The Characteristics of Human Synovial Tissue As Seen with the Electron Microscope

By WILLIAM H. COULTER

Synovial tissue specimens from the joints of nine persons, determined to be normal by light microscopic studies, were examined with the electron microscope. The arrangement of microstructures within the synovial cell indicates a moderate amount of metabolic activity, and appears to be consistent with the production of mucopolysaccharides. A process of active transport was observed between the synovial cell and the joint space. Incorporation of collagen into the general architecture is also described, and the technical problems are considered.

Specimens de tissu synovial ab le articulationes de novem personas esseva examinate per microscopia electronic post determination de lor normalitate per microscopia optic. Le disposition del microstructura intra le cellula synovial reflecte un grado moderate de activitate metabolic e pare esser apte al production de mucopolysaccharidos. Un processo de transporto active esseva observate inter le cellula synovial e le spatio articular. Le incorporation de collageno in le architectura general es etiam describite. Problemas technic es discutite.

LIGHT MICROSCOPY has provided information concerning both the general architecture of the synovial membrane[13,16,24,25,49] and its histochemical properties.[29] Cytologic fine structure measuring less than 0.2 μ cannot be resolved by the methods of light microscopy due to the relatively long wave length of visible light. The primary purpose of this investigation was to determine the nature of normal human synovial microstructure as a basis for a study of pathologic specimens. Observations on various other cell types will be considered for comparison with the synovial cell, to provide a basis for evaluating its special characteristics.

In addition, a simple solution to the special problem of orientation of this tissue is described, by which the interested observer can obtain consistent results in microtomy.

MATERIALS AND METHODS

Synovial membrane specimens were obtained at operation from the knees of nine persons. All were males ranging in age from 15 to 76, with a mean age of 51. In three, arthrotomy was done for the removal of torn menisci and in six, vascular disease was the cause for amputation. All samples appeared to be normal by both gross inspection and light microscopic studies. Joint fluid of normal viscosity, cellularity, and mucin clot characteristics was obtained in one case, and in the others the fluid adherent to the joint lining appeared normal in color and viscous characteristics. In no case was there a clinical history

of joint disease or effusion. Several of the specimens were from the same samples used by Castor[7,9] in the study of synovial tissue with the light microscope.

Fixation of specimens with osmium tetroxide was accomplished in the manner of Palade[33] approximately 45 minutes after termination of the blood supply. Because the synovial membrane is a surface tissue, optimal fixation of the cells as described by Farquhar[18] was impossible.

Orientation of the synovial membrane presented a challenge. The problem was not only to insure that the intimal lining surface was included in the sections, but also to obtain these sections at right angles to the surface. The membrane with approximately 1 mm. of attached subsynovial connective tissue was excised in plaques 1 cm. square. When fixation, washing, dehydration in ethyl alcohol and infiltration with catalyzed methacrylate was accomplished, the plaques were cut into strips 1 mm. wide with the aid of a dissecting microscope. These strips were then suspended and embedded in size 00 gelatin capsules. This was accomplished by cutting a slit in a small piece of heavy paper, 3 x 10 mm. One end of the tissue strip was inserted in the slit, and then the tissue-and-paper unit was placed in the capsule, previously filled with catalyzed methacrylate. In this way the tissue is held in place by the force of the paper against the inner sides of the capsule (fig. 1). This method provided a long continuous tissue block which was, nevertheless, correctly oriented and adequately penetrated by osmium tetroxide. It is felt that this method would be useful in the study of other surface membranes.

Sectioning was done with a Porter-Blum microtome using glass knives. Viewing was accomplished with an RCA type EML-1 microscope.

OBSERVATIONS

Many of the microstructures described in other tissues were observed in normal human synovial tissue.

