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Abstract

The synovial membranes from 16 rheumatoid patients treated with intramuscular injections of gold sodium thiomalate were observed by light and electron microscopy with special reference to the distribution of gold particles in the tissue. 1) Light microscopic study revealed that the gold demonstrated as cytoplasmic granules by OKAMOTO'S histochemical method were contained in the synovial lining cells and in the macrophages around lymph-follicles and blood vessels in the subsynovial layer. In the well-developed villi on the surface of rheumatoid synovial membrane, large macrophages with gold granules infiltrated into the lymphoid cell accumulation of small lymph-follicles. 2) The deposition of gold in the synovial tissue increased with the increase of the doses of gold administered. 3) Electron microscopic observation indicated that gold particles are contained in the numerous lysosomes in the Type A and intermediate lining cells. The macrophages around lymph-follicles and blood vessels also possessed a large amount of gold particles gathered in the lysosomes of these cells. 4) Macrophages containing gold particles in their long cytoplasmic extensions were found often in a close contact with plasma cells of various differentiation stages. A direct cytoplasmic connection was observed between the two kinds of cells but an artifact could not be excluded. 5) The effect of gold salt in the treatment of RA was discussed from the immunological view point.

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A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE DISTRIBUTION OF GOLD SODIUM THIOMALATE IN THE RHEUMATOID SYNOVIAL MEMBRANES

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Recently, the study on the pathophysiology of regional inflammation in rheumatoid arhtritis (RA) has greatly developed on the basis of the remarkable progress made in the field of immunology. Gold salts which are now widely used for the treatment of RA, serve also as the tools for the immunological approach to the disease as well as the curative agents.

Absorption, distribution and excretion of gold were recently studied by using redioactive ^{198}Au (1, 2, 3, 4), revealing that the gold of the salts administered intramuscularly was accumulated in the inflammatory rheumatoid joints (2, 3, 4). Histological investigations by GAUNT and TUCKER (5) revealed the deposition of the gold particles in the synovial cells, but the details of Au deposition in relation to the cell organellae is obscure, as few electron microscopic observation has been carried out on the rheumatoid tissues having gold deposition.

Studies of the gold deposition in tissue and cell in detail may give an important information for the mechanism of supressing effect of the gold salt on rheumatoid inflammation. The purpose of this paper is to describe the distribution of gold in rheumatoid joint tissue of the patients treated with gold salts as revealed by light and electron microscopy with special reference to the relationship between the macrophages responsible for phagocytosis of gold particles and lymphoid cells for antibody formation.

MATERIALS AND METHODS

The specimens used for the present observation were of 32 samples; 29 from the synovial membranes of 19 RA patients which had the symptoms defined as typical RA according to the criteria of the American Rheumatism Association (6) and 3 from non-RA patients. Sixteen of RA patients were treated with gold therapy by intramuscular injections of gold sodium thiomalate according to the method of FREIBERG. Three RA patients and 3 non-RA cases recieved no gold injection. Twenty-six specimens were obtained at surgical

operations, 3 from knee joints by punch biopsy under arthroscopy, (Table 1), and other specimens were obtained from non-RA joints of amputated leg with malignant bone tumor.

Table 1 Specimens from various joints of RA

	Elbow	Wrist	Finger (DIP)	Hip	Knee	Ankle	Total
Female	3	5	1	1	13	1	24
Male	1	3			1		5
Total	4	6	1	1	14	1	29

The specimens were obtained from 19 RA patients (female 14, male 5), and 16 of them were treated with gold therapy by intramuscular injection of gold sodium thiomalate.

Individual specimens were divided into 3 parts; two for light microscopy and one for electron microscopy. For the light microscopy the tissue was fixed either in a 10% neutral formalin or a Carnoy's fluid and embedded in paraffin. All the specimens were stained with hematoxylin eosin and also stained by the histochemical methods by OKAMOTO and others (7) and HASHIMOTO (8) for the detection of gold. The specimens fixed in Carnoy's fluid were stained by methyl-green-pyronin staining. Berlin blue method for iron demonstration was also carried out.

For the demonstration of acid phosphatase activity the synovial tissues were frozen-sectioned in a cryostat, stained by the method of Gomori and observed under light microscope.

Besides these, thicker sections of Epon embedded tissue were obtained and stained by OKAMOTO's method for gold for the light microscopy.

According to the light microscope pictures appearing on the hematoxylin eosin-stained sections, all the RA samples were divided into the 6 types defined by KODAMA (9) and histochemical and electron microscope observations were carried out in connection with the light microscope picture.

KODAMA's classification of RA is as follows:

a. Simple synovitis type; a simple inflammation in the synovial membrane showing the proliferation of the lining cells.
b. Arteritis type; predominated by arteritis with lymphocyte infiltration in perivascular area.
c. Fibrinoid type; predominated by fibrinoid degeneration.
d. Coating type; type having distinct fibrinoid membrane coating the synovial surface.
e. Follicular type; predominated by proliferation of lymph-follicles.
f. Fibrosis type; predominated by proliferation of connective tissue elements.

For the electron microscopy, the tissues were fixed in a buffered 1% osmium tetroxide for 80—90 minutes, dehydrated in ethanol and 100% propylenoxide, embedded in Epon 812, sectioned with glass knives with Porter-Blum microtome, mounted on uncoated copper grids, stained with lead citrate by the method of KARNOWSKY (10) and observed with HU-11, HU-7 and

JEM-7 electron microscopes, using accelerating voltages of 50 kv.

RESULTS

Histological Findings

In four specimens obtained from the patients, who were treated with gold sodium thiomalate injections within 200 mg in total dosage without any detectable clinical effect in the general and local symptoms of RA, a small amount of gold was demonstrated by the OKAMOTO's method as granules incorporated in the macrophages in the subsynovial tissue of these specimens. In the arteritis type, the gold granules were recognized within the cytoplasmic processes of macrophages around the small vessels and sometimes of a few cells just under the lining layer (Figs. 1, 2). Large lining cells remarkably increased in number in the surface layer and formed various villi and folds, but they rarely contained gold particles (Fig. 3). Similar perivascular deposition of gold was recognized, though not so clearly as in the arteritis type, also in the follicular and fibrinoid coating types; such a distribution of gold around the lymph-follicles as will be described in the cases treated with higher doses does not yet occur (Fig. 4).

In most specimens, which were obtained in surgical operations of the patients in the course of intramuscualr injections of aurothiomalate over 200 mg in total dosage, that showed the distinct anti-rheumatic effects clinically, histological findings were as follows:

Arteritis type: The main lesion of the synovial tissue in this type was represented by perivasculitis of the small arteries and arterioles. There may be further found hyperemia, thrombus formation and fibrous hypertrophy around the affected vessels. Lymphocytes, plasma cells and large macrophages infiltrated around the vessels. A moderate amount of gold was incorporated in the cells in the infiltrative areas (Fig. 5). Gold incorporation was especially conspicuous in large macrophages located at a two- or three-cell distance from the vessel wall. The endothelial cells of the affected vessels contained gold particles.

Fibrinoid type: In this type, fibrinoid hypertrophy and degeneration spread widely in the synovial membrane, and such changes also occurred in the arterial wall. Macrophages laden with gold containing granules were scattered among the infiltrating cells in the fibrinoid tissue (Fig. 6). Fig. 7 shows that gold was markedly positive in the inflammatory cells around the hypertrophied wall of the arterial capillary.

Coating type: A mossy fibrinoid layer covered the synovial membrane

and there were detected no gold deposition in this layer. Together with numerous plasma cells and lymphocytes, macrophages infiltrated in the subsynovial tissue which showed a considerable amount of gold containing granules within the cytoplasm (Fig. 8).

