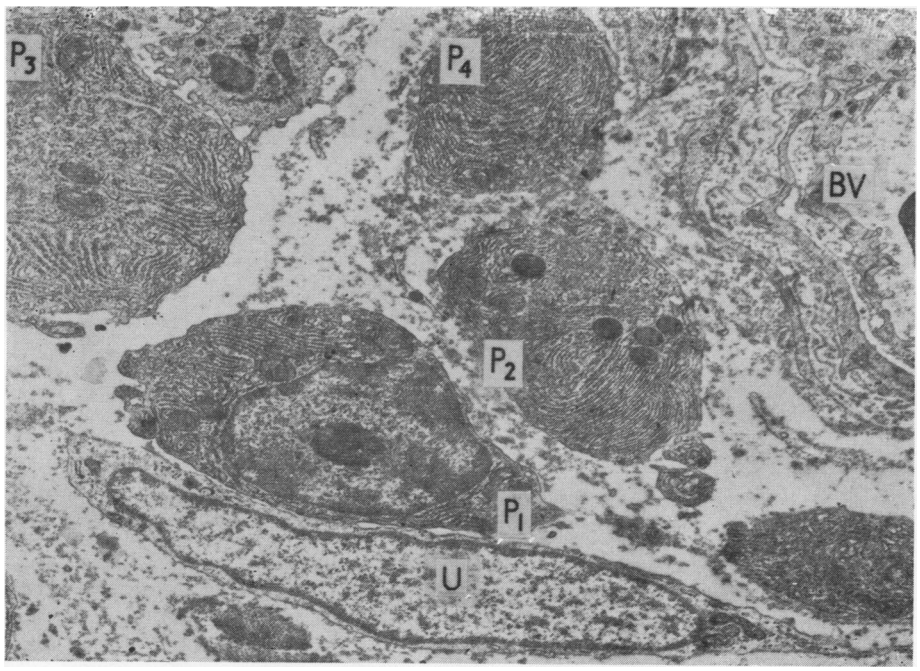

FIG. 2 *Electron micrograph of young plasma cells (P_1 to P_4) near subsynovial blood vessel (BV). Note spoke-like distribution of chromatin in P_1, extensive Golgi zones in P_2 and P_3, dense lysosome-like inclusions in P_2. U = undifferentiated cell.* × 7,250.

