

The Microscopic Structure of Normal Human Synovial Tissue

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Microscopic study of normal synovial tissue from 16 humans of varying age disclosed an architectural pattern which did not vary significantly with age, sex or intra-articular location. Except for fibrous synovium, the tissue samples were similar in vascularity, number of surface cell layers and in the differential composition of the surface cells. Histochemical procedures delineated a pericellular surface reticulin network but failed to establish the locus of hyaluronic acid synthesis.

Le studio microscopique de normal histos synovial ab 16 humanos de varie etates revelava un configuration architectural que non variava significativamente con etate, sexo, o sito intra-articular. A parte specimens de histo fibrose, le specimens esseva simile in vascularitate, numero del stratos de cellulas superficial, e composition differential del cellulas superficial. Studios histochimic delineava un systema de reticulina al superficie pericellular sed non succedeva a establir le loco del synthese de acido hyaluronic.

SEVERAL AUTHORITATIVE studies form the foundation of current concepts of normal synovial morphology which serve as a baseline for evaluating pathologic changes.¹⁻⁵ With the development of the joint needle biopsy technic, specimens of synovial tissue from patients with rheumatic diseases are more commonly available for pathologic examination. While experience with this technic has led to the appreciation that some disorders, such as gout or tuberculous synovitis, may be recognized with a reasonable degree of certainty, the pathologic picture often is not specific for any one rheumatic disease.^{6,7} A detailed study of the micro-structure and histochemistry of normal synovial tissue might aid in interpreting the histopathologic picture in this tissue in the rheumatic disease states. The objective of this report will be to extend the observations of earlier workers, by employing semi-quantitative methods and more recent histochemical technics.

MATERIALS AND METHODS

Human synovial tissue was obtained from the knee joint at the time of amputation in 15 instances and at the time of arthrotomy on another occasion. This material was obtained from 5 women and 11 men with ages ranging from 14 to 68 years and an average age of 49 years. Above-the-knee amputations were performed for peripheral arterial occlusive disease in 11 persons and for malignant neoplasms in four, while the arthrotomy was done to remove a torn meniscus. Inclusion of a specimen as "normal" depended on the following criteria: (1) absence of known chronic joint disease; (2) absence of effusion at time of joint dissection; (3) absence of significant gross articular cartilaginous defects; and (4) a grossly thin, pliable, white synovial membrane. Tissue was removed by sharp dissection

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from the marginal joint areas, the suprapatellar bursa, the patellar synovial fold and, in several instances, from the posterior popliteal pouch. Eighty-two specimens were removed for study. The tissues were variously fixed with Zenker-formol, Bouin's fluid, formalin-alcohol-acetic acid, 10 per cent formalin, 80 per cent ethyl alcohol, 100 per cent ethyl alcohol, Carnoy's fluid, B-20 (Allen's modification of Flemming's fluid), and additional material was prepared by the freeze-drying procedure. The dehydrating agents used included graded ethyl alcohol and redistilled ethyl cellosolve, while clearing was accomplished with chloroform, acetone or aniline oil. Paraffin sections were cut 3 to 4 μ in thickness and mounted from water or diacetin on glass slides, both with and without albumin fixative.

The general histologic features of the synovium were outlined with the Masson trichrome stain. Toluidine blue O and azure A were used to demonstrate metachromasia, employing the pH range 3.5 to 7.0. Toluidine blue O was used at concentrations of 0.1 per cent to 0.5 per cent and azure A at 0.006 per cent. The periodic acid-Schiff procedure with and without Alcian Blue was used to reveal aldehyde-yielding structures. The Wilder silver method was used for reticular fibers, and the Gomori aldehyde technic to delineate elastic fibers.

Synovial specimens were classified on the basis of the predominant structure of the "subintimal"* connective tissue as: (1) fibrous, (2) fibro-areolar, (3) areolar, (4) areolo-adipose, and (5) adipose. The average depth of the synovial cell layer in number of cell layers was estimated visually and the range for each specimen recorded. The number of cross sections of subintimal blood vessels was counted over 10 high power microscopic fields (430 \times) and the range of values recorded. The cellular structures beneath the synovial surface for a distance of 70 μ (measured with an ocular micrometer) were identified as (1) synovial "intimal" cells, (2) mast cells, (3) endothelial cells and (4) "unclassified" connective tissue cells.

