ELECTRON MICROSCOPY OF
THE HUMAN SYNOVIAL MEMBRANE

PETER BARLAND, ALEX B. NOVIKOFF, and DAVID HAMERMAN

From the Departments of Pathology and Medicine, Albert Einstein College of Medicine, New York

ABSTRACT

The structure of the lining cells at the surface of the synovial membrane facing the joint cavity has been studied by electron microscopy. The long cytoplasmic processes of these cells appear to be oriented toward the surface of the membrane, where they overlap and intertwine. The matrix of the lining cells contains dense material but no fibers with the periodicity of collagen. The lining cells are divided into two cell types or states of activity on the basis of their cytoplasmic contents. Type A is more numerous and contains a prominent Golgi apparatus, numerous vacuoles (0.4 to 1.5 microns in diameter) containing varying amounts of a dense granular material, many filopodia, mitochondria, intracellular fibrils, and micropinocytotic-like vesicles. Type B contains large amounts of ergastoplasm with fewer large vacuoles, micropinocytotic-like vesicles, and mitochondria. The probable functions of these cells are discussed in the light of current knowledge of the metabolism and function of the synovial membrane.

INTRODUCTION

Stained with hematoxylin and eosin and viewed with the light microscope, the surface of the synovial membrane facing the joint cavity shows large cells with prominent cytoplasmic processes (1). These cells form a lining 1 to 3 cells deep and appear similar to fibroblasts in the more basal part of the synovial membrane. However, by their detailed structure and metabolic activity these lining cells may be distinguished from the fibroblasts. The long cytoplasmic processes of the lining cells contain numerous vacuoles (1). Cytochemical stains show higher levels of several oxidative enzyme activities in the lining cells than in underlying fibroblasts (2). Acid phosphatase (2) and N-acetyl-β-glucosaminidase (3) activities are almost completely restricted to the lining cells. Finally, material staining metachromatically with toluidine blue, presumed to be hyaluronate

since the metachromasia was abolished by streptococcal hyaluronidase, is limited to the intercellular matrix of the lining cells (4).

Analyses of homogenates of normal human synovial membrane show low levels of glycolysis and aerobic metabolism (5). The suggestion from staining reactions is that this metabolic activity resides predominantly in the lining cells. Since it is known that synovial cells synthesize hyaluronate (6), it is possible that the major metabolic activity of the lining cells is to synthesize hyaluronate of synovial fluid and to carry on active interchange of other components of synovial fluid.

Electron microscopy can supplement histologic and cytochemical studies and provide additional clues to the function of the lining cell. Electron micrographs of lining cells of the calf, dog, guinea pig, rabbit, and cat have been published (7–9).

MATERIALS AND METHODS

Synovial specimens were obtained from patients by means of open biopsy or by closed biopsy using the Polley-Bickel needle. Prior to fixation, the surface of the synovial membrane facing the joint cavity was identified by inserting a straight surgical needle into the capsule and forcing it through the tissue until it emerged at the surface. The tissue was fixed in cold buffered 1 per cent osmium tetroxide in 0.14 M sucrose (10) for 2 hours, washed in veronal-acetate buffer, pH 7.2, and stored in cold 4 per cent formalin buffered at pH 7.2. Under the dissecting microscope, 1 mm blocks were cut from the synovial surface marked by the surgical needle. The blocks were dehydrated by increasing concentrations of ethanol and embedded in a 1:7 mixture of methyl and butyl methacrylate containing 0.75 per cent uranyl nitrate (11). Polymerization was accomplished overnight at 60°C in the presence of 1 per cent benzoyl peroxide. The embedded blocks were so trimmed that transverse sections of the membrane were generally obtained on sectioning. Thick sections (2 μ) were studied by phase microscopy or after toluidine blue staining to locate the lining cells. The blocks were then retrimmed and sectioned at 300 to 600 A with an LKB ultratome. The sections were mounted on carbon-coated grids and sandwiched between the grid and a second layer of carbon (12). Prior to sandwiching, some sections were stained with saturated aqueous potassium permanganate (12), or 10 per cent aqueous phosphotungstic acid or 10 per cent phosphomolybdic acid (13). Sections were studied in an RCA EMU-3B microscope, using 100 kv.

Frozen sections of the same synovial membrane, both unfixed and following overnight fixation in cold formol-calcium, were studied for enzyme activities by methods described elsewhere (14).

RESULTS

Cytochemical studies, particularly with stains for DPNH-diaphorase activity, reveal more clearly than hematoxylin and eosin staining the location and structure of the lining cells. Formazan deposits from oxidative enzyme activity are found almost exclusively in the lining cells. These cells are clearly polarized: from each cell one or more cytoplasmic processes extend toward the luminal surface of the synovial membrane. At the free surface of the synovial membrane the processes appear to overlap and intertwine to form an apparent limiting margin.

Under relatively low magnifications of the electron microscope, the lining cells form a discontinuous outer margin of cells, 2 to 3 cells in depth, merging gradually with the underlying connective tissue. There is no evidence of a basement membrane separating the lining cells from the other elements of the synovial membrane. Occasionally a lining cell is seen close to a capillary. Mitotic figures have not been observed.

Cytoplasmic processes of the lining cells are usually directed toward the membrane surface, tapering as they approach the surface. In most areas these processes overlap to form a loose network (Fig. 1). However, occasionally the cytoplasmic processes of adjacent cells are closely apposed to each other (Fig. 2). In such apposed areas, interdigitations are sometimes seen. Many of the intercellular spaces among the loosely arranged processes are readily traced to the surface, where they are frequently in direct continuity with the joint cavity.

The intercellular matrix of the lining cells contains amorphous material, similar to that observed in cytoplasmic vacuoles (to be described below), and numerous thin, non-periodic branching filaments. No collagen fibers are observed.

In the more basal layers of the synovial membrane the matrix contains less amorphous material, and collagen fibers with typical 640 A periodicity are seen. These fibers stain with phosphotungstic acid. Fewer cells are noted and these have the typical appearance of fibroblasts.