Follicular type : Numerous lymphoid cells formed rather small lymph-follicles in the proliferated villi, and large ones in the subsynovial layer. Gold containing macrophages characteristically surrounded the large follicles which included numerous plasma cells (Figs. 8, 9). The small lymph-follicles in the villi were infiltrated by macrophages which extended their gold containing cytoplasm among the lymphoid cells (Figs. 10. 11). Macrophages around the vessels also possessed gold positive particles. This follicular type was characterized by that the lining cells of the synovial membrane also incorporated gold in a form of cytoplasmic granules positive in OKAMOTO's reaction.

Fibrosis type : Fibrosis generally followed the inflammatory changes described under the names of other types. Large fibrocytes with very long cytoplasm were observed in the proliferation of connective tissue, and some of them contained a marked amount of gold positive granules, However, a deposition of gold on the collagen fibers as described by ADAM *et al.* by electron microscopy (11) was not confirmed (Figs. 13, 14).

The synovial membranes from the patients injected over 1000 mg

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Figs. 1, 2, 3 and 4. Light micrographs of the synovial membranes from the rheumatoid patients treated with gold sodium thiomalate injections within 200 mg in total dosage. A small amount of the gold granules was recognized around the small vessels (Fig. 1 ×400) and just under the lining layer (Fig. 2, ×400). Large lining cells increased in number in the surface layer and formed elongated villi and folds but did not contain gold granules (Fig. 3, × 400). Fig. 4 showed a lymph-follicle which is not yet surrounded by the cells incorporating gold granules. × 300.

Fig. 5. Arteritis type : Lymphocytes, plasma cells and macrophages infiltrated around the vessels. The gold granules (g) are incorporated in the macrophages located at a two-cell or three-cell distance from the vessel wall. × 400.

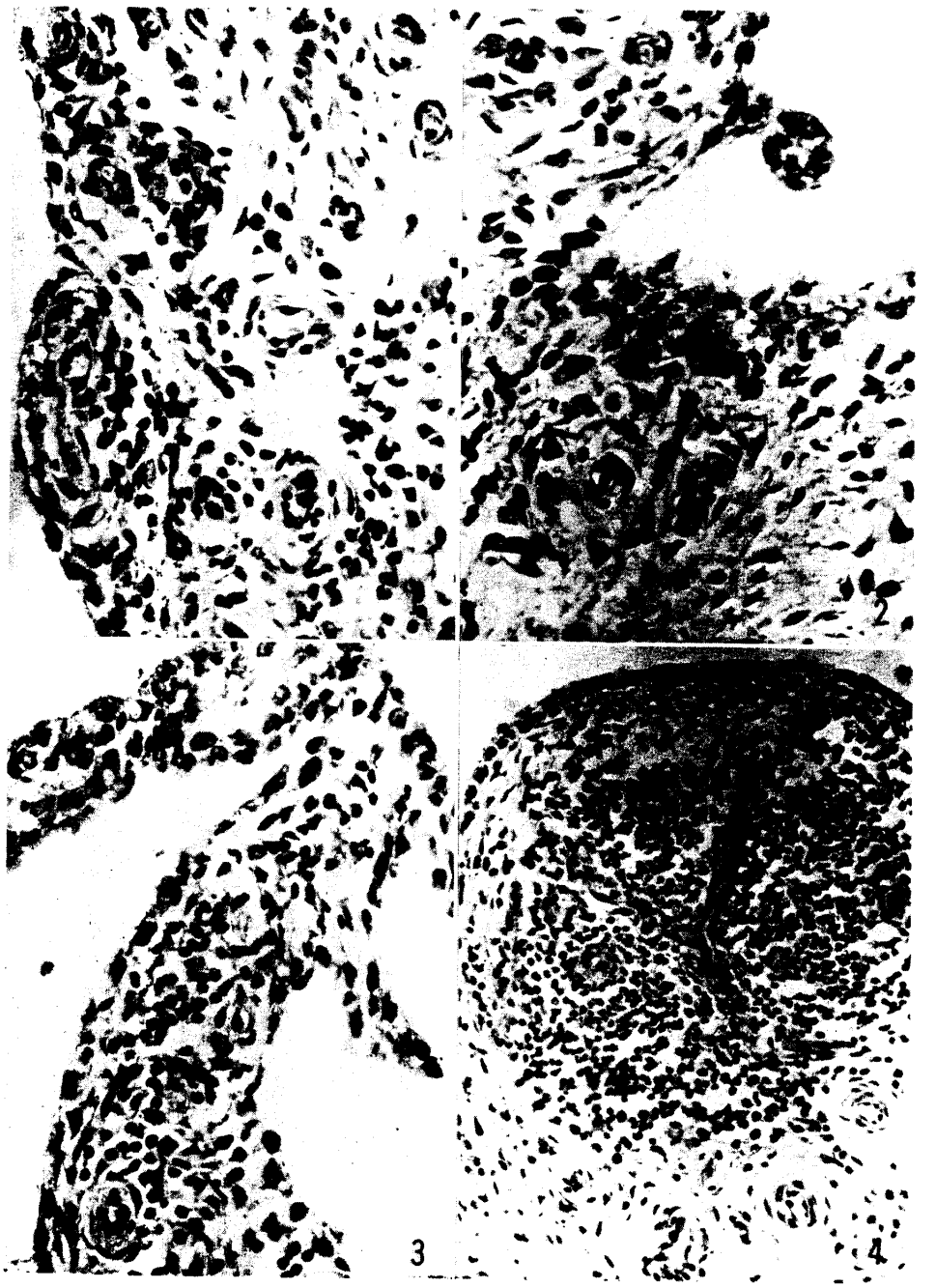
Fig. 6. Fibrinoid type : The infiltrating cells in the fibrinoid degeneration contain the gold granules (g). × 400.

Fig. 7. A marked deposit of the gold granules (g) is observed in the cells around the hypertrophied wall of the arterial capillary. × 500.

Fig. 8. Coating type : Fibrinoid layer covers the synovial membrane. Gold is seen distributed in the subsynovial layer. × 300.

Figs. 9 and 10. Follicular type ; Gold (g) conatining macrophages characteristically surround the large lymph-follicle (Fig. 9, ×100) which include numerous lymphoid cells (Fig. 10, ×400).