OBSERVATIONS

General Architecture

General histologic information derived from Masson trichrome-stained sections of synovial tissue is summarized in tabular form. Table 1 shows that in the material sampled, areolar synovium and its gradations were frequently encountered, while fibrous synovium was uncommon. Statistical analysis[†] supports the concept that synovial tissue type is related to intra-articular location. This is probably a reflection of the high frequency of adipose synovial samples obtained from the patellar synovial fold. The depth of the synovial surface, measured as the number of intimal cell layers, is recorded in table 2 in relation to synovial tissue type. An analysis of variance revealed no evidence that age affected the thickness of the normal synovial surface cell layer. Significant ($P < .01$) variation in cellularity, however, occurred in relation to different synovial tissue types, reflecting the prominent hypocellularity of the fibrous synovial samples. Table 2 also records the data on the frequency of subintimal blood vessel cross sections per high power microscopic field in the various tissue types. Again, age was not related to variation in vessel frequency, whereas there was a significant ($P < .01$) relation between vessel frequency and synovial type. This, again, appeared to reflect the hypovascularity of the fibrous synovium.

*"Subintimal" as used here refers to the area immediately beneath the synovial surface, "intimal" cells.

†This analysis (Rank Sum Test) was "person specific" rather than "specimen specific," since some individuals contributed more than one specimen.

Table 1.—*Frequency of Histologic Synovial Types in Relation to Intra-Articular Location*

Type of synovium	Number of samples	Marginal area	Supra-patellar bursa	Post-popliteal pouch	Patellar synovial fold
Fibrous	4	2	1	1	0
Fibroareolar	15	10	2	1	2
Areolar	31	18	7	4	2
Areoloadipose	11	3	4	0	4
Adipose	11	0	2	0	9
Total	72	33	16	6	17

Table 2.—*Frequency of Synovial Cell Layers and Vessel Cross Sections in Relation to Synovial Tissue Type*

Type of synovium	No. of intimal cell layers			No. of subintimal blood vessel-cross sections per H.P.F.		
	No.* obs.	Mean mid-range	Absolute range	No.* obs.	Mean mid-range	Absolute range
Fibrous	6	0.7	0-3	4	1.0	0-7
Fibroareolar	12	1.6	0-4	12	3.4	0-12
Areolar	13	2.0	0-4	12	5.1	0-12
Areoloadipose	6	1.8	0-3	6	3.8	0-8
Adipose	7	2.4	1-5	7	4.0	1-8

*An observation is here defined as a mean mid-range by tissue type by person; e.g., one person might contribute three tissue types, hence contributing three observations, while several examples of one tissue type from a single person are treated as one observation.

†The mid-range for any given section is calculated as the average of the absolute range and the mean of several such calculations is the "mean mid-range."

In 15 patients, a total of 55 one hundred cell differential counts were made to define the cellular makeup of the synovial surface. Table 3 suggests that for a depth of 70 μ from the joint-lining surface, the cellular milieu consisted of roughly equal numbers of specialized connective tissue cells (the synovial "intimal" cells), and "unclassified" connective tissue cells ("fibroblasts," histiocytes and primordial mesenchymal cells). Approximately 9 to 10 per cent of the cellular population consisted of capillary endothelium, and nearly 3 per cent of the cell population were typical mast cells. Of the various cell types, only the mast cell was significantly affected ($P < .05$) by tissue type, apparently due to its rarity in fibrous synovium. Endothelial cell frequency appeared significantly decreased ($P < .05$) with advancing age. With these two exceptions, the general cellular makeup of the synovium appeared to be unaffected by tissue type or age.

The synovial membrane is a specialized connective tissue, and like all connective tissues is most conveniently subdivided for purposes of analysis into cellular, ground substance and fibrillar components.