With higher magnifications, the lining cells may be differentiated into two cell types or states

FIGURE 1

A somewhat oblique transverse section of a normal human synovial membrane. At the top is the joint space (*JS*). Beneath it, the synovial surface is composed of overlapping cytoplasmic processes of the lining cells. Such organization of lining cells separated by relatively wide intercellular spaces is much more common in the normal synovial membrane than the more closely knit arrangement seen in Fig. 2. Some of the processes (*CP*) extend many micra before terminating or leaving the plane of the section. A finely granular material (*GM*) is abundant in the intercellular space (*S*) between the cytoplasmic processes. No collagen is visible. × 7600.
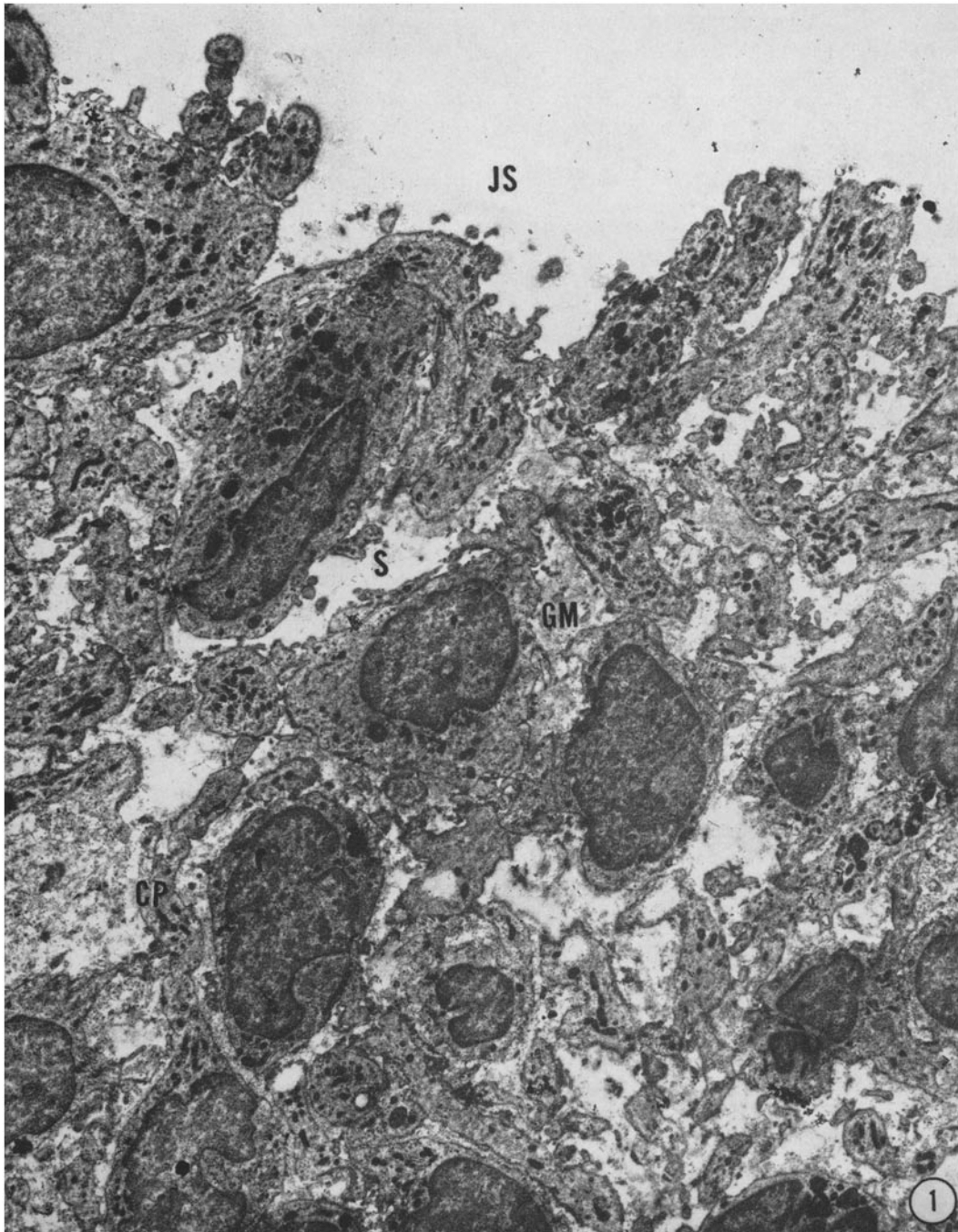
of activity, each with different fine structure (Fig. 2).

The predominant cell type A (Fig. 4) shows:

1. Numerous large vacuoles, 0.4 to 1.5 μ in diameter, most prominent within the cytoplasmic processes. These vacuoles are bound by thin "single" membranes and contain a dense granular material resembling the material in the intercellular spaces. This material does not stain with phosphotungstic acid (which stains collagen) or phosphomolybdic acid (which stains liver glycogen).

2. Many branched aperiodic fibrils, 50 to 75 A in diameter, in the cytoplasmic ground substance. These fibrils are numerous beneath the cell membrane and lie roughly parallel to it. They do not appear to bear a spatial relationship to the endoplasmic reticulum or Golgi apparatus (Fig. 5). Similar fibrils are seen in the intercellular space.

3. Numerous filopodia, finger-like projections, extending into the extracellular matrix. These often enclose portions of the extracellular matrix (Fig. 6). While the filopodia may be found over the entire cell surface, they are most numerous in the distal parts of the cytoplasmic processes.

4. Many vesicles, 0.1 to 0.3 μ in diameter, most numerous in the cytoplasmic processes. Their continuity with the cell membrane has been seen on numerous occasions (Figs. 3 and 10), and it is tentatively assumed that they are micropinocytotic vacuoles. Their contents have a low electron opacity that is not increased by treatment of sections with solutions of metal salts. In some places they are seen adjacent to the larger and denser vacuoles.

5. Numerous mitochondria, usually elongated in the axis of the cytoplasmic process, containing typical cristae and outer membranes. Intermediate forms between mitochondria and denser vacuoles were not encountered.