Nucleus

The nucleus (figs. 6 and 7) of the synovial cell is heavily granular and considerably more osmiophilic than the cytoplasm. Nuclear density is greatest
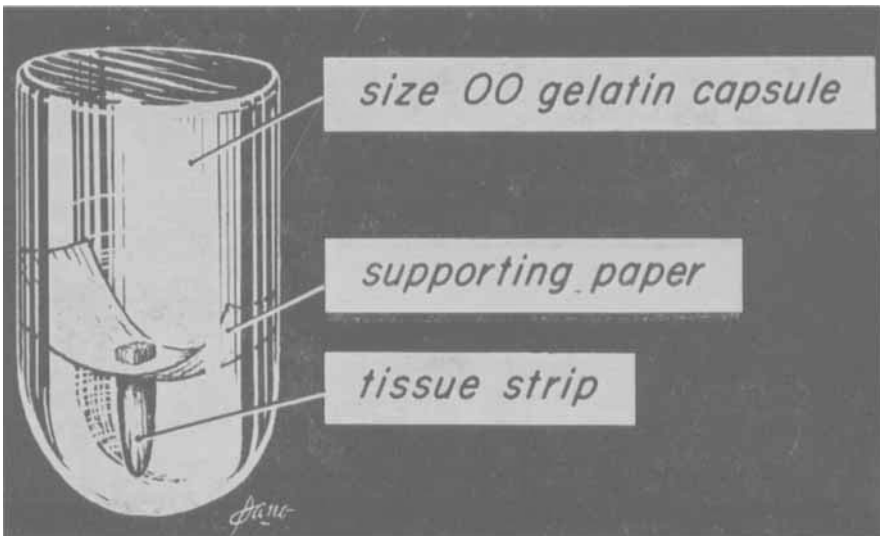

Fig. 1.—Schematic diagram showing the method of tissue orientation, which proved reliable in providing tissue sections at right angles to the synovial surface.
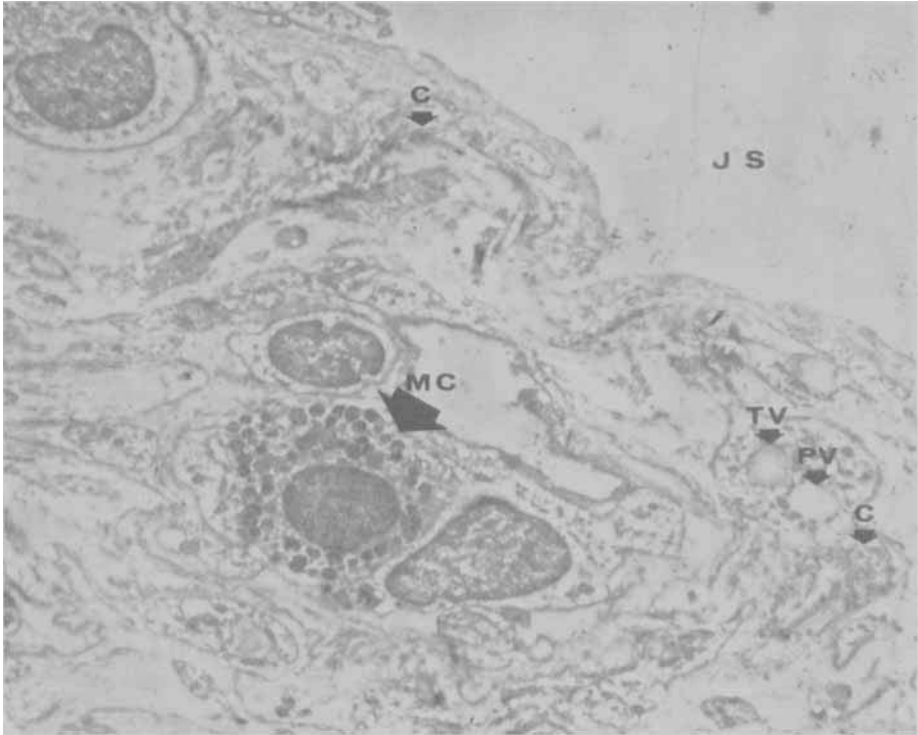
Fig. 2.—Low-power section of the synovial surface, showing the joint space (JS). A mast cell (MC) is shown in close proximity to a synovial cell. Note distinct differences in nuclear density between the two cell types. Collagen fibers (C) may be seen nearly in contact with the joint space (JS). Vacuolar structures of both the thick-walled type (TV) and phagocytic type (PV) are demonstrated. Magnification x3500.

along the periphery and, in tangential sections, this density appears to be uniformly perforated by the underlying less dense nuclear material. The perforations, an average of 500 Å in diameter, are approximately 0.1 μ apart.