Cellular Components

Synovial intimal cells.—These constituted the major cell type of the superficial zone facing the joint cavity (figs. 1, 2). These cells formed a compact interlacing layer varying from 1 to 3 cells in depth and extended 20 to 40 μ beneath the synovial surface. The intimal cells were generally ellipsoidal

Table 3.—Frequency of Various Cells Observed to a Depth of 70 μ Beneath the Joint Lining Surface

Cell type	No. obs.*	Mean frequency (%)	S.D. %
Intimal cell	23	49.0	± 13.4
Unclassified			
connective tissue-cell	23	38.8	± 13.6
Mast cell	23	2.8	± 1.4
Endothelial cell	23	9.3	± 3.7

*An observation is here defined as a mean percentage by tissue type by person, e.g., one person might contribute both fibrous and areolar tissue, thus contributing two observations.

with frequent processes extending long distances from the main cell body. The major axis of the cell bodies commonly measured 8 to 12 μ and the minor axis 6 to 8 μ . Intimal cell nuclei were oval or egg-shaped with minor axes of about 4 μ and major axes of 6 to 7 μ . The nuclear membrane was a distinct structure and was frequently invaginated to form a concavity facing the major portion of the cell cytoplasm. Chromatin in the interphase nuclei was seen in coarse .2 to .3 μ aggregates evenly dispersed over a pale nucleoplasm. Usually one nucleolus, 0.5 to 1.0 μ in diameter was present, although double nucleoli were not uncommon. The Feulgen nuclear reaction colored the nucleoplasm a pale pink and the chromatin aggregates a dark violet with notable variation in the intensity of staining from cell to cell. Mitotic figures were not seen.

The synovial intimal cell cytoplasm was moderately to deeply stained by the acid fuchsin of the Masson trichrome procedure. So visualized, a network of branching cell processes with finely granular fuchsinophilic cytoplasm was found to make up a major portion of the inner cellular zone. Cells 40 μ below the joint surface occasionally sent processes which appeared to terminate at the surface. Vacuoles occurred both in the main body of the cytoplasm and in the long cell processes. These vacuoles measured up to 3 μ in diameter, and their contents were not stained by any procedure used in this study. Rarely, the negative image of the Golgi apparatus was seen in the cytoplasm opposite an indented nucleus. Toluidine blue O or azure A usually stained a sparse lacy network of cytoplasm metachromatically. Incubation of chemically fixed sections with testicular hyaluronidase for two hours did not significantly alter cytoplasmic metachromasia, whereas incubation with ribonuclease at 37 C. for one hour resulted in a marked decrease in metachromatic staining. In five specimens prepared by freeze-drying, incubation with ribonuclease-free testicular hyaluronidase yielded obvious reduction of intracellular metachromasia in two, and equivocal change in the remaining three specimens.* Acid hydrolysis of chemically fixed sections with 1 N hydrochloric acid at 60 C. for 10 minutes removed most of the metachromatic material. Alcian Blue, presumed to react with tissue acid radicals, did not stain synovial cell cytoplasm in the pH range of 2 to 3, but did in the range from pH 4.25 to 5.0. Extraction of

*Ribonuclease-free testicular hyaluronidase, 5000 TRU/ml. of incubation solution in 0.05 M acetate buffer, pH 5.7.