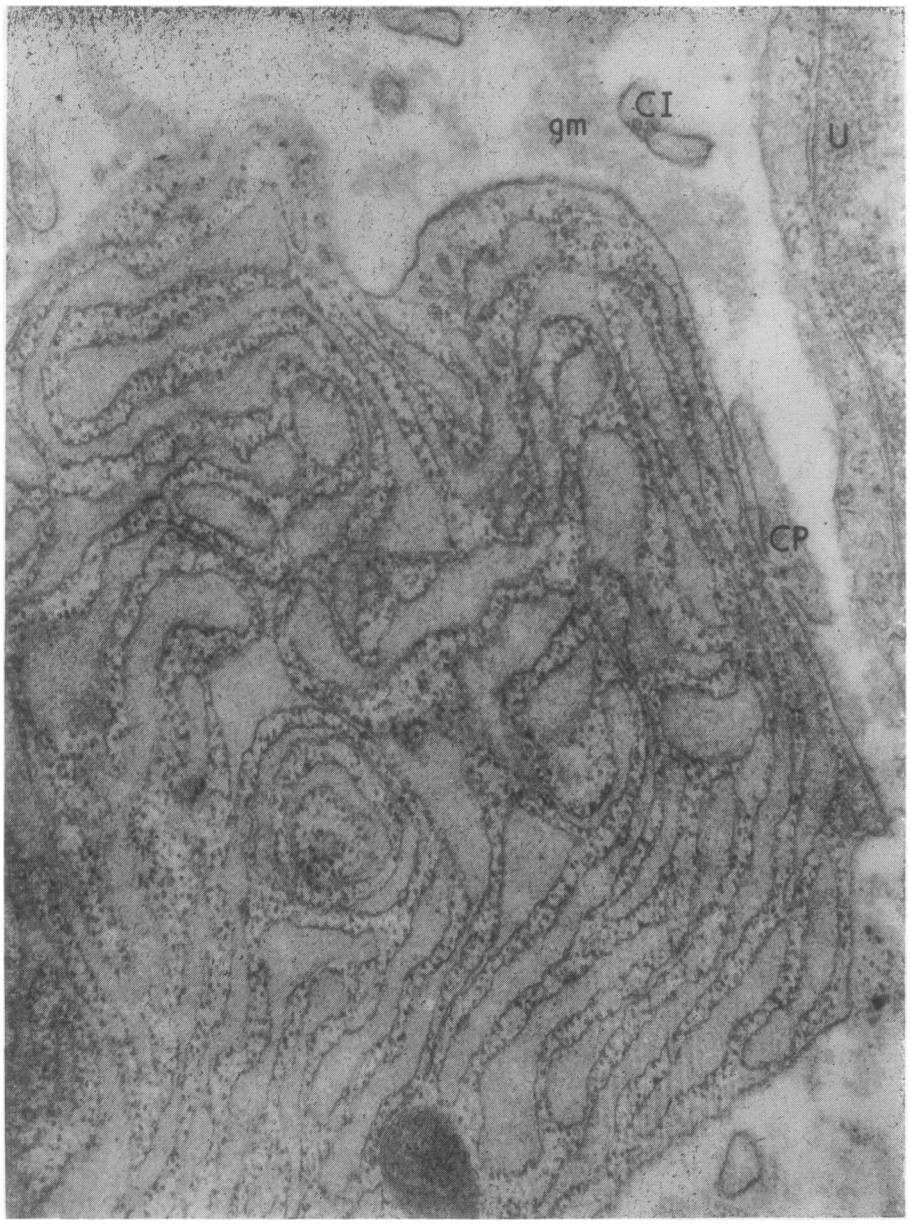

FIG. 3 *Dilated ergastoplasmic cisternae of plasma cell containing moderately osmiophilic substance. U = undifferentiated cell. Cytoplasmic process (CP) and islets (CI). Fine granular material (gm) in extracellular space.* × 46,000.

There were also many oval to elongated cytoplasmic islets around these plasma cells, sometimes at some distance from the cell body. It is suggested that these islets are formed by separation from the cytoplasmic processes. The processes and the islets both contained rough endoplasmic reticulum cisternae, mitochondria, and free ribosomes. The islets gradually lost their surrounding membrane and became amorphous, moderately osmiophilic masses dispersed in the intercellular spaces (Fig. 4).

The endoplasmic reticulum cisternae of some plasma cells were dilated so that the cells seemed to be divided into numerous polygonal sectors. The more the cisternae are dilated the fewer are the ribosomes seen in their membranes.

Some plasma cells contained Russell bodies, which are osmiophilic round structures within the cisternae of the endoplasmic reticulum, of widely varying number and size (Fig. 5).

Most plasma cells with many small Russell bodies or a few large ones had ordinary plasmocyte organelles (Fig. 6). Some plasma cells containing Russell bodies showed obvious features of degeneration, such as decrease and degranulation of the endoplasmic reticulum, swelling of the mitochondria, hydration of cytoplasm, and margination of the nuclear chromatin. In such cases, it seemed that the Russell bodies lay in the cytoplasm and not in the endoplasmic reticulum cisternae.