The perinuclear envelope consists of an inner and outer membrane. The outer membrane appears to be continuous with the endoplasmic reticulum. Complete perforations of both membranes of the nuclear envelope, described as "pores" by Watson,[55] were not visualized in this study.

Cell Membrane

In general, the outer wall of the synovial cell was typical of that seen in the majority of other cells. It consisted of a thin, osmiophilic line containing the structures of the cytoplasm. Contrary to the findings of Langer and Huth,[27] little interdigitation of human synovial cells was noted.

Numerous micro-invaginations were noted along this outer surface, particularly those portions of the cell in contact with the joint space. These small vesicles measure approximately 500 Å in diameter and appear to be taken into the interior of the cell as seen in the process of pinocytosis.
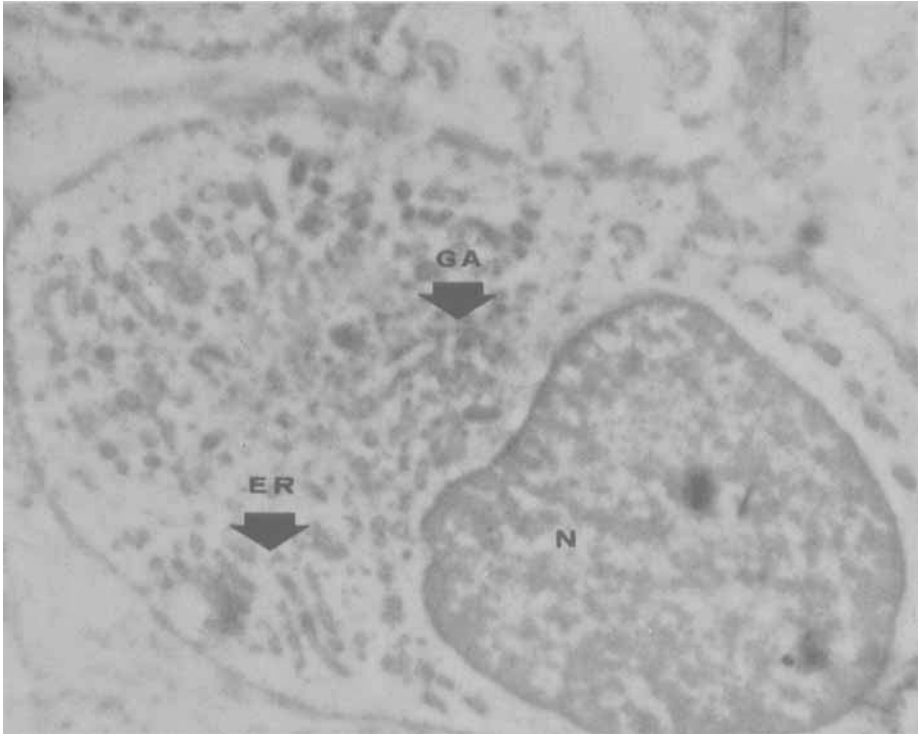
Fig. 3.—Synovial cell showing "cysternae" or elongated endoplasmic reticulum (ER) and Golgi area (GA) in relation to a scooped-out portion of the nucleus (N). Osmiophobic "Golgi vacuoles" may be seen. Magnification x15,000 (reduced).

Cytoplasmic Components

The cytoplasmic ground substance, a light grey granular material, closely resembles that reported by others in various cell types.

Mitochondria (fig. 4): Mitochondria of the synovial cell appeared similar to those of other tissues.[34] Christae mitochondriales were not prominent and the fine double membrane described by Sjöstrand[50] was visualized only with difficulty. The size and number of mitochondria varied (10 to 35 per average cell section) from cell to cell. Their distribution was random.