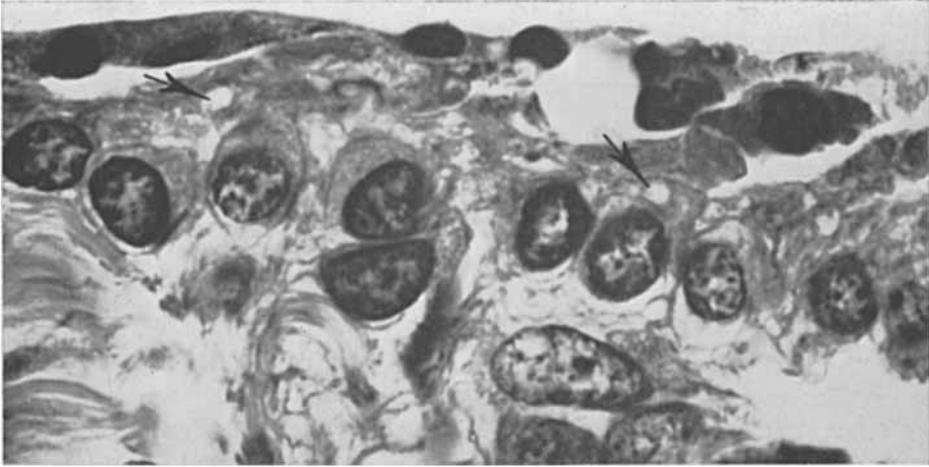
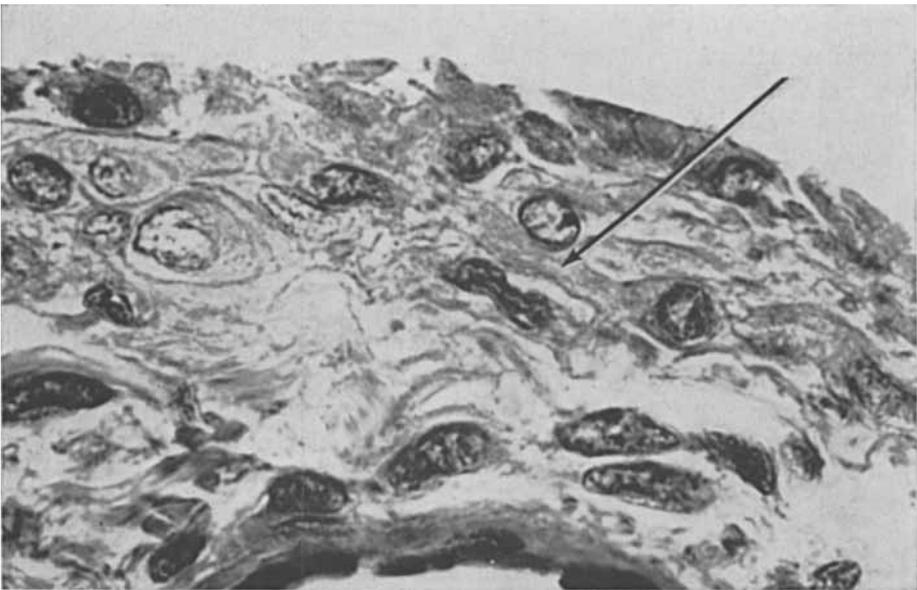


Fig. 1 (at top).—Areolar synovium from patellar synovial fold of a 65 year old male showing cytoplasmic vacuoles (arrows). Masson trichrome stain 1540 \times .

Fig. 2 (at bottom).—Areolar synovium from 65 year old male demonstrating long cytoplasmic processes. Masson trichrome stain 1080 \times .



chemically fixed sections with 1 N HCl for ten minutes at 60 C. did not decrease cytoplasmic uptake of Alcian Blue at pH 5.0. Treatment of chemically fixed sections with testicular hyaluronidase did not yield a consistent effect on Alcian Blue staining of the synovial cell cytoplasm. The periodic acid leucofuchsin reactions failed to color the cytoplasm significantly. Prolonged staining with Southgate's mucicarmine yielded variable results with faint to moderate staining of cell cytoplasm. Preferential staining of the superficial

synovial zone was usually observed, and vascular endothelium was irregularly deeply stained with this technic.

Mast cells.—Tissue mast cells composed nearly 3 per cent of the cellular population of the synovium and were located immediately beneath the intimal cell layer, and adjacent to capillaries and fat cells. The shape of the mast cell varied from spherical to elongated ellipsoid, without prominent cell processes. Over-all cell size, nuclear and nucleolar dimensions resembled those of the synovial intimal cell. Heavy cytoplasmic granulation frequently obscured nuclear detail. These granules measured approximately 0.3 to 0.5 μ in diameter and were usually arranged in a compact fashion within the limits of the cytoplasm. Occasional cells were seen in association with granules dispersed into the surrounding interfibrillar areas. Whether this occurred in vivo or during tissue preparation is uncertain. The PAS-positive "reticulin" mesh seen about the synovial lining cells was usually absent around the tissue mast cell.