6. Smooth membrane pairs and small vesicles, characteristic of the Golgi apparatus, were encountered near the apical aspect of the nucleus and in the more distal parts of the cytoplasmic processes. A moderately dense granular material is present in some dilated ends of the Golgi cisternae (Figs. 7 and 8). This appears similar to the material in the 0.4 to 1.5 μ vacuoles that are found close to the Golgi apparatus.

Cell type B (Fig. 9) is less numerous than type A and contains:

1. Abundant ergastoplasm that shows narrow cisternae and small sacs containing a homogeneous, non-dense material. On the surface of the membranes are particles 100 to 150 A in diameter, presumed to be ribonucleoprotein (RNP) particles.

2. Occasional mitochondria that appear smaller and contain fewer cristae than those in the type A cells.

3. Vacuoles and vesicles, such as are found in cell type A, are present but they are far less numerous than in the type A cells.

We have carefully studied the areas of approximation of the cytoplasmic processes, as well as the adjacent cell membranes of the cell bodies, for thickening of the plasma membrane, condensation of submembranous cytoplasm, and increased density between the two cell membranes characteristic of the desmosomes or adhesion plates found in many other membranes. None of these morphological features was noted.

FIGURE 2

Transverse section of human synovial membrane. The joint space is seen above (*JS*). These cells are more closely applied than is generally the case. Two types of lining cells are present: type A, recognized at this magnification by its numerous cytoplasmic vacuoles, 0.5 to 1.5 μ in diameter, partially filled with a dense granular material; and type B, containing abundant ergastoplasm. Some dense aperiodic fibrous material (arrow) is present in the intercellular matrix, but no collagen is visible. × 7600.

FIGURE 3

A cytoplasmic process from a lining cell of a human synovial membrane. The process contains vacuoles presumed to be micropinocytotic vesicles. The membranes of the vesicles occasionally show continuity with the cell membrane, as at the arrows. The cytoplasm also contains granular vacuoles (*V*) and many fine aperiodic fibrils (*F*). × 59,000.
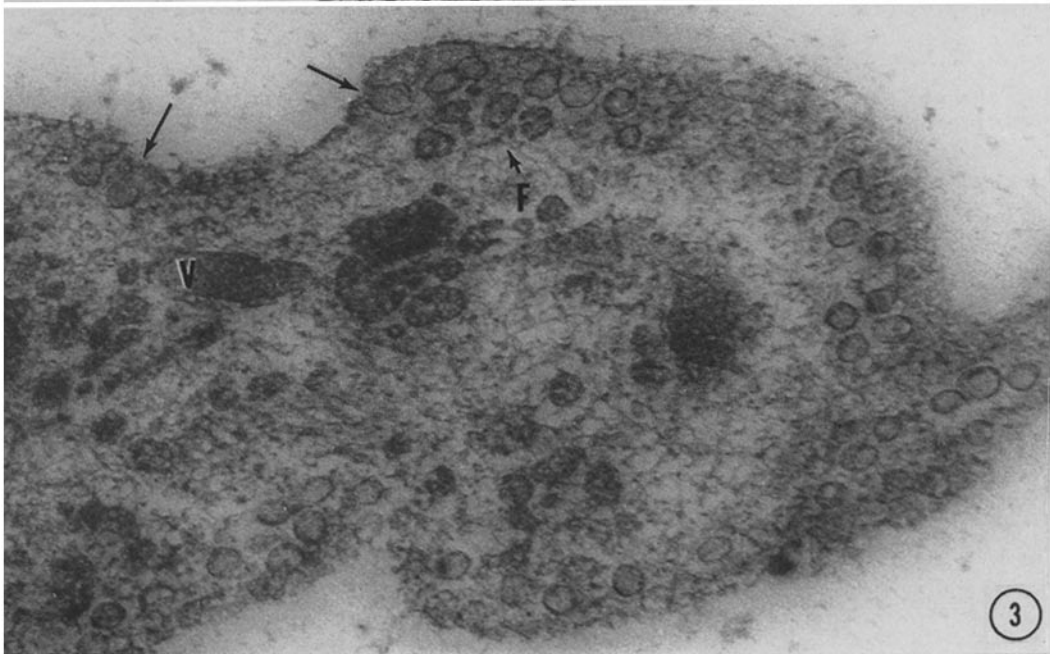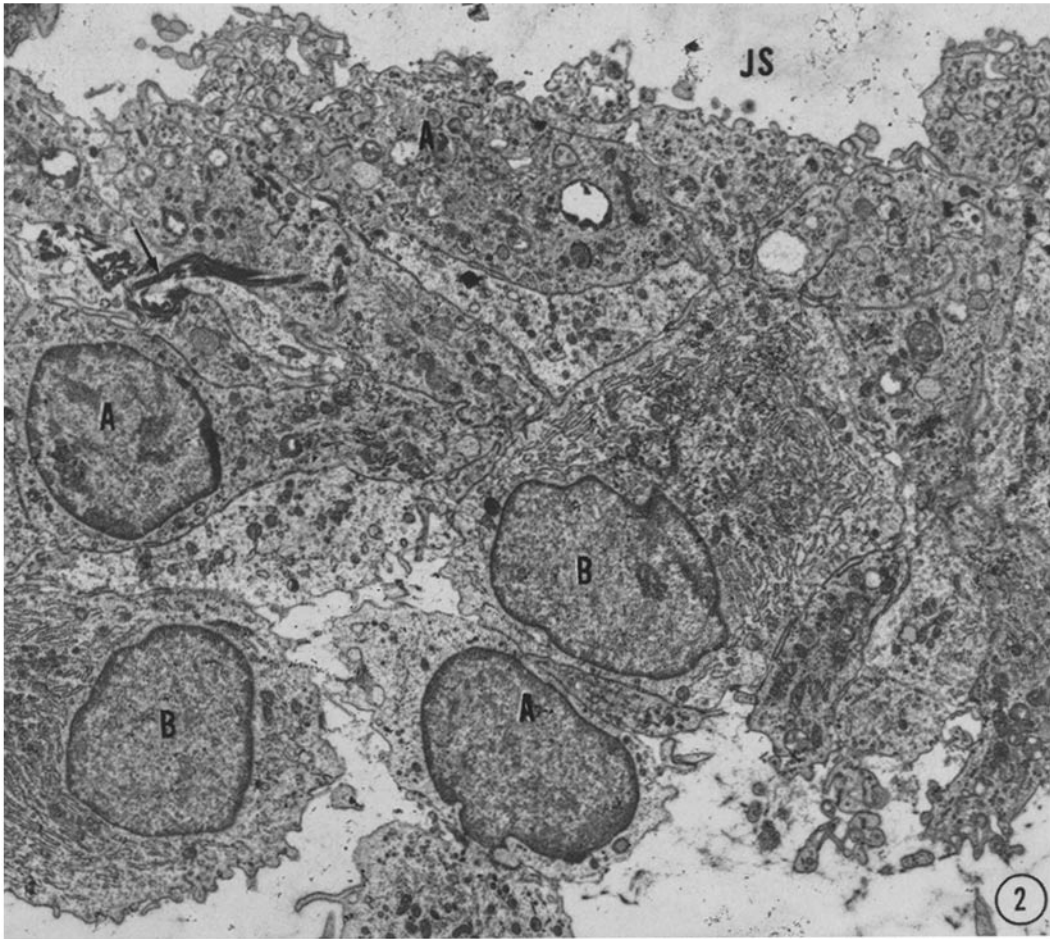
DISCUSSION