Endoplasmic reticulum (fig. 3): "Ergastoplasm" or endoplasmic reticulum of both types described by Palade[38] was found. "Smooth-surfaced elements," most frequently seen at the periphery of the cells, were more variable in number than the ribonucleic acid-containing "rough-surfaced elements."[45] Some of the smooth-surfaced structures were seen in contact with the plasma membrane. Frequently there was moderate alignment of "cysternae" or elongated endoplasmic reticulum parallel to the plasma membrane. Kemp[23] notes this to be characteristic of a protein-producing cell. Occasionally there appeared to be continuity between the endoplasmic reticulum and nuclear membrane.

Golgi apparatus (fig. 3): This structure is prominent in the synovial cell
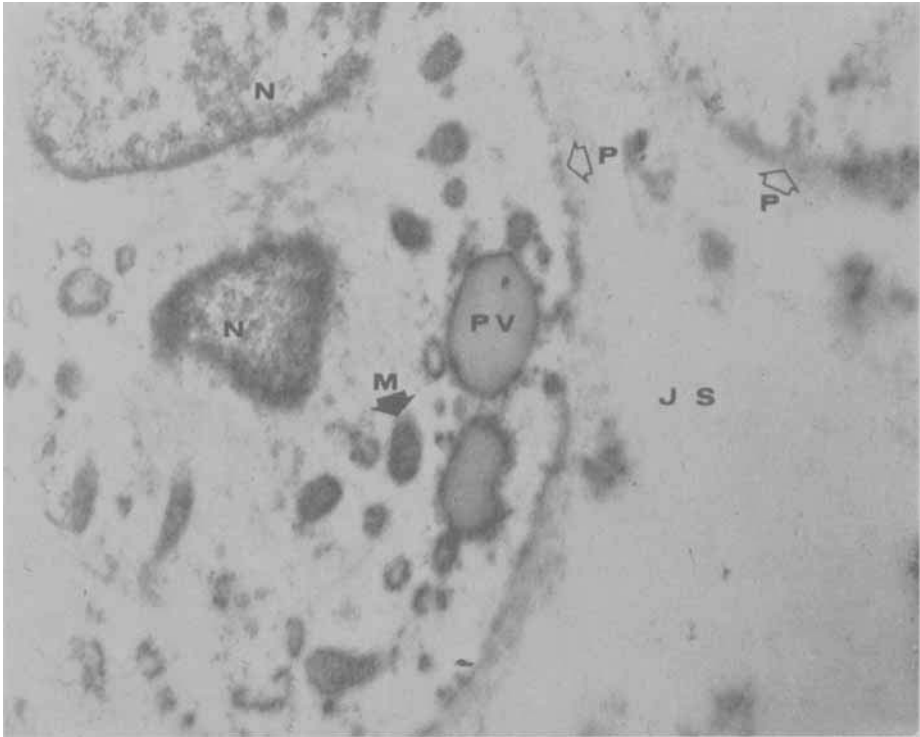
Fig. 4.—Two "phagocytic" vacuoles (PV) of a synovial cell. An inclusion may be seen in one vacuole. M designates mitochondrion with visible christae. P denotes pinosomes, evidence of active ingestion of materials from the joint space (JS) by the cell.

and appears to be continuous with the endoplasmic reticulum as described by Palay.[39] It is characterized by a concentration of fine vesicles oriented close to a scooped-out portion of the nucleus. Osmiophobic structures resembling "Golgi vacuoles"[3,11] have been observed.

Vacuoles (fig. 5): Two types of "vacuoles" were observed in the synovial cell. Most frequently seen was a membrane-enclosed vacuole containing a uniform substance of moderate osmiophilic properties. These vacuoles were occasionally seen with inclusions and resembled those described by Parks[41,42] in the hepatic sinusoidal endothelial cell, and Odor[32] in the mesenteric mesothelial cell. Felix and Dalton[19] describe a similar structure in the free macrophages of the mesentery, and give evidence for phagocytic function of these structures. The second type of vacuole was of the same general size (0.75 μ) but contained a strongly osmiophilic substance which lined its interior and was about 0.1 μ in thickness. These vacuolar structures are considerably less dense in their centers, and most closely resemble the lipid droplets seen in cells of the intestinal mucosa. This is compatible with findings in synovial tissue-culture,[8,10] where lipid-staining technic reveals droplets in the cytoplasm.