Tinctorial properties of human synovial mast cell granules were the expected ones: (a) metachromasia with toluidine blue O and azure A, (b) strongly positive PAS reaction (c) deep staining with Alcian Blue at pH 2.7 and (d) deep red violet staining with the Gomori aldehyde fuchsin procedure. The metachromasia and PAS reactivity of the mast cell granules was unaffected by pretreatment of chemically fixed sections with testicular hyaluronidase, ribonuclease, 1 N HCl and 0.1 N NaOH. Mast cell granules in frozen-dried specimens also remained highly metachromatic after incubation with ribonuclease-free testicular hyaluronidase.

Unclassified connective tissue cells.—Beneath the synovial intimal cell layer, in addition to the mast cells with their striking morphologic characteristics, there was a population of less spectacular connective tissue cells. These cells are the "unclassified" connective tissue cells alluded to earlier and were undifferentiated from the standpoint of cytology. Perhaps 40 per cent of the cells closely associated with the joint cavity in a spatial sense fell into this category. These "unclassified connective tissue cells" were heterogeneous, being composed primarily of so-called "fibroblasts," histiocytes and, to a lesser extent, of vascular smooth muscle cells and primordial mesenchymal cells. Probably one-half to two-thirds of the "unclassified" subintimal cells belonged morphologically to the fibroblast group. These cells usually possessed elliptical nuclei and little stainable cytoplasm. Chromatin density and nucleolar size resembled that found in intimal cells, but cytoplasm was scanty if visible at all. The pericellular fibrillar encasement noted about intimal cells was poorly developed or lacking entirely. Tissue macrophages made up a significant portion of the remaining "unclassified" cells. They had smaller nuclei with coarse, deeply stained chromatin and frequently marked nuclear folding. The so-called primordial mesenchymal cells were similar in appearance and were distinguished primarily by their position outside the endothelial layer of capillaries. Due to the occasional marked development of small arterioles near the synovial surface, smooth muscle cells also formed a minor portion of the cell population.

Endothelial cells represented approximately 10 per cent of the synovial cell

population and lined the synovial capillaries, arterioles and venules. Both endothelial and smooth muscle cells were rendered prominent by the methods for demonstrating reticulin, which tended to outline these cells.

Ground Substance

Intercellular substance was sometimes demonstrated with metachromatic dyes applied to chemically fixed tissues. One tenth per cent toluidine blue O buffered to pH 6.5 to 7.0 with 0.05 M phosphate buffer resulted in capricious staining. Similar findings were noted with 2×10^{-4} M azure A. In approximately one-half of the specimens examined, no intercellular substance was stained with the metachromatic dye; most of the remaining specimens showed a variable degree of intercellular metachromasia, and less commonly orthochromatic staining of intercellular substance was noted. Neither Alcian Blue at pH 5.0 nor the McManus PAS procedure resulted in significant intercellular staining. The possibility of leeching out or diffusion of potentially stainable acidic materials during preparation of chemically fixed tissue renders the observations difficult to interpret. However, in frozen-dried synovial specimens, in which molecular diffusion was minimized, prominent intercellular metachromasia was demonstrable in the intimal zone in three of five specimens. Incubation of sections showing such intercellular metachromasia with ribonuclease-free testicular hyaluronidase for one hour at 37 C. resulted in absence of metachromatic staining in experimental sections.

Fibrillar Components

Collagen.—Collagen fibers provided the major structural support for the synovial intimal cells and their associated blood and lymphatic vessels. While the arrangement of the supporting connective tissue varied greatly, the more common architecture was exhibited by the areolar type of synovium (fig. 3). Three zones were distinguished in areolar synovium; a superficial zone composed of fibers measuring 0.3 to 1.2 μ in diameter, a loose middle stratum carrying the major vascular elements, and a dense deep meshwork of coarse fibers measuring 3.0 to 7.5 μ in diameter which blended into the joint capsule.

The over-all depth of the superficial zone varied from 30 to 120 μ . Thin collagen fibers at the surface of the superficial zone were usually oriented parallel to the intimal cell layer and lay along the long axes of the synovial cells. These fibers tended to intermesh at right angles, forming a "platform" for the cell layer.

In the midzone the fibrillar elements became sparser, coarser and tended to be oriented perpendicularly or at an angle to the lining cell layer. In many specimens the middle zone was either poorly developed or entirely lacking.