Fig. 11 shows a diagrammatic view of the normal human synovial membrane based on our cytochemical and electron microscopic observations. We conceive that the lining cells form an intricate meshwork between the joint cavity and the underlying capillary bed. Unlike Lever and Ford (7), we have encountered no areas in which the lining cells are absent in these studies or in earlier cytochemical studies (2). On the other hand, we find that areas in which the lining cells are closely apposed, as described by Langer and Huth (9), are quite uncommon (Fig. 2). Generally, the cells and their processes are separated by wide intercellular spaces, as described by Lever and Ford (7) and Luse (8). Adhesion plates or desmosomes, characterizing sheets of epithelial cells, are not found. A basement membrane separating the lining cells from the more basal capillaries and other connective tissue cells is not observed. Although the cell processes approach one another more closely immediately beneath the joint space, even here interruptions are clearly evident. Thus, direct continuity between joint cavity and intercellular spaces is readily observed in thin sections. The matrix of the lining cells appears to be free of mature collagen fibers and contains abundant amorphous material (cf. 7) and fine fibrils, usually nearer the cell surfaces.

This picture of the synovial membrane structure is readily correlated with what is known concerning its function. The synovial membrane appears predominantly concerned with the production of hyaluronate of synovial fluid. Hyaluronate forms part of the matrix of the lining cells, and our observations are consistent with the view that hyaluronate is formed and secreted by the lining cells. Proteins present in synovial fluid, as well as small molecules such as glucose, urea, and electrolytes, are derived from plasma within capillaries directly below the lining cells (Fig. 11).

Passage of proteins and smaller molecules from these capillaries to the joint cavity is influenced by a physical barrier of overlapping cytoplasmic processes of lining cells, and by a chemical barrier of hyaluronate in the intercellular matrix. Ogston and Phelps (15) have recently shown that some solutes are sterically excluded from the volume occupied by hyaluronate chains. In the normal synovial membrane, hyaluronate chains may prevent the passage of serum proteins of high molecular weight from capillaries into the joint cavity.

Synovial lining cells, particularly the type A cell, may be active in the uptake of material. Vaubel (16), working in tissue culture, thought he could distinguish synovial cells (synovioblasts) from fibroblasts by the ability of the former to take up droplets of neutral red. Uptake of material by type A cells may be achieved by phagocytosis and pinocytosis. As suggested in Fig. 6, filopodia appear to phagocytize material in the extracellular milieu. The cell membrane appears to be coated by a fibrillar and granular material of medium density and of fairly uniform thickness (200 to 400 A). This material also lines both the spaces partly enclosed by filopodia and the inner surface of vacuoles that lie adjacent to the plasma membrane (Fig. 6). In the ameba a morphologically similar "extraneous" coat at the cell membrane has been described, and may be a mucopolysaccharide (17) to which protein molecules (ferritin) are adsorbed prior to being pinocytosed (18). Fig. 3 demonstrates the small vesicles that are present in abundance below the cell surface. Without the use of electron-opaque markers, the suggestion that these are micropinocytotic vacuoles is clearly tentative. Their relation to the 0.4 to 1.5 μ vacuoles remains to be studied, as does the possible relation of these vacuoles to the acid phosphatase-rich lysosomes. The role of the synovial lining cells in active interchange of material was emphasized by Langer and Huth (9).