Collagen (fig. 2): Fibrous structures identified as collagen were observed
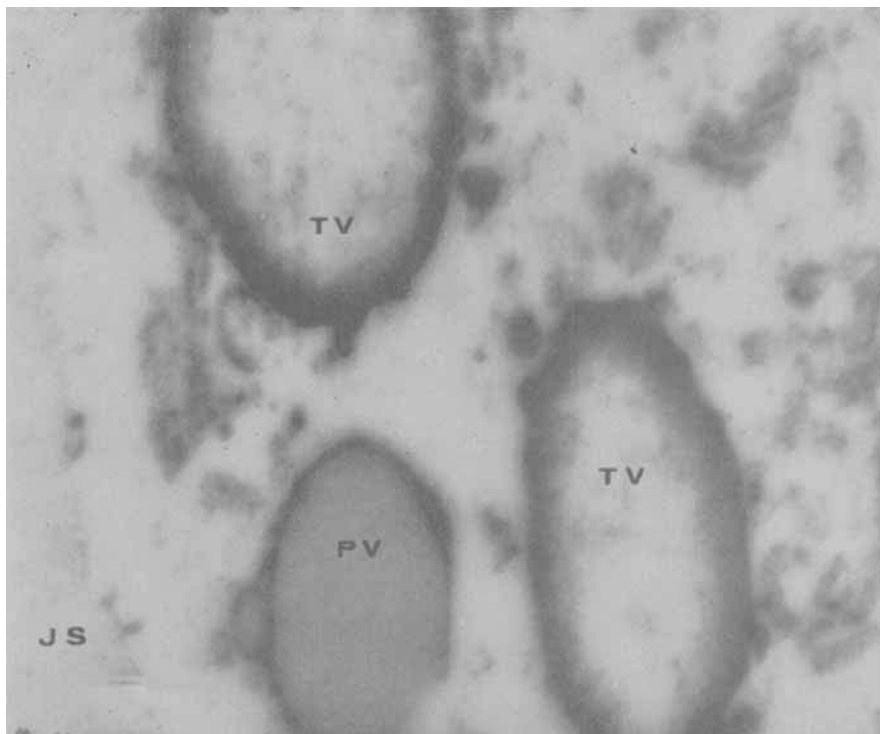
Fig. 5.—Unusual view of thickwalled vacuoles (TV) and "phagocytic" vacuole (PV) in close proximity. Magnification x50,000 (reduced).

to be in close proximity to individual synovial cells, and in communication with the joint space itself. Periodicity of these fibers could be demonstrated. A distinct band of collagen was commonly seen separating the synovial membrane from subsynovial layers. This is in agreement with findings using the light microscope.[29]

Mast cells (fig. 2): Mast cells were frequently seen below the synovial membrane, and occasionally within the membrane itself. These cells appear similar to those described with similar technic by Smith and Lewis.[52] In the present study, however, the cytoplasmic granules were not packed tightly around the nucleus. The usual cytoplasmic components described above were also observed, but they appeared to be secondary to the numerous granules. Granule size averaged 0.15 μ in diameter, with the largest measuring 0.25 μ.

DISCUSSION

A method for orientation of surface tissues has been described which has proven consistent in yielding sections which are at right angles to the synovial surface. The possibility exists that this method might be helpful in the study of other surface membranes.