A heavy external fibrous layer anchored the synovium to the joint capsule. This stratum was relatively acellular, consisting of dense collagenous tissue interrupted at intervals by vascular elements. Adipose tissue in varying amounts was found in all three of the strata described.

Elastic fibers.—Elastic fibers as stained by the Gomori aldehyde fuchsin procedure were rare in the synovium. In five of nine cases surveyed, occasional

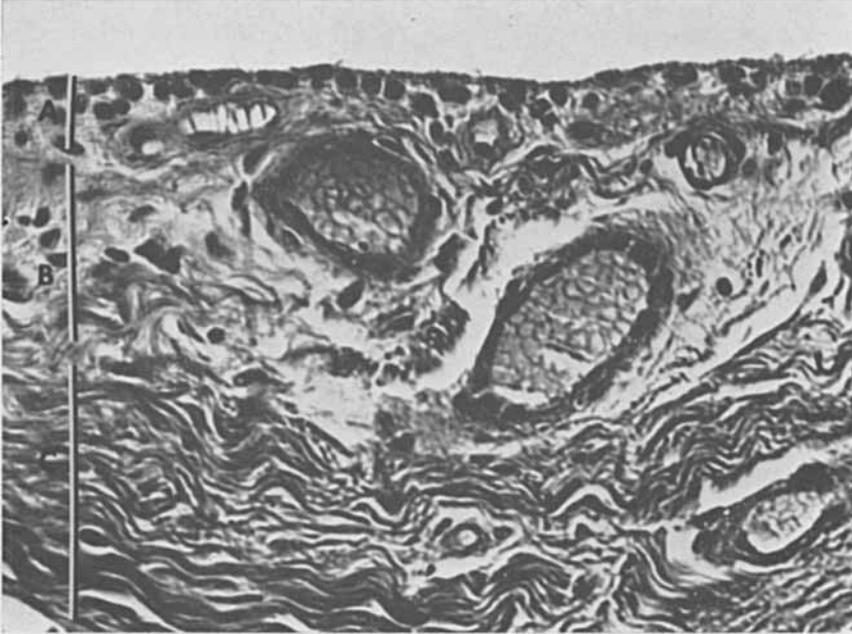


Fig. 3.—Areolar synovial tissue from the marginal area of the knee of a 58 year old male. Three zones are illustrated: (A) superficial “intimal” cell zone, (B) loose, vessel-laden “middle zone,” (C) external fibrous layer. Masson trichrome stain 148 \times .

aldehyde fuchsin-stained fibers were found, usually in the external fibrous zone with occasional examples in the midzone. The five persons with detectable synovial elastic fibers ranged in age from 54 to 67 years, while three of the four patents without (detectable) elastic fibers were 35 years of age or less.

Reticular fibers.—Fibers and structures exhibiting the morphologic and tinctorial properties of reticulin were numerous in the superficial cellular layer of the synovium. Reticular fibers were prominent in the inner 20 to 40 μ of the superficial zone, forming a complex network which appeared to enmesh the synovial lining cells. The fibers were 0.15 to 0.30 μ in diameter and tended to curl and branch. Frequently they were so closely applied to the synovial cell cytoplasm and its processes that the cell appeared to be encased by the reticular mesh. Capillary endothelial cells and fat cells were also enclosed in a reticular mesh, while the major portion of the subintimal connective tissue cells appeared to lack this fibrillar enclosure.

Synovial reticular fibers were well demonstrated with the Wilder silver impregnation technic (fig. 4). The fibers were markedly colored with the McManus procedure (fig. 5) and to a lesser extent with the Hotchkiss modification of the PAS procedure. In the course of the Masson trichrome procedure the reticular fibers were moderately stained with aniline blue. Alcian Blue stained synovial reticulin well under some conditions. One tenth per cent Alcian Blue in 3 per cent glacial acetic acid (pH 2.7), as recommended by Mowry, colored synovial reticulin poorly if at all. However, if the dye was