FIGURE 4

Type A lining cell from a normal human synovial membrane. The supranuclear Golgi zone (G) is partially enclosed by the line marking the rectangular area enlarged in Fig. 7. The numerous and tortuous filopodia (F), abundant vacuoles with granular material (V) (see also Fig. 7), and Golgi apparatus (G) suggest an active interchange with the extracellular matrix. Many mitochondria (M) with numerous cristae are also present; the ergastoplasm (E) is scant. The ovoid, irregular nucleus (N) with finely scattered chromatin is typical of these cells. A dense material is present in the extracellular matrix, frequently next to the cell membrane (arrows). × 15,000.
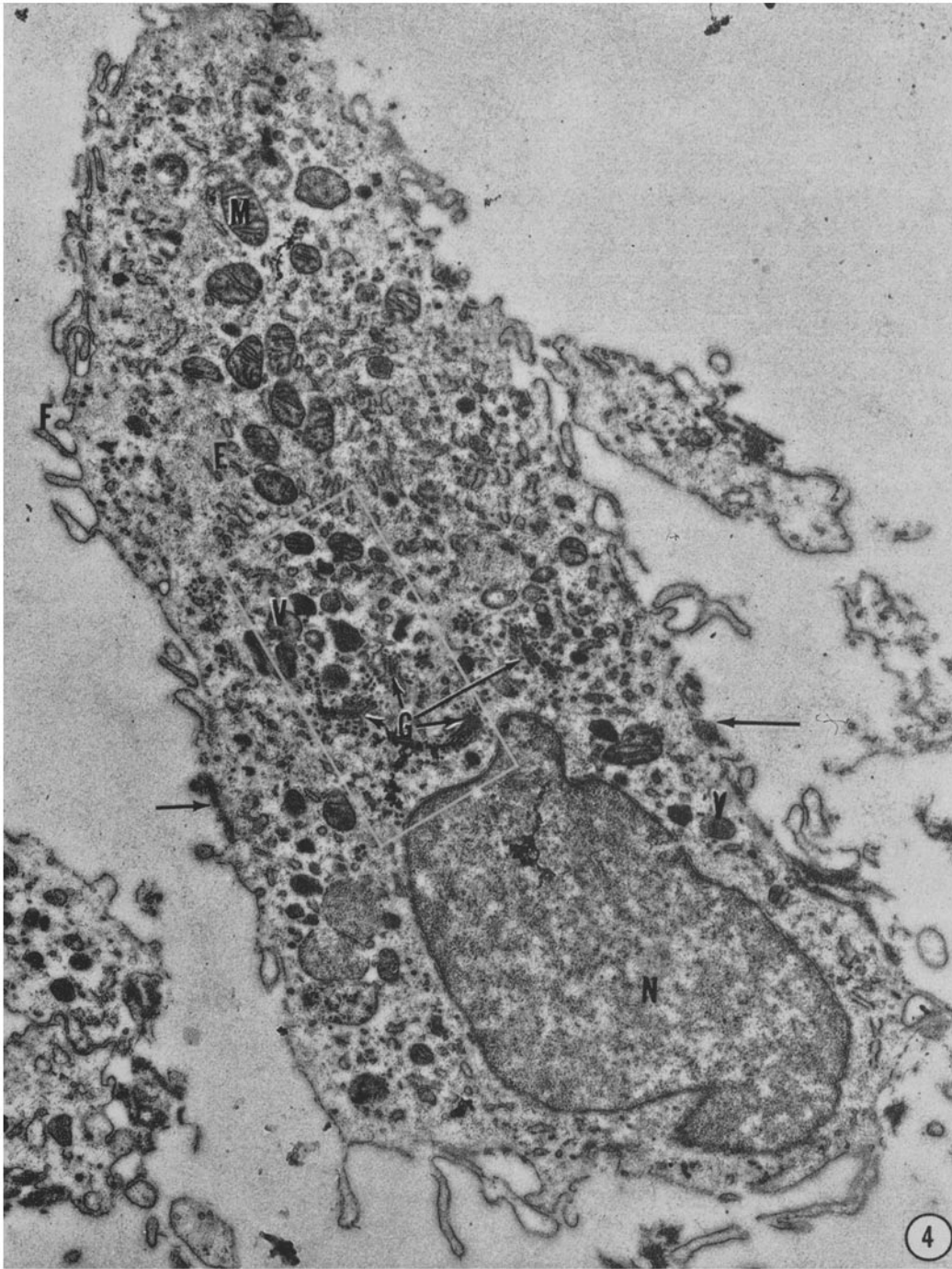
The less numerous cell type B contains abundant ergastoplasm. Such ergastoplasm, in other cells such as the exocrine cells of the pancreas and the plasma cells, is thought to synthesize proteins to be discharged from the cell. It remains to be determined whether the type B cells synthesize and secrete protein. All the known proteins of joint fluid appear to arise from plasma. About 2 per cent protein, firmly bound to the hyaluronate of synovial fluid, has been described but its origin is not known (19).

It may be expected that the structural differentiations of the two cell types, or functional states manifested by a single cell type, as described here, are correlated with different activities of the synovium. It appears likely that the lining cells are the major source of hyaluronate, that they play a role in the turnover of the components of synovial fluid, and that their secretory products in the matrix influence the passage of molecules between blood and joint cavity.

REFERENCES

1. Castor, C. W., The microscopic structure of normal human synovial tissue, *Arth. and Rheumat.*, 1960, **3**, 140.
2. Hamerman, D., Stephens, M., and Barland, P., Comparative histology and metabolism of synovial tissue in normal and arthritic joints, *in* Inflammation and Diseases of Connective Tissue, (L. D. Mills and J. H. Moyer, editors), Philadelphia, W. B. Saunders, 1961.
3. Pugh, D., and Walker, P. G., The localization of N-acetyl-β-glucosaminidase in tissue, *J. Histochem. and Cytochem.*, 1961, **9**, 242.
4. Hamerman, D., and Ruskin, J., Histologic studies on human synovial membrane. 1.

Figure 5

Part of a cytoplasmic process of a type A lining cell. The intracytoplasmic fibrils, 75 to 150 A in width, lie adjacent to and roughly parallel with the cell membrane. They appear to branch frequently and show no regular periodicity, though points of increased density occur commonly, especially at the bifurcation of a fibril (A). Similar fibrils are present in the extracellular matrix, usually adjacent to the cell membrane (B). Also present in the cytoplasm are vacuoles (V) containing a dense granular material, and a prominent Golgi apparatus (G). The cell membrane appears to be coated by a fibrillar and granular material of medium density (M). Arrows point to two vacuoles the membranes of which are continuous with the plasma membrane. × 35,000.

Figure 6

Portions of cytoplasmic processes of two lining cells in normal human synovial membrane. The numerous filopodia are presumed to be engulfing portions of the extracellular matrix, resulting in the formation of cytoplasmic vacuoles; see V. In most areas the cell membrane, and large vacuoles forming from it, appear to be coated by a fibrillar material of medium density, 200 to 400 A in width. Its fibrillar nature is seen clearly at the short arrows. Several other types of vacuoles are seen: some small with moderately dense material (A), others small with denser material (B), and larger ones with dense material (C). It is possible that a type A vacuole is continuous with the larger vacuole at the long arrow. The dense material of vacuoles B and C may have been engulfed from the extracellular matrix. × 45,000.