Figs. 11 and 12. A small lymph-follicle in the proliferated villi is infiltrated by macrophages which extend their gold containing cytoplasm among the lymphoid cells (Fig. 11, ×400). Fig. 12 shows fine gold granules (g) in the cytoplasm of macrophages at high magnification (×1000).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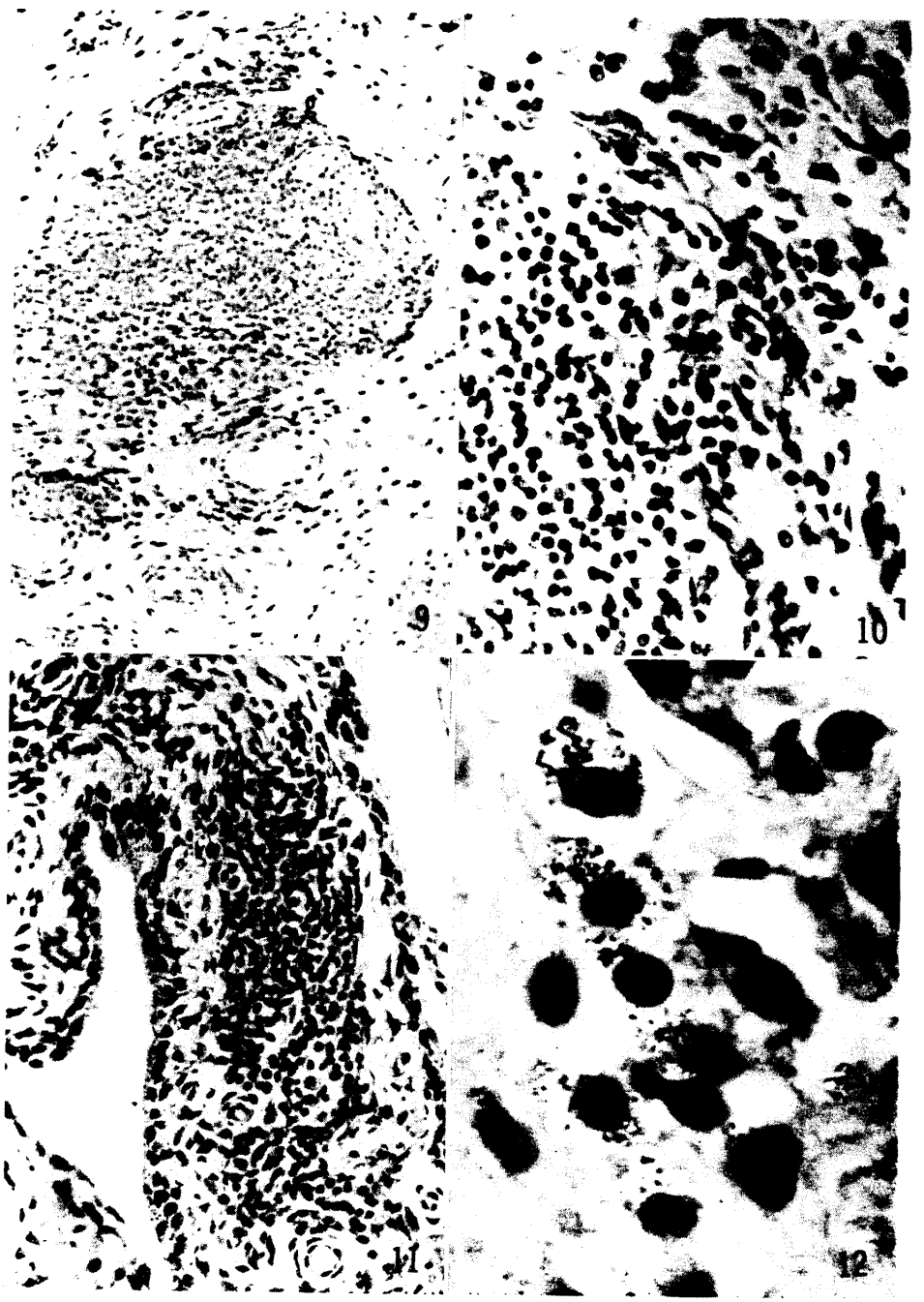
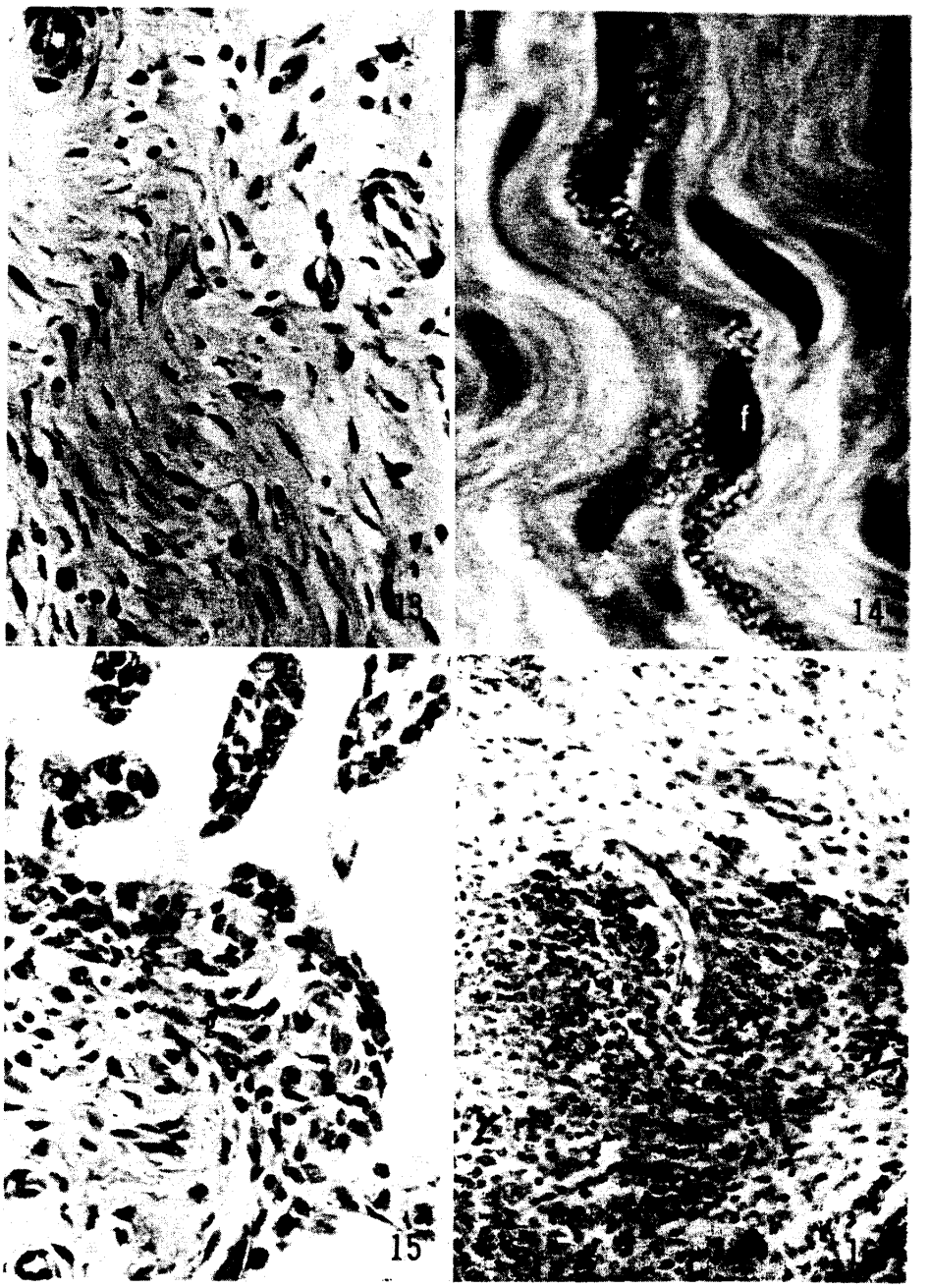




Figs. 13 and 14. Fibrosis type: The gold granules are positive in the cytoplasm of fibrocytes in the proliferation of connective tissue (Fig. 13, ×400). High magnification of these cells shows a considerable amount of gold granules (g) in the cytoplasm, but there is no deposition of gold on the collagen fibers (Fig. 14, ×1000).

Figs. 15 and 16. The synovial membranes show a considerable amount of gold (g) deposition in the lining (Fig. 15) and subsynovial layer in the gold injection of large dosage. ×400.

showed a conspicuous amount of gold incorporated in the cellular elements mentioned above including the lining cells and the macrophages in and around the lymph-follicles (Figs. 15, 16).

Some of these specimens were secured as long as eight months after the final injection of gold and still contained numerous gold conatining granules. The amount of the gold detected in the synovial membranes increased, in general, with the increase in doses of the gold administered. The distribution of gold in the different elements of the synovial wall varied considerably according to the six different types of rheumatoid inflammation.