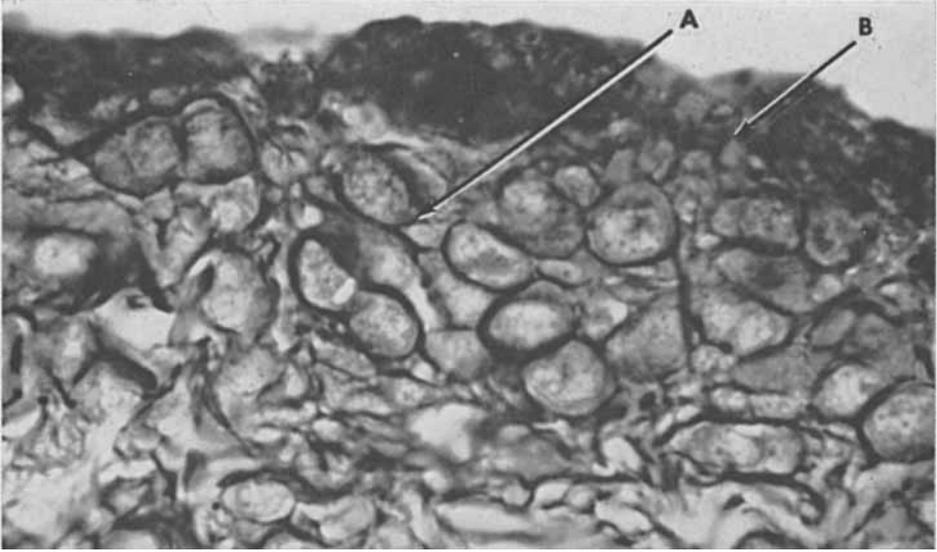


Fig. 4.—Areolar synovium from the suprapatellar bursa of a 65 year old male, demonstrating pericellular argyrophilic network. (A) branching argyrophilic fiber, (B) precipitated joint fluid. Wilder silver strain, hematoxylin and eosin 1080 \times .

buffered from pH 4.25 to pH 5.0 with 0.05 M phosphate buffer, marked staining occurred in both synovial and pericapillary reticulin. This fibrillar material was orthochromatically stained by toluidine blue O and azure A. Neither testicular hyaluronidase nor trypsin affected the tinctorial properties of synovial reticulin.

DISCUSSION

The normal variability of synovium from different areas in a joint must be considered when interpreting pathologic material obtained by blind needle biopsy. From a practical standpoint it is noteworthy that 90 per cent of the specimens of articular tissue examined in this study from multiple areas of the knee joint were of either the areolar or adipose types of a gradation between these types, as opposed to the less common fibrous synovium. It should be further noted that the "vascularity" and intimal cell layer "cellularity," importantly altered in synovial pathologic states, were essentially the same in these common synovial types. In addition, the cellular "differentials" were similar in these preponderant synovial varieties, in contrast to fibrous synovium with its deficiency of mast cells. The demonstrated uniformity of normal synovial micro-structure irrespective of age or biopsy site should allow one to study subtle deviations with greater confidence.

Attempts to demonstrate elastic fibers in synovium other than in the internal vascular tunics has met with varying success. Key¹ working primarily with dog and rabbit synovium, spoke of "a few fine elastic fibers scattered through the fibrous matrix," while Fawcett⁸ stated that the synovium is "rich in elastic fibers." In our human material, the Gomori aldehyde fuchsin procedure delineated a few distinct fibers in two-thirds of the specimens examined. Most of