Figure 7

Enlargement of area outlined in Fig. 4, showing a portion of the extensively developed Golgi apparatus (G) and its close relationship to the vacuoles (V) of the dense type. Mitochondria (M) and nucleus (N) are also seen. × 35,000.
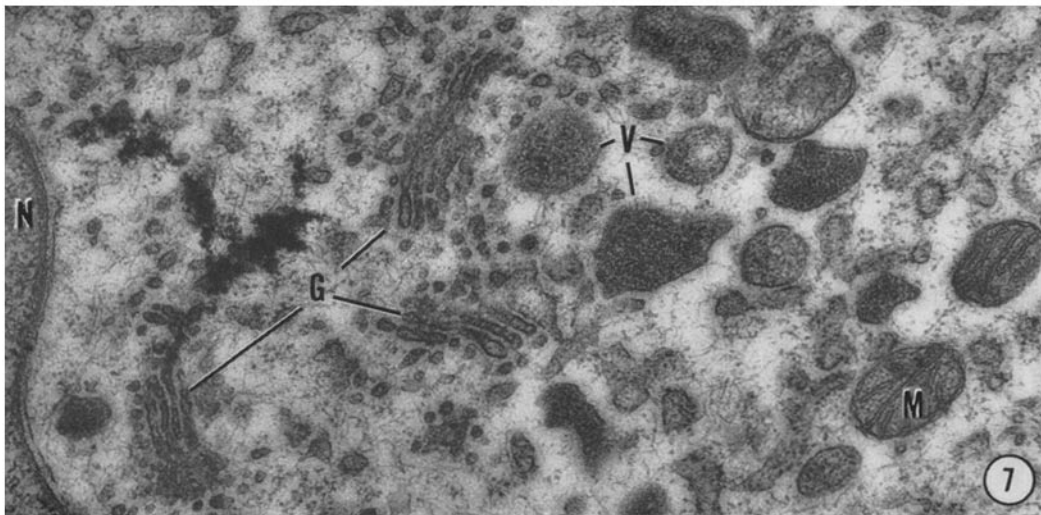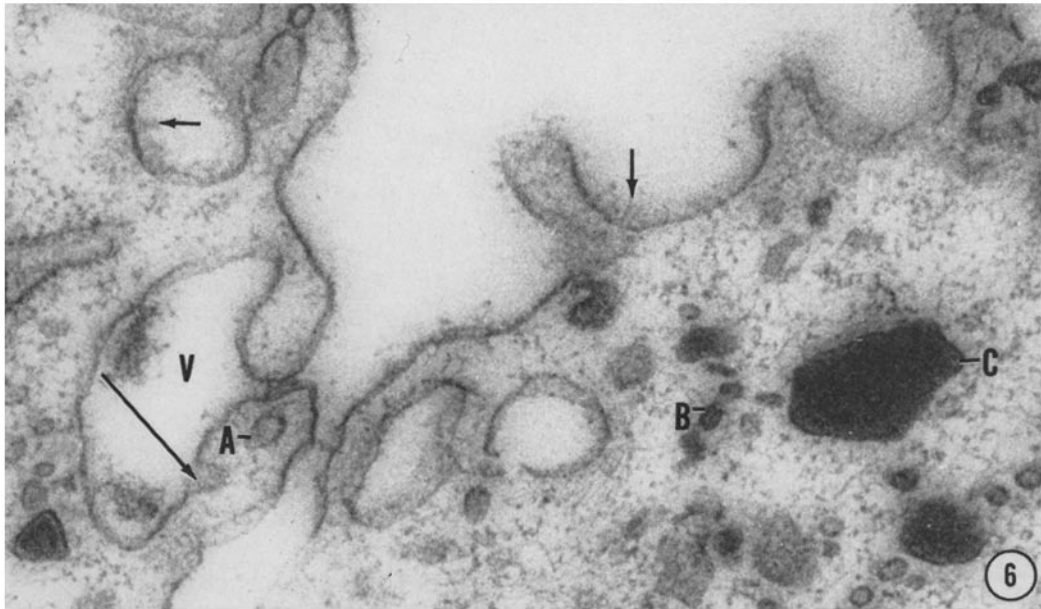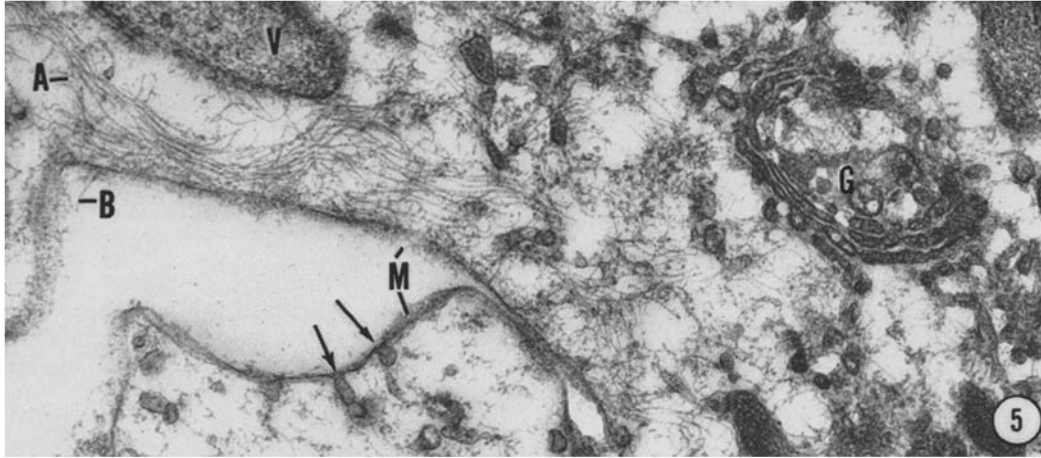
Metachromatic staining and the effects of streptococcal hyaluronidase, *Arth. and Rheumat.*, 1959, **2**, 446.

5. Thomas, D. P. P., and Dingle, J. T., Studies on human synovial membrane *in vitro*. The metabolism of normal and rheumatoid synovia and the effect of hydrocortisone, *Biochem. J.*, 1958, **68**, 231

6. Yielding, K. L., Tomkins, G., and Bunim, J. J., Synthesis of hyaluronic acid by human synovial tissue slices, *Science*, 1957, **125**, 1300.

7. Lever, J. D., and Ford, E. H. R., Histological, histochemical and electron microscopic observations on synovial membrane, *Anat. Rec.*, 1958, **132**, 525.