Areas of disintegration in the plasma cells were

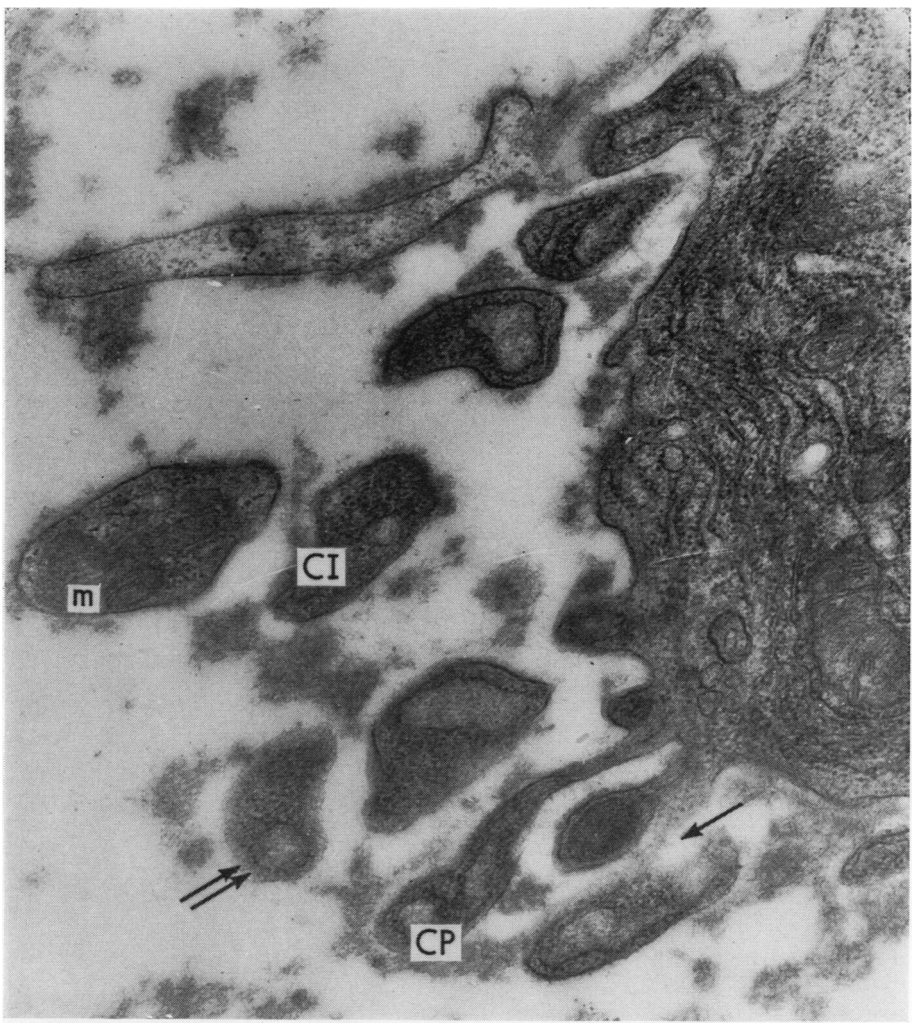

FIG. 4 *Cytoplasmic processes (CP) and islets (CI) of a plasma cell, containing dilated ergastoplasmic cisternae, free ribosomes, and mitochondria (m). One such process is breaking away from the plasma cell body (arrow). Some islets have indistinct plasma membranes (double arrow).* × 51,300.

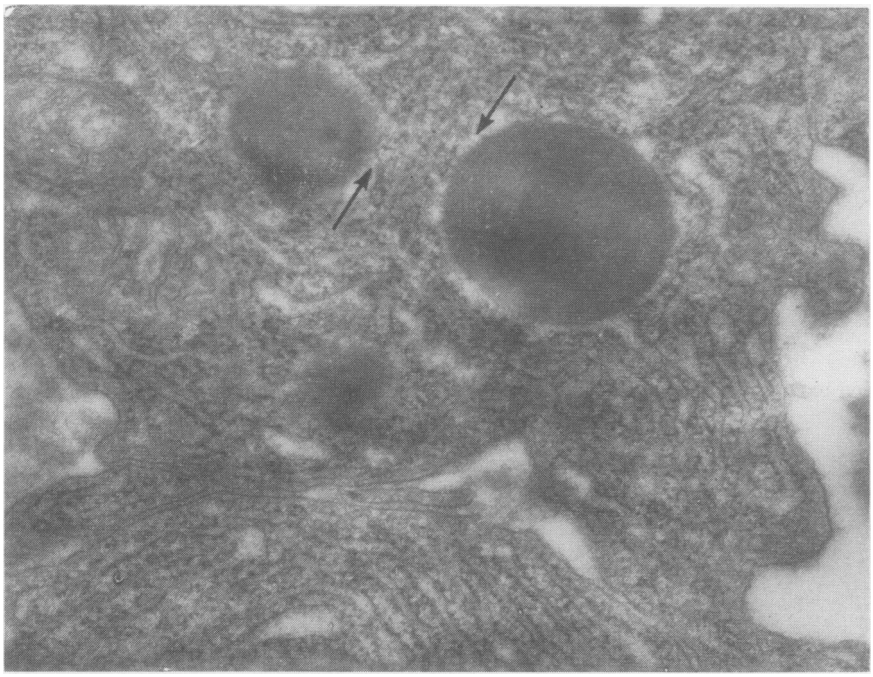

FIG. 5 *Three Russell bodies at different stages of formation within the ergastoplasmic cisternae. Fine fibrils extend from the ergastoplasmic membrane to the Russell body surface (arrows).* × 37,700.