Pinocytosis, according to recent work,[5] suggests a high rate of active transport. It is thought by many to explain the entrance of macromolecules into the cell. Mucopolysaccharides on the cell surface apparently act as selective bind-
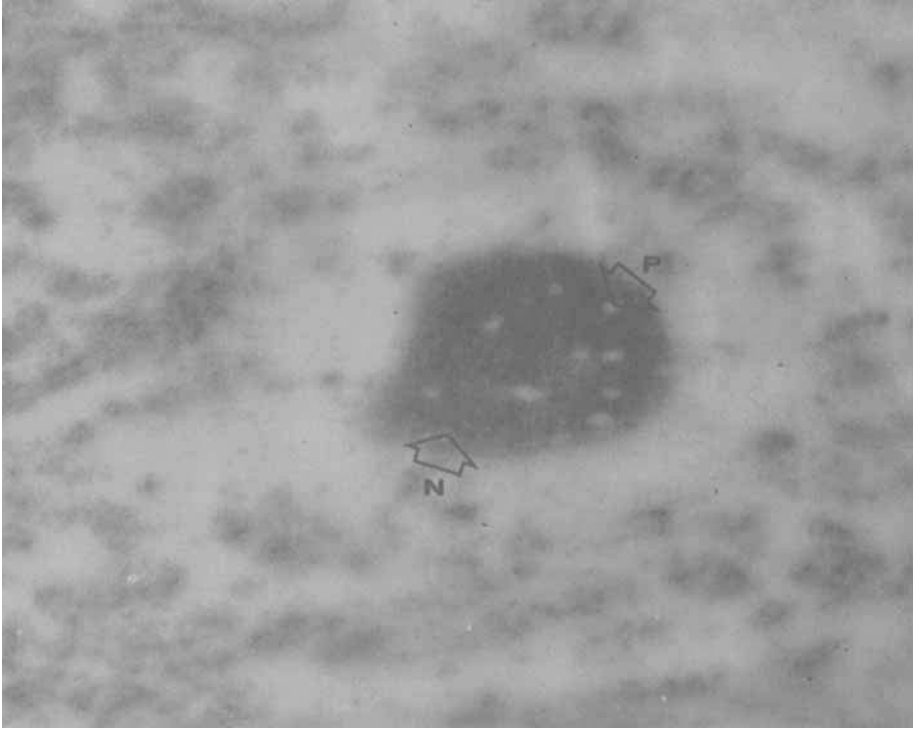
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Fig. 6.—High power tangential section of synovial cell nucleus. Note the small, constant-sized perforations (P) in the dark-staining peripheral nuclear material (N). Magnification x90,000 (reduced).

ing sites for the specific proteins needed in the cell's metabolic processes. These essential materials are then brought to the interior of the cell via the "pinosome." In the case of the synovial cell, this is felt to be clear evidence of metabolic interaction between the intimal lining surface and the joint fluid.

Due to the seemingly traumatic assignment of the synovial membrane, one would expect to find considerable interlocking of the intimal lining cells. Although Langer and Huth[27] note this to be true in several species, interdigitation was not found to be prominent among human synovial cells.

In vitro studies by Bahr[1] indicate that the electron density achieved by osmium tetroxide fixation is probably due mainly to unsaturated double bonds and sulfhydryl groups. In the synovial cell, the nucleus probably represents a concentration of such structures.

A perinuclear membrane similar to that of the synovial cell has been noted by Watson[55] to exist in the parenchymatous cells of the liver, primary spermatocytes, lymphocytes and reticular cells of the spleen. He suggests that such a perinuclear space which is continuous with the endoplasmic reticulum may be a pathway of exchange between nucleus and cytoplasm.