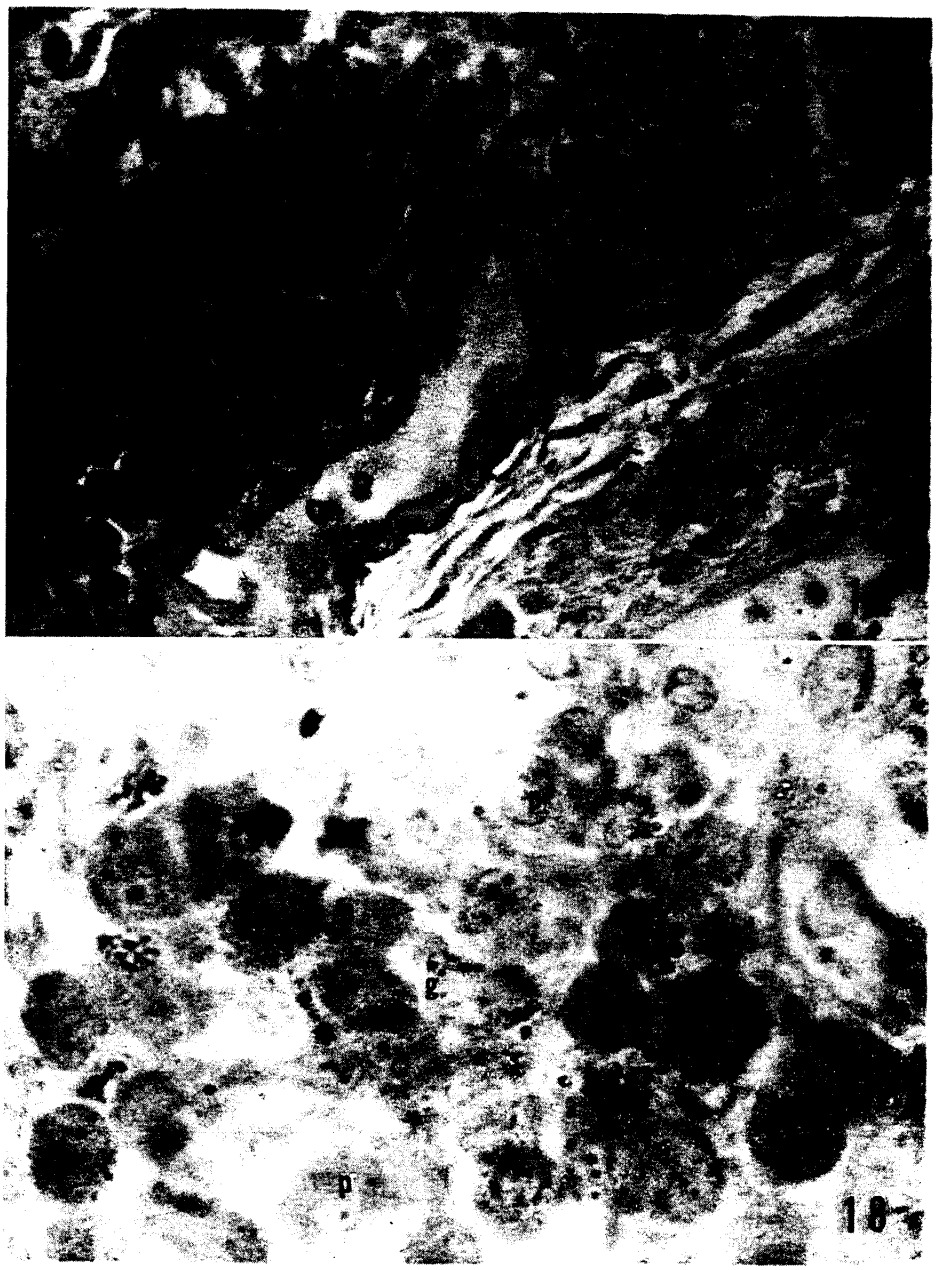
Light microscopic observation of thicker sections from the tissue embedded in Epon for electron microscope gave less OKAMOTO's gold reaction than in those from paraffin embedded ones. However, cytoplasmic granules with a positive reaction could be proved in the macrophages around the small vessel. Round or oval cells with eccentric nucleus and dark cytoplasm including no gold positive granules were identified with plasma cells by the electron microscopic observation of the adjacent ultrathin sections (Figs. 17, 18).

Electron Microscopy

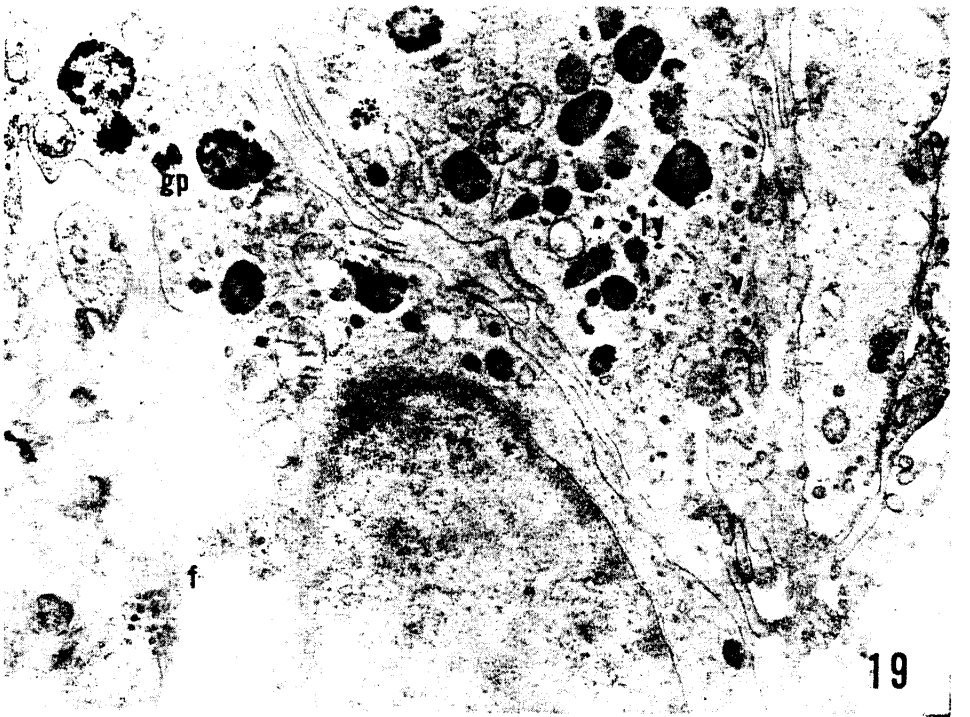
Lining Layer

The lining layer of the rheumatoid synovial membrane observed in the present study showed a remarkable increase in the number of the intermediate cells by GHADIALLY and ROY (12), as compared with the normal synovial membrane ; the increase of the type A cells rich in smooth surfaced endoplasmic reticulum and having well developed Golgi body by BARLAND *et al.* (13) was less conspicuous and type B cells rich in rough endoplasmic reticulum (13) appeared unincreased. The lining cells in RA were closely packed with each other and extended their cytoplasmic processes into the intercellular spaces. Type A cells and the intermediate cells in the synovial membrane from the patients treated with gold salts included various forms of granules to be classified under the names of lysosomes. These lysosomes which varied in diameter from 120 to 700 mμ contained numerous round or needle-shaped particles of an extremely high electron density measuring 10—60 mμ in diameter. These particles, as described later (see Discussion), were believed to correspond to the gold incorporated into the cytoplasm.

Figs. 17 and 18. Light micrographs of thicker sections from the electron microscopic specimens. Fig. 17 shows the lymphoid cell accumulation around the vessel and collagenons fibers. The small granules are identified as gold (g) in the macrophages (×1000). Round or oval cells with eccentric nucleus, including no gold positive granules, are identified as plasma cells (p) by the electron microscopic observation of the adjacent sections (×1200). →