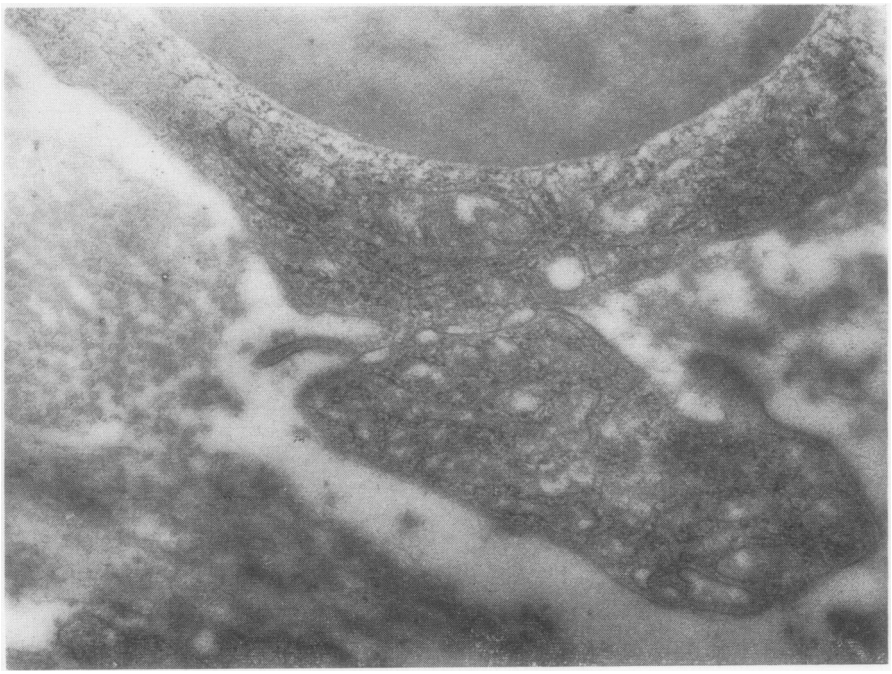

FIG. 6 *Part of a giant Russell body in a plasma cell. Note that the organelles are not degenerative.* × 32,300.

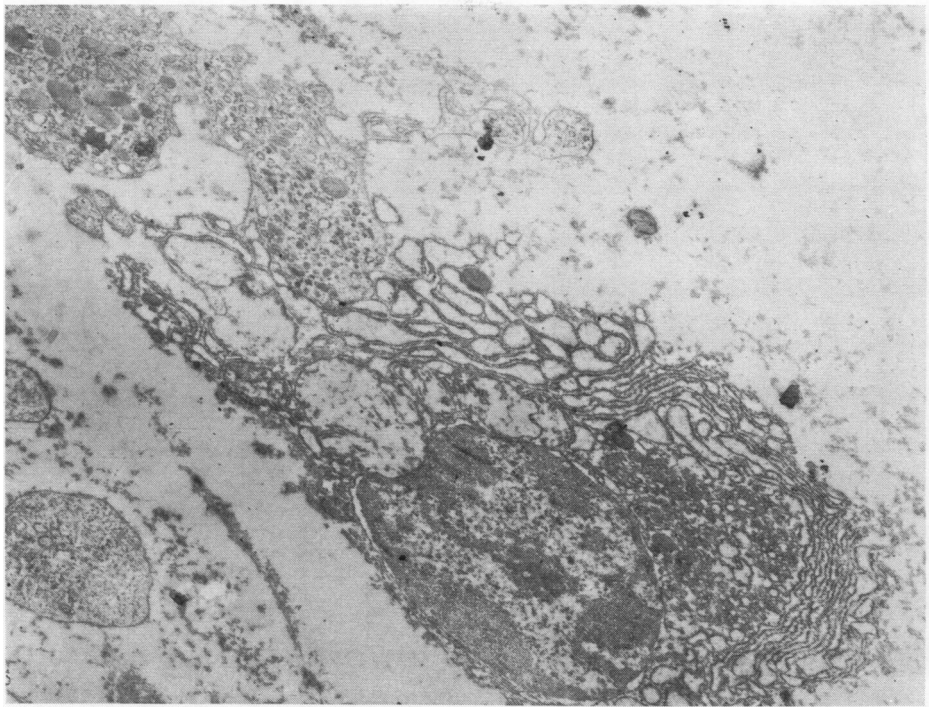

FIG. 7 *Disintegrating plasma cell with no plasma membrane. Part of the macrophage with many lysosomes is seen top left.* × 8,000.

found in some plasmocyte infiltrates (Fig. 7). The altered organelles of the disintegrating plasma cell emerged from the cells through breaks in the outer membrane. Membranous frameworks (Fig. 8), clumps of granular substance, nuclear fragments, Russell bodies, etc., could be seen between the intact or altered plasma cells. Even the Russell bodies lying free in the extracellular space were mostly surrounded by cytoplasmic debris and fragments of plasma membranes.