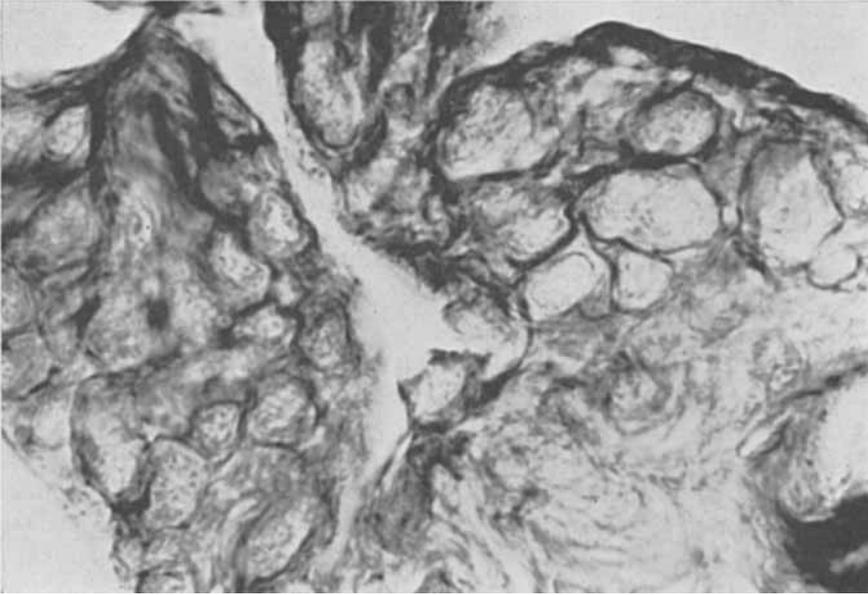


Fig. 5.—Areolar synovial tissue from the suprapatellar bursa of a 57 year old female, demonstrating pericellular fibrillar network. McMannus PAS followed by Alcian Blue (0.1 per cent in 3 per cent acetic acid) 1080 \times .

the fibrils seen were well below the synovial surface, and in no case did the density of stained elastic fibers approach that seen in human dermis.

Identification of reticulin in synovium has been based on the demonstration of argyrophilic fibers.⁹ Since there is doubt concerning the specificity of silver methods for demonstrating reticulin,¹⁰ further characterization of these fibers seemed important, particularly in view of the recent demonstration of an antigenic substance with the same general distribution.¹¹ In support of the reticular nature of this argyrophilic mesh are the following findings: (1) small fiber diameter, (2) fiber branching, (3) marked staining with the periodic acid-Schiff procedure, (4) affinity for acid aniline blue, (5) affinity for Alcian Blue and (6) resistance to tryptic digestion.

In spite of evidence from tissue culture and tissue slice experiments which indicates that total synthesis of hyaluronic acid may be effected by the cellular components of the synovial tissue,¹²⁻¹⁶ there has been no unequivocal demonstration of the cellular site of origin. Both the tissue mast cell¹⁷ and the synovial lining cell¹⁸⁻²⁰ have been suggested as the source of joint fluid hyaluronic acid. The only cell to show consistent and marked metachromasia is the mast cell. This cell, a relatively minor component of the synovial cell population, exhibited cytochemical characteristics consistent with the presence of sulfated acid mucopolysaccharides.²¹ The cytoplasm of intimal cells, quantitatively the largest protoplasmic element of the synovium, shows some metachromasia, indicating the presence of high molecular weight polyanionic material. However, within the limits of the methods, predigestion with crystalline ribonuclease or extraction with 1 N HCl at 60 C. removed most of the metachromatic

material. While this does not conclusively prove that ribonucleic acid is the agent responsible for the metachromasia, it would be difficult to interpret such evidence as favoring cytoplasmic hyaluronic acid as the responsible chromotrope. On the other hand, the preliminary hyaluronidase experiments on frozen-dried tissue would suggest that part of the intracellular metachromasia may be due to a hyaluronidase-labile mucopolysaccharide, such as hyaluronic acid or chondroitin sulfate A or C. The cytoplasmic Alcian Blue uptake at pH 5.0, unaffected by degradative procedures, suggests the presence of acid radicals other than ribonucleic acid. This does not distinguish acid mucopolysaccharides from acidic proteins, although the latter is more likely.

The numerical preponderance, location, cytologic characteristics and evidence from enzymatic digestion of frozen-dried tissue suggest that synovial intimal cells are involved in mucopolysaccharide synthesis. It is not possible, however, to exclude important participation by the other cell types found in the synovium. The significance of extracellular metachromasia in the superficial synovial zone is not clear. Interpretations of this observation include extracellular storage of mucopolysaccharide, and the possibility that mucopolysaccharide synthesis is carried out in part in an extracellular location under the influence of one or more cell types.

SUMMARY

Specimens of normal synovial tissue from the knee joints of 16 humans of varying ages were examined by several histologic and histochemical procedures. Data regarding the distribution and tinctorial properties of the cellular, fibrillar and intercellular components are presented.

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