Figs. 19 and 20. Electron micrographs of the lining cells including various lysosomes (ly) of 120—170 mμ in diameter. These lysosomes contain numerous round particles (gp) with an extremely high electron density measuring 10—60 mμ in diameter, which are considered to correspond to the gold incorporated into the cytoplasm. These are type A lining cells which include numerous lysosomes and small vesicles (v). ×12000. f: fibrils


gp
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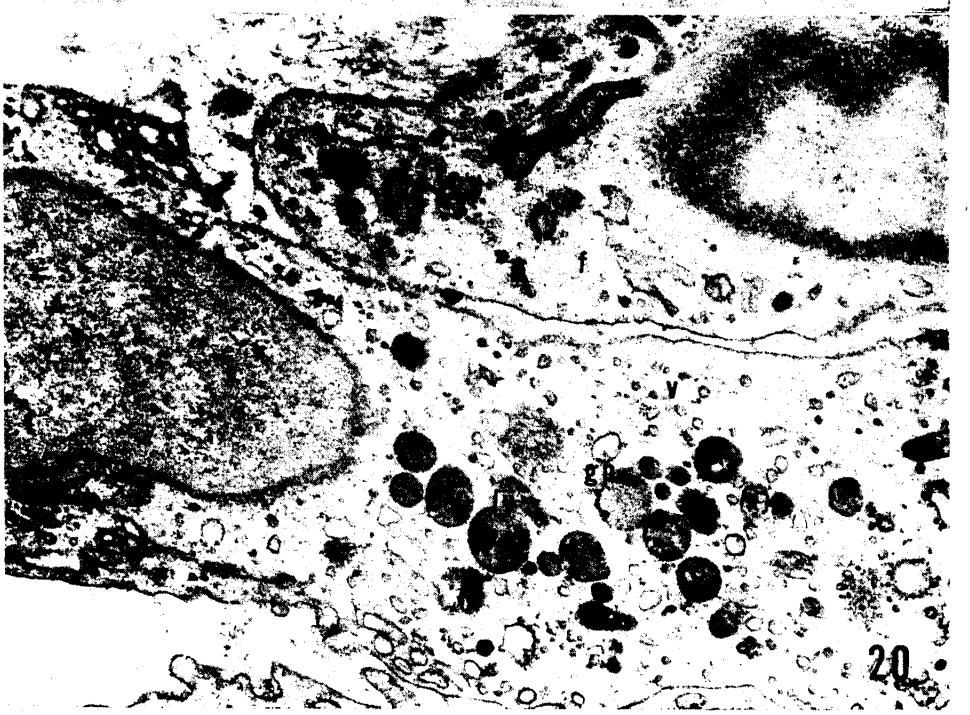
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The type A and intermediate cells also contained many pinocytotic vesicles, occasionally including the same electron dense particles as in the lysosomes. These cells showed, further, numerous filopodia or finger-like processes protruding into the extracelluar spaces. Fine aperiodic fibrils (about 100Å thick) spread in the cytoplasmic matrix and were also seen in the intercelluar spaces. No electron dense particles were detectable in these spaces (Figs. 19, 20).

Subsynovial Layer

As the rheumatoid changes in the subsynovial layer, it was possible to observe hyperemic blood vessels and inflammatory cells infiltrated around them, lyphoid cell accumulation in the lymph-follicles and proliferated fibrillar components, though the six different types of rheumatoid lesions could not be identified at the level of electron microscopy.

The endothelial cells of small vessels in the subsynovial tissue were bounded closely to each other and contained fine filamentous fibers in the whole cytoplasm, numerous pinocytotic vesicles lining up along the cell surfaces and small amounts of smooth surfaced vesicles, mitochondoria and the elements of endoplasmic reticulum. In these cells lysosomes occurred occasionally but included few electron dense particles regarded as gold. Erythrocytes often filled the lumen of the vessels; they did not carry any particles on their surface (Fig. 21). Occasionally neutrophils and lymphocytes included various vesicles and lysosomes, but no gold particles were detected in their cytoplasm (Fig. 22). The basement membrane of the vessels consisted of a layer of aperiodical fibrillar substance showed no gold either.

There were recognized numerous macrophages especially around the blood vessels and lymph-follicles. They contained, besides numerous cytoplasmic vesicles, lysosomes of various size and contents. Numerous round or needle-shaped particles (10—60 mμ) of a very high electron density were recognized in these cytolysomes and were regarded as gold particles.

There was recognized in the rheumatoid subsynovial layer a more or less marked proliferation of fibrillar components of the extracellualr spaces. The collagenous fibers with their characteristic periodicities and microfibrils without these increased throughout the tissue. The former ran in parallel with each other and the latter diversely or interwoven with each other, sometimes forming a fibril-mass shaping "writing brush" as described by KOBAYASHI (14). There were recognized any dense particles neither on the collagenous fibers nor on the microfibrils (Fig. 23).

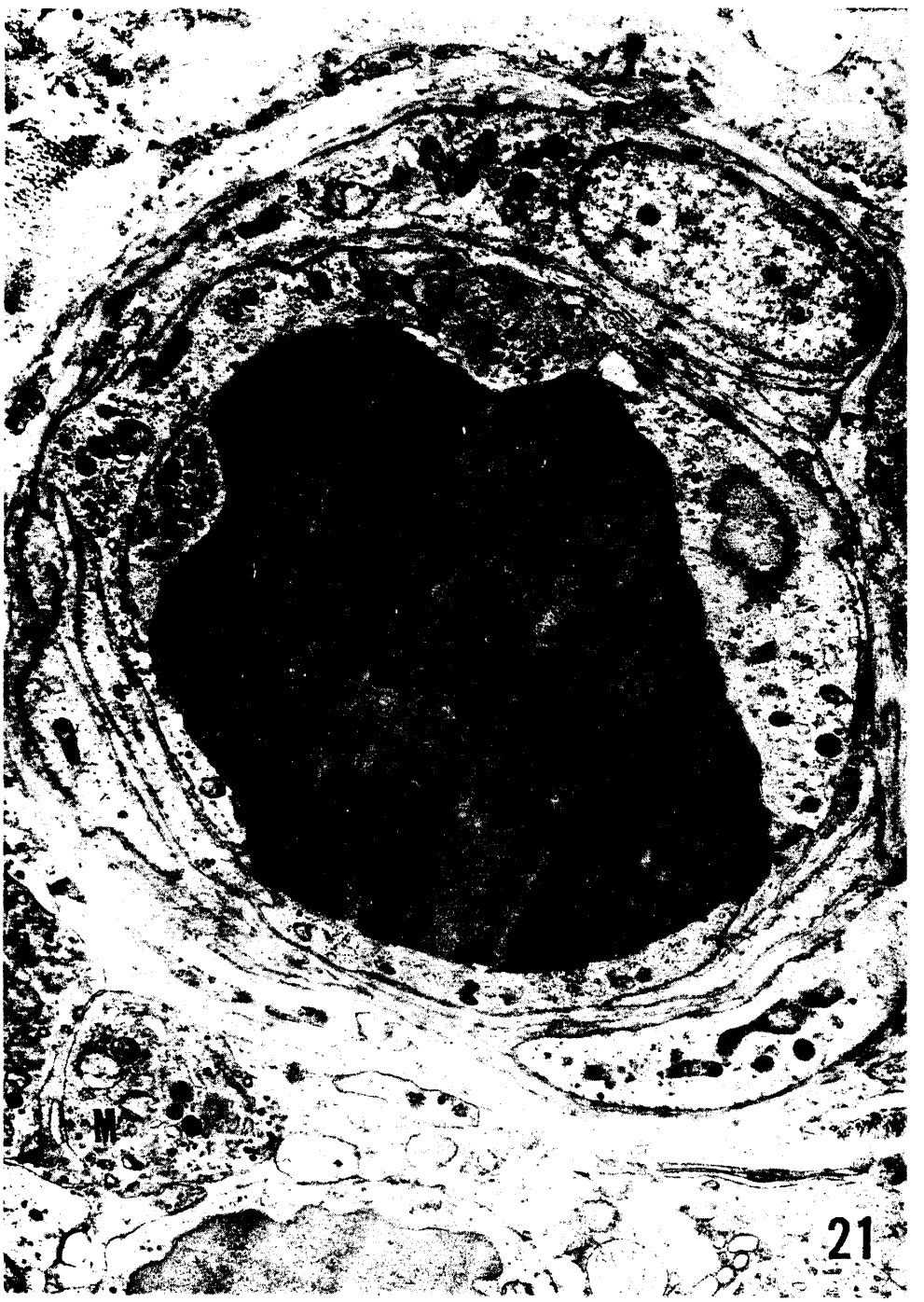
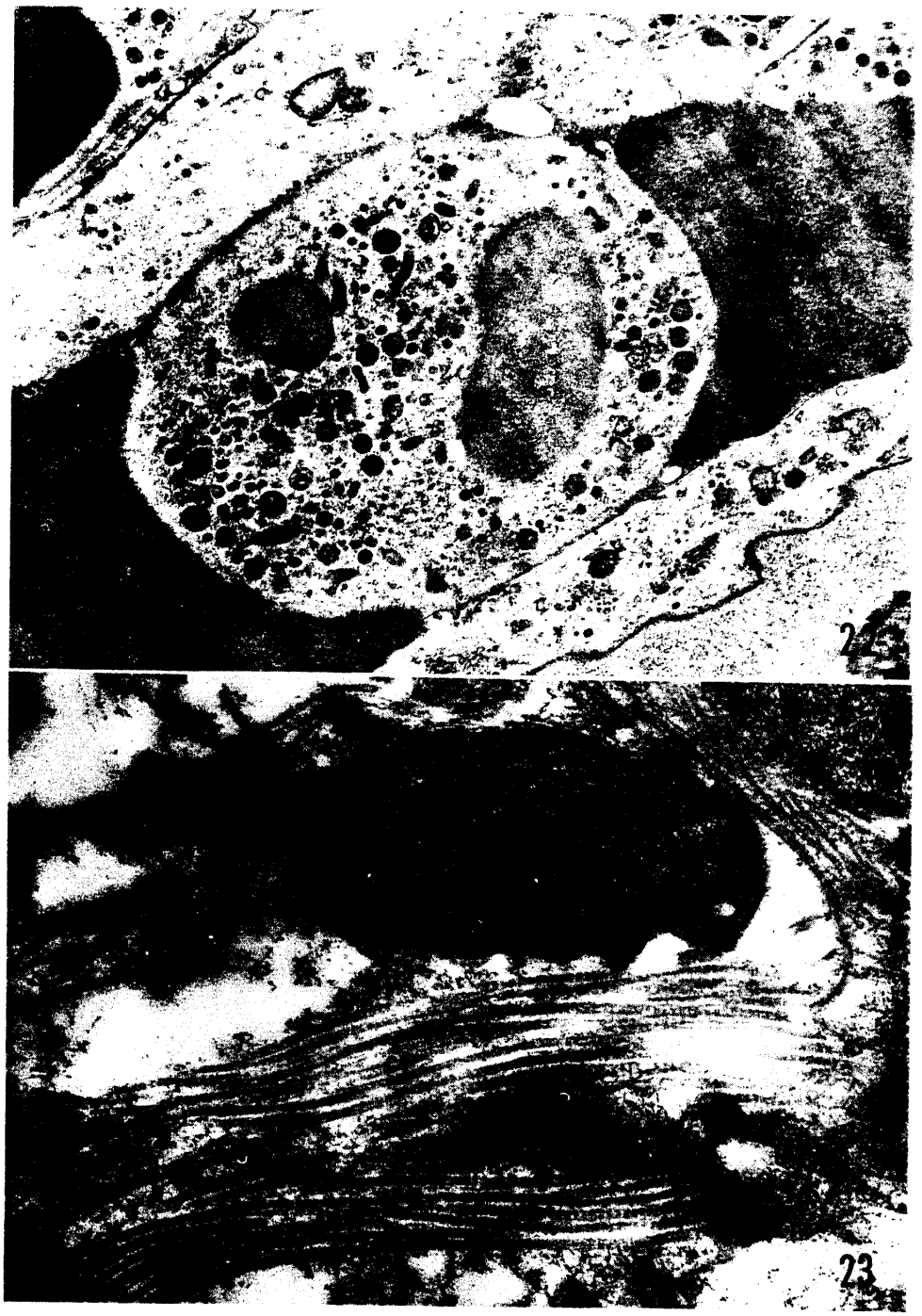