8. Luse, S. A., A synovial sarcoma studied by electron microscopy, *Cancer*, 1960, **13**, 312.

9. Langer, E., and Huth, F., Untersuchungen uber den submikroskopischen Bau der Synovialmembran, *Z. Zellforsch.*, 1960, **51**, 545.

10. Caulfield, F. B., Effects of varying the vehicle for OsO₄ in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.

11. Ward, R. T., Prevention of polymerization damage in methacrylate embedding media, *J. Histochem. and Cytochem.*, 1958, **6**, 398.

12. Lawn, A. M., The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy resin, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 197.

13. Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

14. Hamerman, D., and Blum, M., Histological studies in human synovial membrane. 2. Localization of some oxidative enzymes in synovial membrane cells, *Arth. and Rheumat.*, 1959, **2**, 553.

15. Ogston, A. G., and Phelps, C. F., The partition of solutes between buffer solutions and solutions containing hyaluronic acid, *Biochem. J.*, 1961, **78**, 827.

16. Vaubel, E., Form and function of synovial cells in tissue cultures, *J. Exp. Med.*, 1933, **58**, 63.

17. Bairati, A., and Lehmann, F. E., Structural and chemical properties of the plasmalemma of *Amoeba proteus*, *Exp. Cell Research*, 1953, **5**, 220.

18. Brandt, P. W., and Pappas, G. D., An electron microscopic study of pinocytosis in ameba, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 675.

19. Hamerman, D., and Sandson, J., Hyaluronate-protein complex of human synovial fluid studied by iodination with I-131, abstract *Am. Rheumat. Assn.*, 1961.

Figure 8

Part of a lining cell from a human synovial membrane containing a part of the Golgi apparatus (G). The saccular dilatations at the ends of the paired membrane stacks are clearly seen, and a small amount of granular material is recognizable in some dilatations. There appears to be more of this material in the surrounding vacuoles (V). × 35,000.

Figure 9

Type B lining cell from a normal human synovial membrane. The nucleus (N) is eccentric and irregular in outline and its chromatin is finely clumped. In contrast to the type A lining cell, there is an abundant amount of ergastoplasm (E) and fewer mitochondria (M), large vacuoles (V), and filopodia (F). The cavities between the membranes of the ergastoplasm (cisternae) contain an amorphous material of the same density as that of the cytoplasmic ground substance. Much amorphous material of high electron opacity is present in the extracellular matrix in close proximity to the cell membrane (arrow). Less ergastoplasm appears to be present in the process extending from the main body of the cell (left upper quadrant). × 15,000.
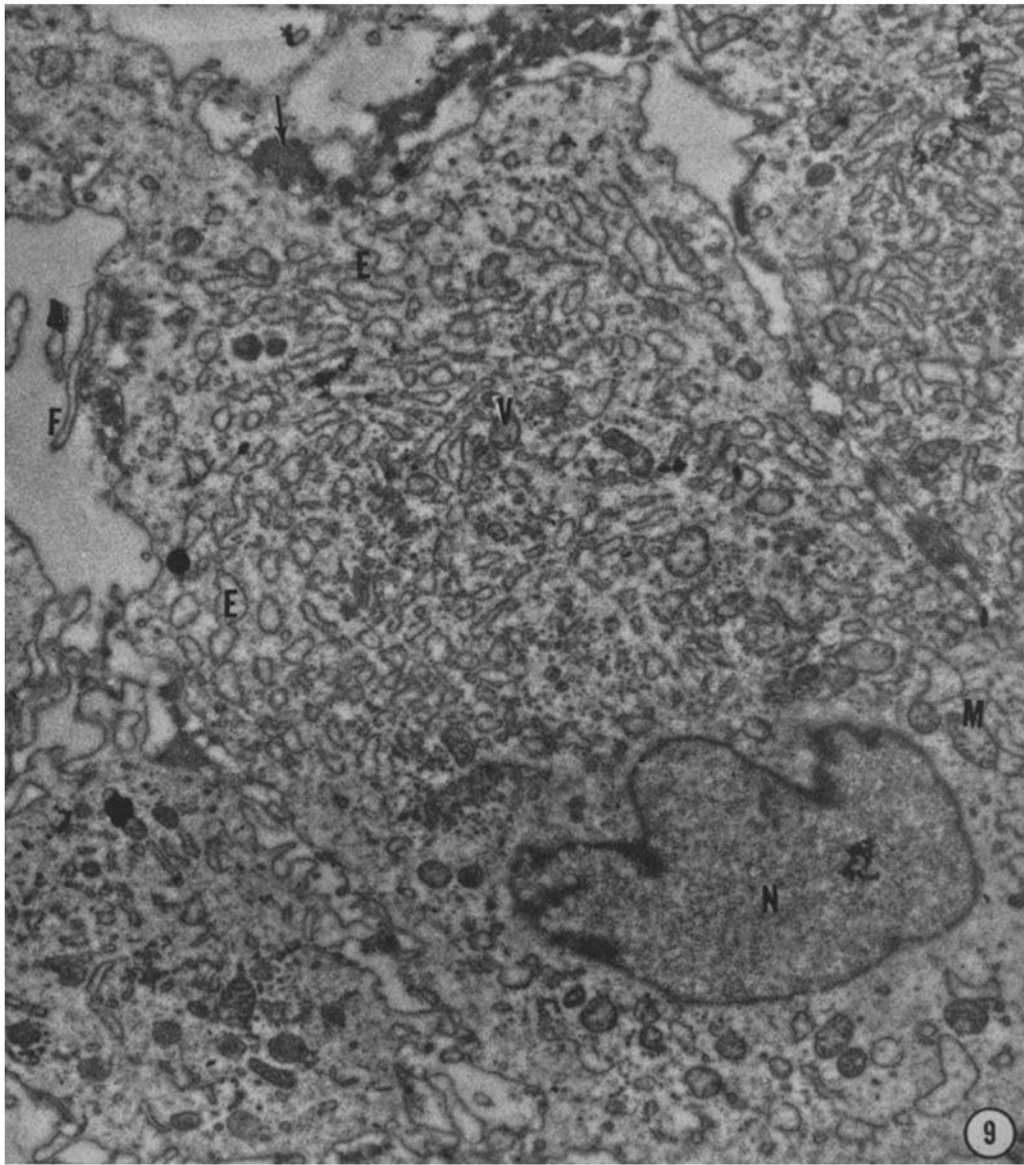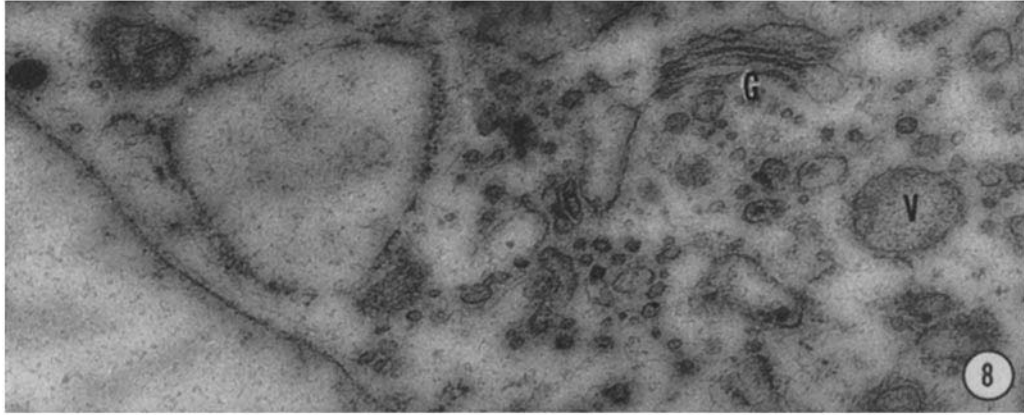
FIGURE 10