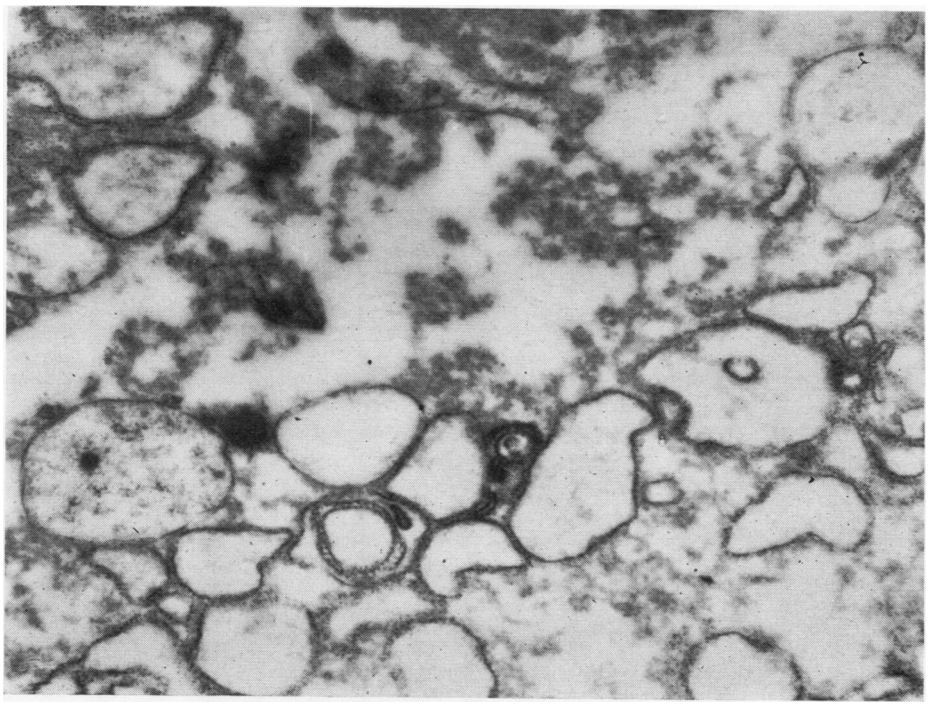

FIG. 8 *Empty vacuoles and granular substance at the site of a disintegrated plasma cell.* × 20,500.