Fig. 21. The small vessel in the subsynovial tissue is filled with erythrocytes (E). The endothelial cells (EC) and the erythrocytes do not contain any particles and the macrophage (M) contains dense particles regarded as gold. ×8000.



Macrophages and Plasma Cells

In the subsynovial layer of the rheumatoid patients, there were found under the electron microscope a considerable number of plasma cells which were mostly gathered in and around the peripheral layer of the lymph-follicles. As was conspicuous by the light and electron microscopic findings the macrophages around the lymph-follicles contained gold positive granules. The relation beween these and the plasma cells was observed under the electron microscope with a special interest.

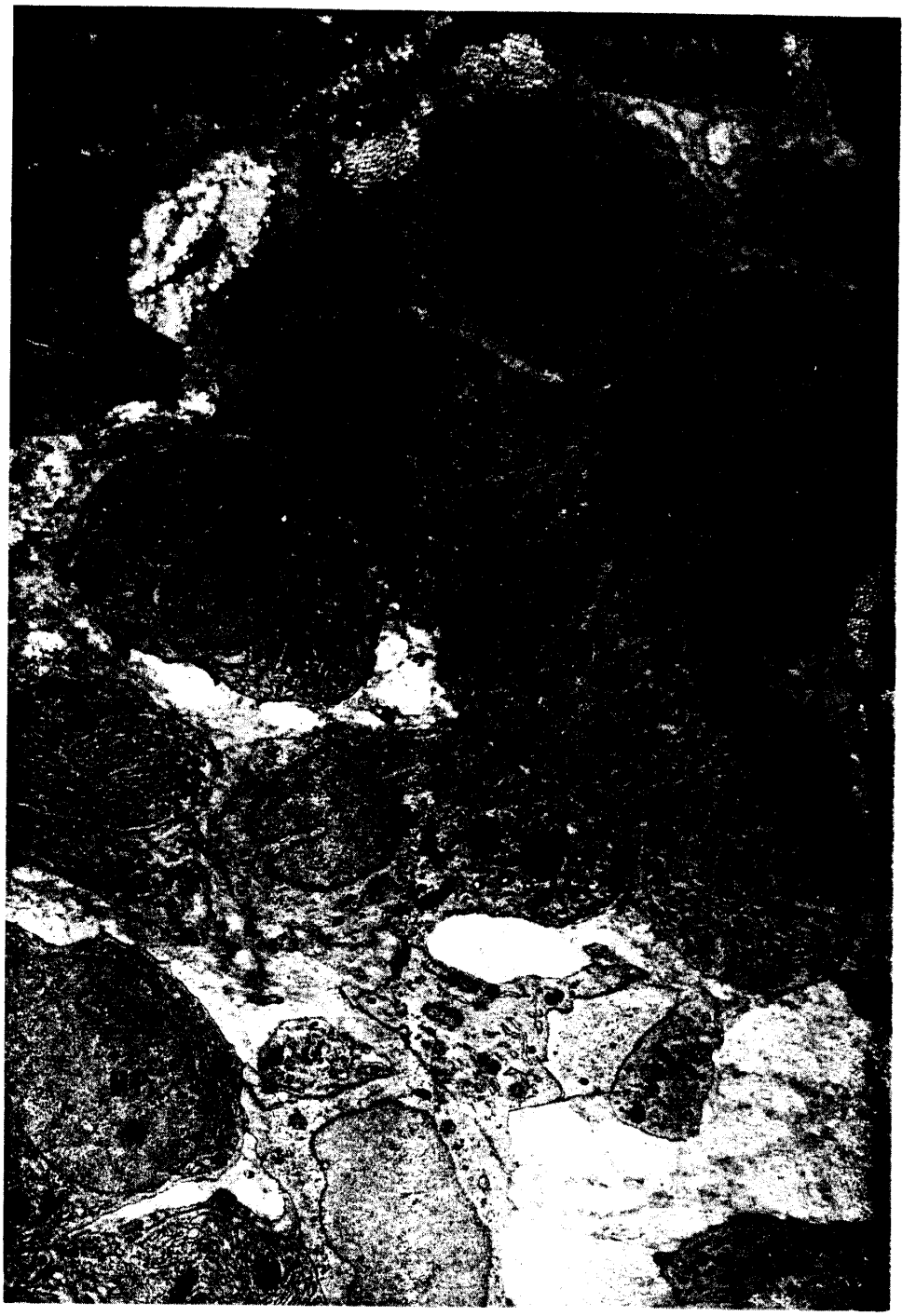
There was repeatedly observed in the present study an accumulation of cells which were identified as lymphoid cells of various stages of differentiation, such as small and large lymphocytes, immature plasma cells and well-differentiated plasma cells. Figure 24 shows about seven of these cells, the cell with a large nucleus and a few mitochondoria and vesicles in the top of the picture appeared to be of a lymphocyte. The two cells containing a large nucleus left below to it were considered as immature plasma cells as they included a small amount of rough endoplasmic reticulnm and Golgi vesicles in the cytoplasm. The nucleus is still located in the center of the cell and occupied most of the cytoplasm. The cells shown in the center and bottom are identified as mature plasma cells as they contain a highly developed rough endoplasmic reticulum and a large Golgi apparatus with increased vesicles. These cells sent out microvillus-like processes from the cell surface. The nucleus now was located eccentrically and fine cytoplasmic fibrils existed in the perinuclear area. Some of these plasma cells (upper right) contained dilated endoplasmic reticulum sacs including electron opaque material, whereas others exhibited flattened sacs bounded by parallel membranes, showing lamellar pattern.