Distal parts of several cytoplasmic processes of lining cells in human synovial membrane. All processes show numerous vacuoles presumed to be micropinocytotic vesicles. They are closely associated with the plasma membrane and in some instances are seen to be continuous with it (arrows). Fibrils are seen in the cytoplasm (A) and in the extracellular space (B). × 25,000.
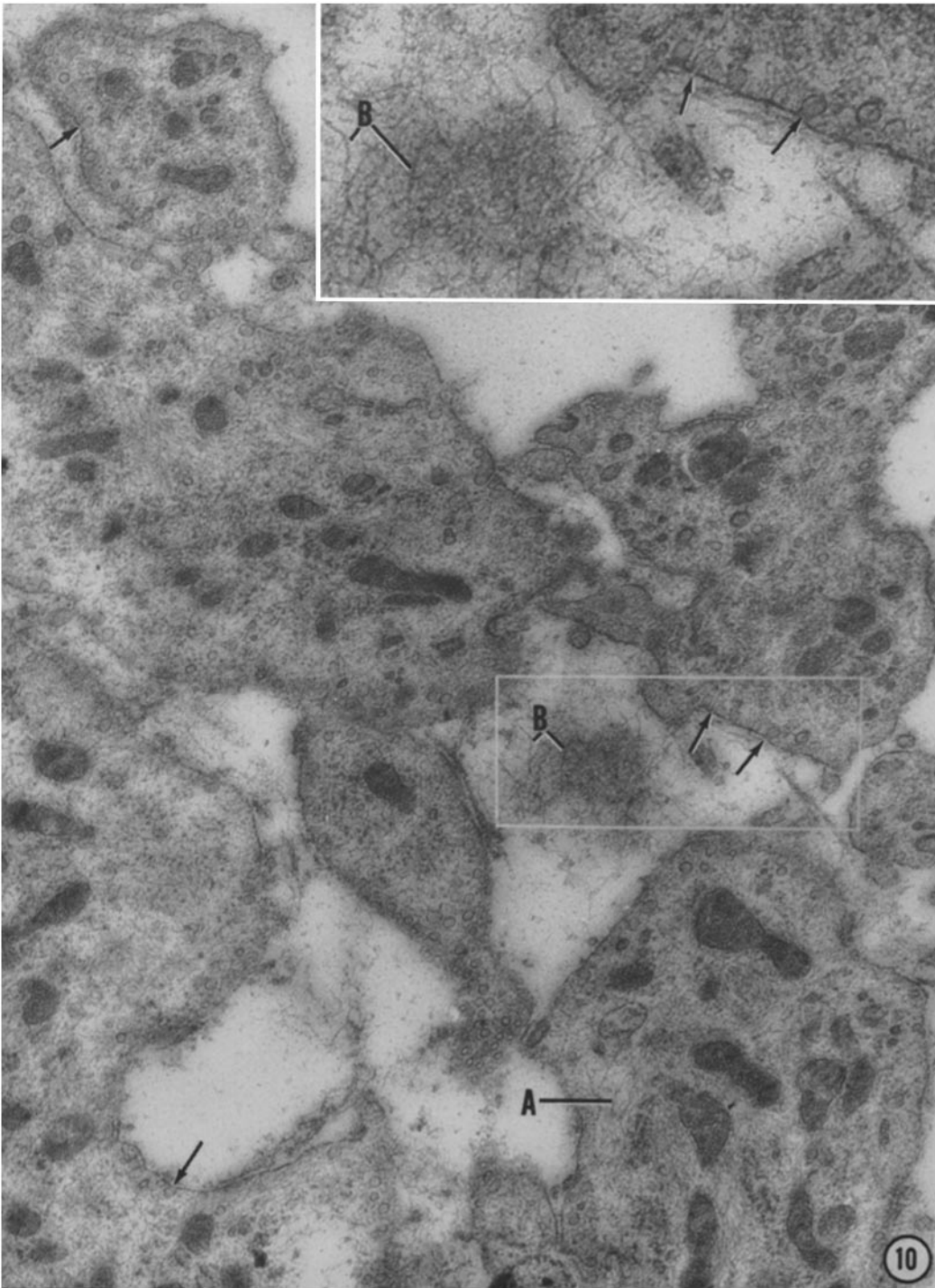
Outlined area is enlarged at upper right. × 50,000.

FIGURE 11

A schematic representaton of the surface of the normal human ⁣
joint cavity.