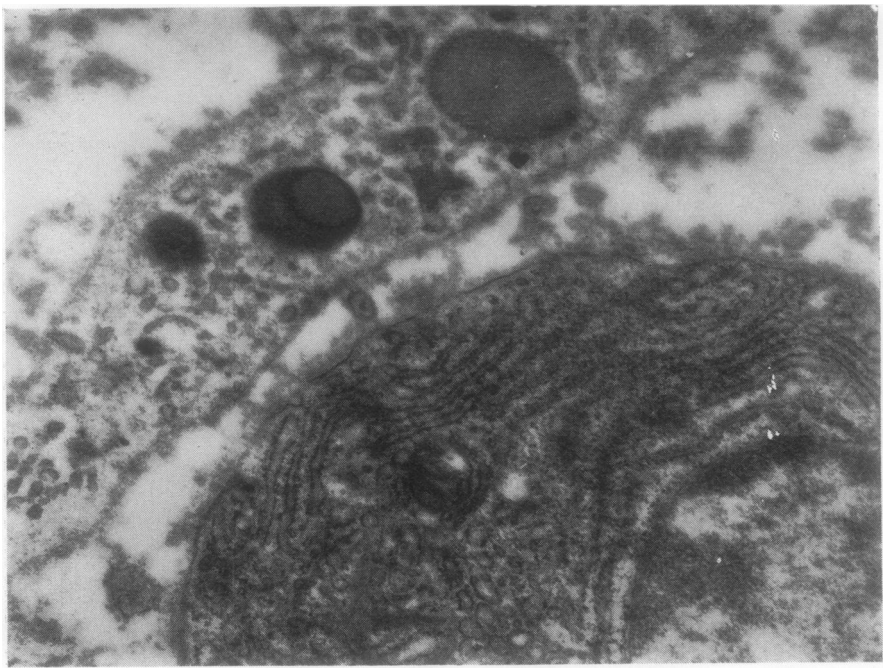

FIG. 9 *Macrophage process with lysosomes in cytoplasm near a plasma cell.* × 44,000.

Macrophages were usually found in the areas of plasmocyte disintegration (Figs 7 and 9). They included cytoplasmic processes, from which as well as from the cell bodies branched numerous filopodia, and large and small vacuoles containing a substance of different electron density. These appeared to be typical phagosomes (Fig. 10), and their contents resembled the extracellular granular material.

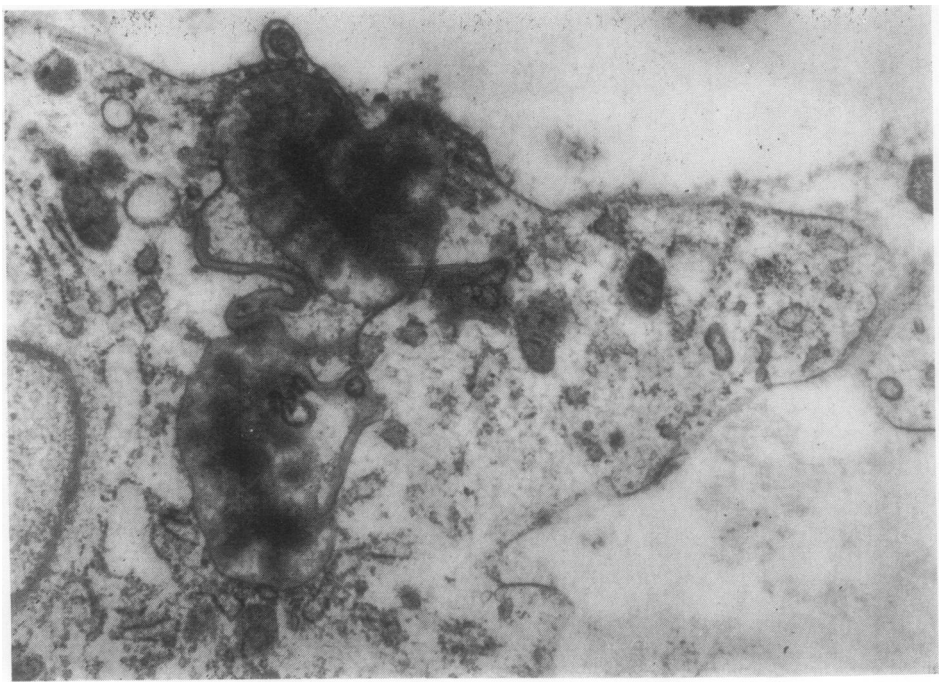

FIG. 10 *Phagosomes in macrophage cytoplasm.* × 20,700.

Discussion

Mellors and others (1959, 1961) showed that fluorescein-labelled aggregated human γ-globulin might be used for the detection of rheumatoid factor *in situ*. Our immunohistochemical findings reaffirm both these results and also our own previous observations (Kazakova and others, 1967) on the localization of rheumatoid factor in rheumatoid synovial tissue. This macroglobulin is found in the mature plasma cells, which may or may not contain Russell bodies, as well as in the extracellular clumps of pyroninophilic material. The latter is most likely a product of plasma cell disintegration. Immature plasma cells rarely contained rheumatoid factor.

The present electron microscopic studies were undertaken with the object of examining the ultrastructure of cells producing γ-globulins including rheumatoid factor in the rheumatoid synovial tissue, and also the modes of γ-globulin release in these conditions.