Macrophages invaded into such lymphoid cell accumulations. They extended their cytoplasmic processes which were so large that they came into contact with three or four lymphoid cells. In these cytoplasmic processes there were recognized, besides small vesicles, a large number of granules of 120—600 mμ in diameter to be identified as lysosomes. These

Fig. 22. Neutrophil in the lumen of small vessel has various vesicles and lysosomes, but no gold particles. ×1000.

Fig. 23. The collagenous fibers (cf) and microfibrils (mf) in the extracellular spaces. There are recognized many particles on them. ×20000.

Fig. 24. Electron micrograph of an accumulation of cells which are identified as lymphoid cells of various stages of differentiation such as lymphocytes (L), inmature plasma cells (IP) and well-differentiated plasma cells. The mature plasma cells (MP) contain highly developed rough endoplasmic reticulum and large Golgi apparatus and eccentric nucleus. The macrophage (M) includes electron dense particles (gp) in the lysosomes (ly) infiltrated among the cells. ×5000.



granules contained many dense particles about 10—50 mμ in diameter were regarded as gold. The elements of endoplasmic reticulum and mitochondoria were relatively few in the cytoplasm of the macrophages (Fig. 25).

Figure 26 shows that there are more enlarged sacs of rough endoplasmic reticulum sacs and a better developed Golgi apparatus in the plasma cell in contact with macrophage, compared with another plasma cell in the same picture which was not in such a contact. It is not known whether this difference is incidental or has a certain meaning.

The macrophages containing dense particles in their lysosomes were very closely attached to the lymphoid cells. In some contact portions between the two elements, there was found a structure which might indicate a direct cytoplasmic continuity, but the author is inclined to interpret it as an artifact made by oblique sectioning, because the membrane structure of the rough endoplasmic reticulum and vesicles near such a connection appeared less clear than in other parts of the cells. In the vicinity of such a close contact with plasma cells, there were recognized no particular changes in the cytoplasm of macrophages, such as an accumulation of vesicles (Fig. 27).

DISCUSSION

According to the general description by FREYBERG (15), gold salts injected intramuscularly are absorbed into the blood, loosely bound with alpha globulin in the plasma as a gold-protein comlex (16), and chiefly excreted in the urine. LAWRENCE reported that when sodium aurothiomalate labelled with radioactive gold was injected intramuscularly into rheumatoid patients, painful joints gave counts 2.5 times as high as symptomless joints. This accumulation of the radioactive gold persisted till long after the level of radioactivity in the blood had become negligible (4).

The histological distribution of the administered gold salts in the rheumatoid synovial membrane has not yet been studied enough. As far as is known, only GAUNT and TUCKER demonstrated under the light microscope a deposition of gold sodium thiomalate, administered by an intramuscular injection, in the synovial membrane of animals and rheumatoid patients (5). The gold granules were noted by these authors only in the cytoplasm of the synovial lining cells. TONNA and his co-workers, using autoradiography, showed that intramuscularly administered ^{198}Au was concentrated, among other sites, in the synovial lining cells of the rabbits

Fig. 25. The cytoplasmic processes of the macrophage (M) are so large that it comes into contact with three to four plasma cells (P). The lysosomes (ly) in the cytoplasm of the macrophage contain a large amount of dense particles. × 5000.

Fig. 26. The plasma cell (P) in contact with macrophage (M) reveals enlarged sacs of rough endoplasmic reticulum (er), in contrast to another plasma cell (P) without a contact. × 120000.

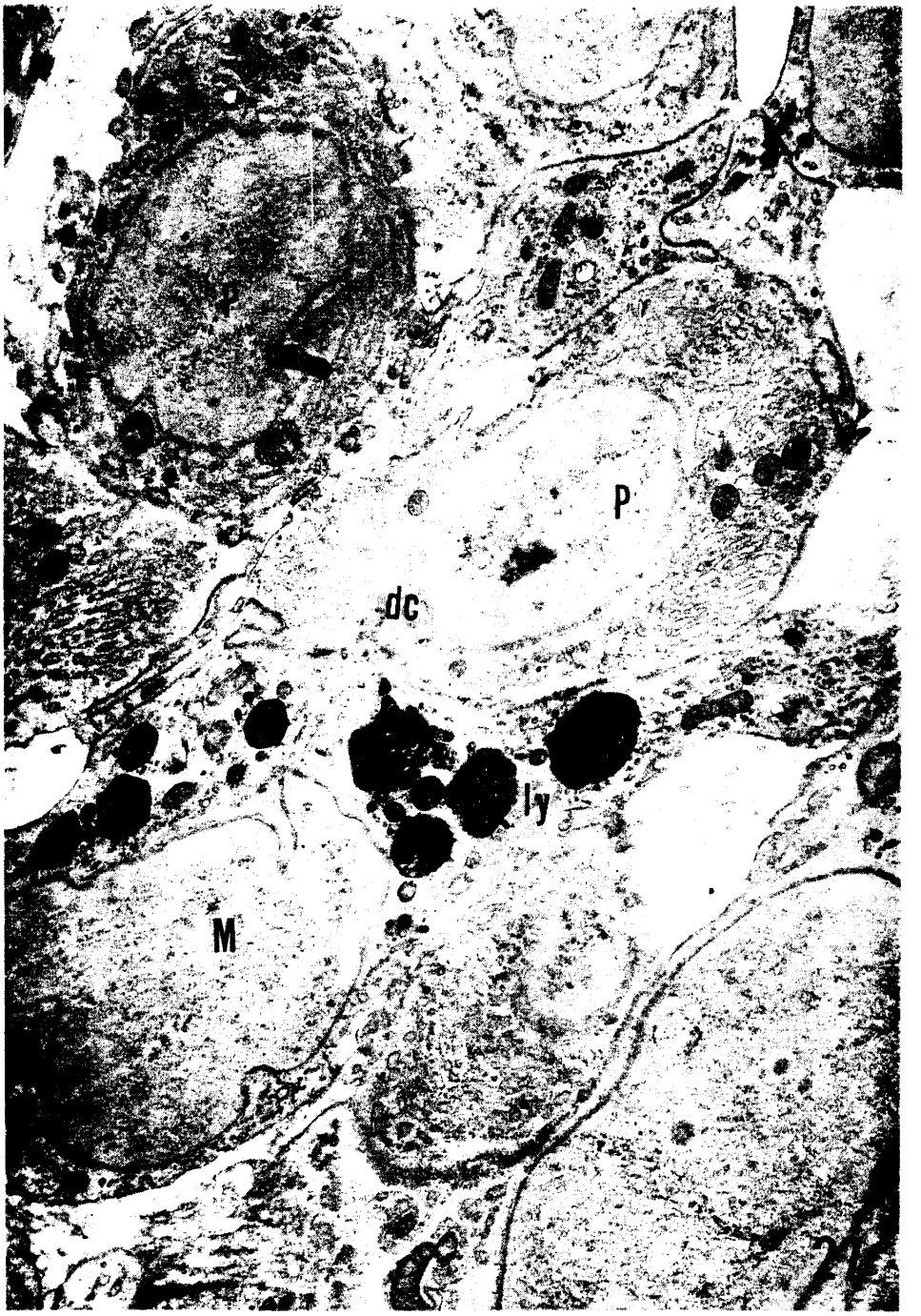


Fig. 27. This electron micrograph may indicate a direct cytoplasmic continuity (dc) between a macrophage (M) and plasma cells (P), but the membrane structure of the rough endoplasmic reticulum and vesicles near such connection appears less clear than other part of the cells. ×12000.