According to Dempsey,[15] the frequency of mitochrondria as seen in synovial cells is probably best interpreted as evidence of moderate metabolic activity.
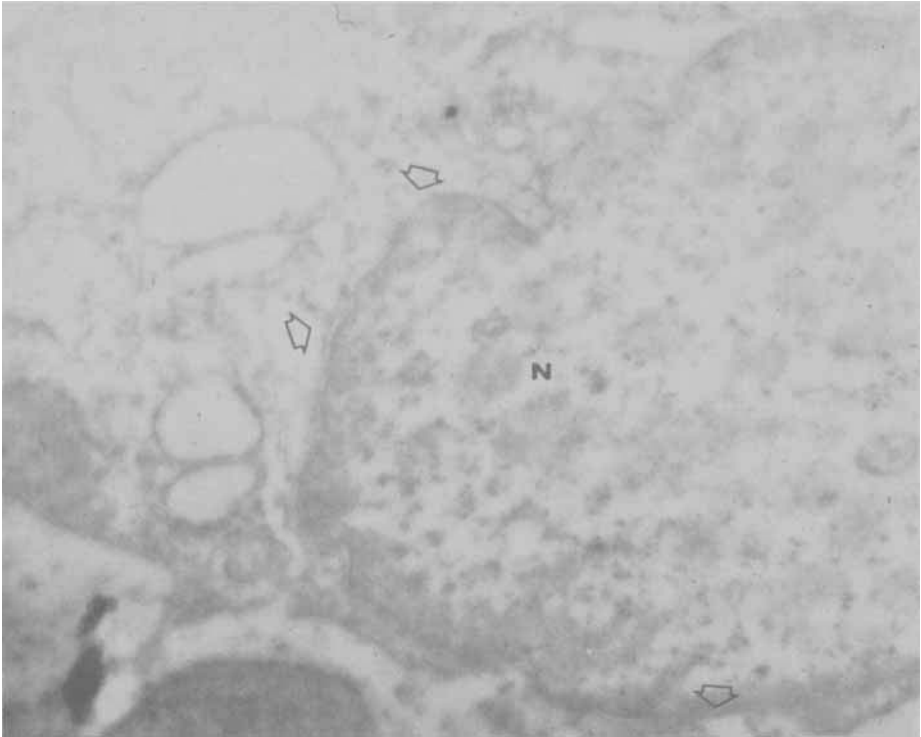
Fig. 7.—Section through synovial cell nucleus (N) showing perinuclear envelope. At certain points the outer membrane of the envelope may be seen to be continuous with the endoplasmic reticulum (arrows). Magnification x75,000 (reduced).

Although this might be explained by the traumatic death and regeneration theory of Efskind,[16] this idea is refuted by the notorious lack of mitotic figures in this tissue. Hence, one is left with moderate metabolic activity without mitosis, which is suggestive of a productive function.

Kemp[23] states that cells which are active in protein synthesis have a characteristic arrangement of cysternae in parallel rows. Such arrangement has been observed in synovial cells. The studies of Castor[8,10] provide conclusive evidence that human synovial cells are active in polysaccharide production. Although no structural configuration has been assigned to the production of mucopolysaccharides, one finds that cells producing glycoproteins and similar large sugar-containing molecules are arranged similarly.[38]

Electron micrographs do not resolve questions concerning the origin of the synovial intimal cell—that is, whether it is more closely related to the fibroblast or the reticuloendothelial system. The general cytoplasmic morphology of the synovial cell appears to closely resemble the splenic phagocyte and the hepatic phagocyte in arrangement of mitochondria and endoplasmic reticulum. It differs markedly from the subsynovial fibroblast, which is conspicuously lacking in these cytoplasmic structures. In this respect, the synovial cell appears to be more active than the mature fibroblast; the available

information[15,23] suggests that it possesses considerable metabolic activity. It would be of interest to compare these findings with those of the metabolically active fibroblast, as in the healing wound.

SUMMARY

1. The synovial membrane of nine persons, determined to be normal by light microscopy, was studied with the electron microscope; a solution to the inherent orientation problem of surface membranes is described.

2. Evidence of pinocytosis points clearly to interaction between the synovial cell and the joint fluid.

3. The mitochondrial and endoplasmic reticular arrangement of the synovial cell are not incompatible with a glycoprotein producing cell of moderate metabolic activity.

4. Two types of vacuoles are observed within the cell; "phagocytic" vacuoles and a second, "thick-walled" structure, probably lipid in nature.

5. A collagenous structure and its incorporation into the synovial membrane is described.

6. Contrary to observations in other species, little interdigitation among human synovial cells is seen.

ACKNOWLEDGMENTS

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