The plasma cells of rheumatoid synovial membrane may have different ultrastructural peculiarities in the same joint and even in the same plasmocyte infiltrate. Some of them are the young (immature) plasma cells that probably do not yet participate in the elaboration of γ-globulin. Others show marked dilation of the endoplasmic reticulum cisternae and accumulations of fine granular material of moderate electron density in their lumina. These cells often included narrow cytoplasmic processes, which usually contain the dilated cisternae of rough endoplasmic reticulum, free ribosomes, and solitary mitochondria, and may have narrowed stems. There are also many cytoplasmic islets around the cells, which seem to correspond to the intercellular pyroninophilic clumps with more or less distinct outlines that are seen in the light microscope. As the islets sometimes appear well away from the cell bodies, it may be suggested that the formation of cytoplasmic processes and their subsequent separation is one of the ways in which γ-globulin is released from the plasmocytes. Although some cytoplasmic islets may be cross-cut processes, most of them, however, must be true islets. Indirect evidence of this is the finding of different stages of disintegration of the islets. Release of γ-globulin from the cellular sites of its formation may occur by way of cytoplasm 'fragmentation'. This observation conforms with that of Ortega and Mellors (1957).

Gamma-globulin may also be released from the plasma cells by the complete disintegration of the cells themselves. This appears to be the only way in which it is released at the stage of plasmocyte differentiation. It has been suggested that Russell bodies result from the condensation of the contents of ergastoplasmic cisternae (Dohi, Hanaoka, and Amano, 1957; Wellensiek, 1957; Thiéry, 1958; Bessis, 1961; Welsh, 1960, 1962), and our findings agree with this idea. Fig. 5 demonstrates three Russell bodies at different stages of their formation. The surface of the larger body is more even and many very fine fibrils stretch to it from the ergastoplasmic membrane.

The plasma cells containing Russell bodies do not always show degenerative organelles, such as swollen mitochondria, degranulated endoplasmic reticulum, etc.; degenerative phenomena are more commonly seen in the presence of the larger Russell bodies, which are formed by the confluence of smaller ones. These bodies may fuse into one giant globule both before and after the disorganization of the endoplasmic reticulum. In the former case, it may be assumed that Russell bodies can 'migrate' along the anastomosing ergastoplasmic cisternae.

We did not observe Russell bodies in the cytoplasmic processes and islets of plasma cells, which suggests that they enter the interstitial space only after the rupture of the plasma membrane.

Extracellular Russell bodies seem to be fairly stable structures, since we have sometimes observed large bodies 'bricked-up' in the fibrous tissue. A confluence of Russell bodies may also occur in the extracellular space; some of them are giant globules such as do not occur intracellularly.

The fate of these extracellular Russell bodies is uncertain, but the immunologically active material (comprising disintegrating plasma cells, Russell bodies, and extensive conglomerates of indefinite form) is constantly present and persists for a long time in the inflamed synovial tissue in cases of rheumatoid arthritis. Pleomorphic conglomerates stain palely and irregularly with a haematoxylin and eosin, PAS, pyronin, and fluorescein-labelled aggregated human γ-globulin. They thus appear to be complexes of plasmocyte disintegration products with serum proteins and glycoproteins.

This immunologically active material may be a cause of the persistence of specific inflammatory processes including the immunological component of inflammation. The special conditions existing within the joints, namely, a closed cavity with a very slow rate of exchange, hamper the removal of this pathological material, but facilitate the spread of the pathological protein complexes within a given joint.

Summary

Plasma cells of the mononuclear cell infiltrates in twenty rheumatoid synovia from patients with positive latex-fixation tests were studied by light, fluorescent, and electron microscopy. Rheumatoid factor was detected in the cytoplasm of mature plasma cells, in flocculates around them, and in both intracellular and extracellular Russell bodies. In ultrathin sections

of tissue from similar areas, plasma cells with the typical parallel arrangement of the rough endoplasmic reticulum, and those with dilated ergastoplasmic cisternae containing a substance of moderate electron density, and occasionally Russell bodies were identified.

Many plasma cells have cytoplasmic processes and some of the latter have narrowed stems and appear to break away from the cells. This is apparently one of the ways in which gamma-globulin is released from the synovial plasma cells.

Certain plasma cells break down and their cytoplasmic organelles are dispersed in the extracellular space. It is assumed that Russell bodies may enter the interstitial space only after rupture of the plasma membrane.

A substance resembling the product of plasma cell disintegration was found in the phagosomes of the macrophages.

Thus, in rheumatoid synovial tissue, immunologically active material (*i.e.* plasma cell disintegration products and their complexes) is constantly present. This may be considered as an endogenic self-reproducing agent capable of maintaining a local immunological inflammation.

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