(17). Also in the present study, the synovial lining cells contained a considerable amount of gold when it had been administered in largedo ses. The gold incorporation in the lining cells may be ascribed to a phagocytotic transport of gold-protein complex from the joint cavity into these cells. In fact, LEWIS and ZIFF showed an incorporation of intraarticularly administered gold granules by the proliferating lining cells (18).

As far as the author knows, there has been neither light microscopic nor electron microscopic observations of gold distribution in the subsynovial layer in pathological changes. The present light microscopic study is characterized by the finding that macrophages in the subsynovial tissue showed more or less marked deposition of gold. Especially the macrophages in and around the lymph-follicles were heavily laden with gold granules : this finding was conspicuous in the follicular type RA. In the arteritis type, on the other hand, the gold deposition occurred mainly in the macrophages around the blood vessels. These findings seem to suggest a possible rôle played by the macophages, in the regional immunological reactions of RA.

The electron microscopic study by BARLAND and his co-workers demonstrated two types of lining cells, Types A and B, in the normal synovial membrane of man (13). Fine structural alterations in rheumatoid synovial membrane have been reported not only in the lining layer but also in the subsynovial tissue elements (19, 20, 21). In the present observation of rheumatoid synovial membrane, there were recognized a considerable number of Type A and intermediate type lining cells; only a few Type B cells were found. This may imply an elevated phagocytotic activity in the lining layer, if one takes it into account that the Type A and intermediate cells contain a large amount of smooth-surfaced membrane elements and lysosomes, whereas the Type B cells only a small amount of them. Each type A, type B and intermediate lining cells may be identified with "M type", "F type" and "F-M type" cells described by HIROHATA and KOBAYASHI (19).

Contrary to the present results, GHADIALLY and ROY (12) described a marked increase in Type B synovial cells associated with a hyperplasia of their rough endoplasmic reticulum in the patients of rheumatoid arthritis and considered that these elements might contribute to the formation of synovial fluid which was unusually increased in the rheumatoid joint. The rôle of the Type B cells played in the protein synthesis was proved by an experiment using ^{3}H-leucine (22). The descrepancy between the findings of GHADIALLY and ROY and the present author with regard to the cell types proliferated may be ascribed to the difference in the activity

of the disease observed. The cases treated by the present author were mostly characterized by synovial hypertrophy which were not accompanied by any conspicuous increase in the amount of joint fluid.

The light microscopic observation of the present study indicated that the staining reaction characteristic of gold occurred in the small cytoplasmic granules of macrophages and, in some cases, of lining cells. This finding may be correlated with the present electron microscopic result that small particles of a high electron density occurred in the lysosomes of macrophages. These particles are believed to be nothing but those of aurothiomalate, because they corresponded well in size and electron density with the aurothiomalate grains themselves spread on the collodion covered grid and observed under the electron microscope.

In the subsynovial layer, the most typical changes are the marked increase of extracellular fibrinoid substance and the infiltration of inflammatory cells. The present observation of rheumatoid synovium also showed an unusual increase of microfibrils and collagenous fibers as described by KOBAYASHI (14). However, neither light nor electron microscopy showed a deposition of gold on these fibers. This result is in descrepancy with that of ADAM and his coworkers who recognized in the electron microscopic study in rat tail that the intramuscularly administered gold deposited as fine granules on the collagen fibers and correlated this finding with the mechanism of the gold therapy of RA.

It is now an international tendency to study the rheumatoid diseases from immunological points of view. The Empire Rheumatism Council showed a significant decrease in RF titer after a gold salt therapy (23). This anti-rheumatic effect of gold suggested that it may affect somehow the immunological reaction in the rheumatoid joints. Gold salts were shown to be concentrated, besides to the general reticuloendothelial system (1), to the site of rheumatoid inflammation (4). It may be speculated that gold salt concentrated within the lysosomes of macrophages and synovial lining cells may inhibit the phagocytotic incorporation of antigen by these cells which are conceived to transmit immunological informations to plasma cells and lymphoid cells. PERSELLIN and ZIFF have demonstrated that when guinea pig peritoneal macrophages were incubated with gold sodium thiomalate, the gold was selectively taken up into the lysosomes and decreased the lysosomal acid phosphatase activity (24). The activity of other enzymes, present in synovial fluid and presumed to be lysosomal in origin, has also shown to be inhibited by gold salt (25). On the other hand, it has been reported that administration of gold salt into hyperimmunized animals did not cause any lowering of their antibody levels (26).

Immunofluorescence study on the synovial membrane from patients with RA showed a positive fluorescence for 7S gamma globulin. More localized occurrence of 19S macroglobulin and rheumatoid factor, as shown by conjugated aggregates, were noted around vessel walls within the synovial membrane (27). MELLOER and his co-workers demonstrated that the rheumatoid factor is present in the cytoplasm of plasma cells of various stages of differentiation in the rheumatoid synovium (28).

Structural units in the lymph nodes of immunized animals consisting of phagocytotic cells and lymphoid cells surrounding them have been observed by several workers (29, 30). In the lymph node culture under administration of tritiated cystidine, lymphocytes surrounding the macrophages were labelled to a greater degree than those showing no topographical relation to the macrophages (31). These results imply that RNA or RNA-antigen complex from stimulated macrophages had to be transferred to the antibody-producing cells. SOHONBERG *et al.* have even described a direct cytoplasmic connection between macrophage and potential antibody-producing cells in lymph nodes and spleen (32). In this connection, the present author paid a special attention to the morphological relation of these cell elements in the rheumatoid synovial membrane. A close juxtaposition of both cells and a cytoplasmic connection between them has been recognized but, as described in Results, the latter findings could not be distinguished from a possible artifact.

SUMMARY

The synovial membranes from 16 rheumatoid patients treated with intramuscular injections of gold sodium thiomalate were observed by light and electron microscopy with special reference to the distribution of gold particles in the tissue.

1) Light microscopic study revealed that the gold demonstrated as cytoplasmic granules by OKAMOTO's histochemical method were contained in the synovial lining cells and in the macrophages around lymph-follicles and blood vessels in the subsynovial layer. In the well-developed villi on the surface of rheumatoid synovial membrane, large macrophages with gold granules infiltrated into the lymphoid cell accumulation of small lymph-follicles.

2) The deposition of gold in the synovial tissue increased with the increase of the doses of gold administered.

3) Electron microscopic observation indicated that gold particles are contained in the numerous lysosomes in the Type A and intermediate

lining cells. The macrophages around lymph-follicles and blood vessels also possessed a large amount of gold particles gathered in the lysosomes of these cells.

4) Macrophages containing gold particles in their long cytoplasmic extensions were found often in a close contact with plasma cells of various differentiation stages. A direct cytoplasmic connection was observed between the two kinds of cells but an artifact could not be excluded.

5) The effect of gold salt in the treatment of RA was discussed from the immunological view point.

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