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Cellular Junctions in Normal and Inflammatory Human Synovial Membrane Revealed by Tannic Acid and Freeze Fracture

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Summary. Cellular junctions between synovial cells and endothelial cells of the microvasculature were examined in 10 normal and 20 inflammatory human synovial membranes by means of tannic acid and freeze fracture, Gap junctions and desmosomes predominated on synovial cells, and tight junctions in the microvasculature.

Comparison between normal and inflammatory synovial membranes did not demonstrate changes in cellular contacts that might be caused by inflammation.

Key words: Human synovial membrane – Cellular junctions – Tannic acid – Freeze fracture.

Introduction

Intercellular spaces are a very important pathway for exchanges between the synovial membrane and articular cavity (Norton and Ziff, 1966) and between the intra and extravascular spaces of the synovia (Dryll et al., 1977b). However, previous ultrastructural studies have paid very little attention to intercellular junctions in synovial membranes. In the rat Roy and Ghadially (1967) described desmosomes connecting synovial lining cells; in cat and rabbit synovial lining Groth (1975) observed desmosomes, hemidesmosomes and gap junctions. In human synovial membrane, Grimley and Sokoloff (1967) were the only observers to notice desmosomes in the synovial lining affected by rheumatoid disease.

In this paper we studied the pattern of cellular junctions in human synovial membrane in order to define what changes in cellular contacts might be caused by inflammation.

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Materials and Methods

A trochar synovial biopsy of the knee was performed in 20 patients with inflammatory arthritis. Clinical diagnosis and the histopathological type of inflammatory synovitis were assessed according to the criteria of a previous study (Dryll et al., 1977a) summarized in Table 1.

Ten normal synovial membranes were sampled during arthroscopy for mechanical disorders of the knee.

Processing for Ultrathin Sectioning

Biopsy specimens sliced into fragments of 1 mm^2 were immediately fixed in 2% glutaraldehyde solution in 0.1 M cacodylate buffer pH 7.3 at room temperature for 90 min. After rinsing in the same buffer, the specimens were post-fixed in 2% O_5O_4 in cacodylate buffer pH 7.3 at 4° C for 90 min and then treated in block according to Simionescu (1975) for 30 min at room temperature with 1% digallic acid ($C_{14}H_{10}O_9$, tannic acid, 1764, Mallinckrodt, chemical works, St Louis, Missouri, U.S.A.) in 0.05 M cacodylate buffer pH 7.0 at room temperature for 30 min.

The blocks were subsequently dehydrated in alcohol and embedded in Spurr resin. Ultrathin sections were cut with a Reichert Om U_3 ultramicrotome, stained with lead citrate and examined with Philips 300 electron microscope at 40 KV. For a semiquantitative evaluation of inter-endothelial junctions in synovial vessels, we took a mean of 75 vessels obtained by examination of an average of 10 blocks in each case. The number of vessels per block was similar in normal and in inflammatory synovial membranes. So as to avoid recounting serial sections through the same vessel, special care was taken to examine only one section from each block. Classification of endothelial junctions was made by two successive observers from photographs having a final magnification of 135,000. Results obtained by the two observers were similar in all cases. Synovial microvasculature was classified as arterioles, venules and true capillaries according to the vessel type classification used by Simionescu et al. (1975).

Processing for Freeze Fracturing

Biopsy specimens sliced into fragments of 1 mm^2 were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3 at room temperature for 90 min. After rinsing in the same buffer the specimens were mounted on specimen carriers, frozen in solid nitrogen and stored in liquid nitrogen until fracturing. Tissues were fractured in a Cryofract, Reichert-Jung freeze etch unit, at $-150°$ C under a vacuum of $1 \cdot 10^8$ Torr.

Fractured surfaces were shadowed with platinum from an electron beam gun followed by a supporting carbon film. Replicas were cleaned in sodium hypochlorite for 24 h, rinsed in distilled water and mounted on 400 mesh grids, they were then examined in a Philips 300 electron microscope at 80 KV. Measurements of cellular junctions were made on photographs having a final magnification on 135,000.

Table 1. Histopathological type of inflammatory synovitis according to clinical diagnosis

Clinical diagnosis	Histopathological type		
	Rheumatoid	Subacute	Sclerous
Classic rheumatoid arthritis	5	2	1
Monoarthritis	0	8	1
Spondylarthritis	0	0	1
Miscellaneous	0	2	0

Results

Ultrathin Sections

Lining and Deeper Layers of Synovial Membrane. Gap junctions were present in all the inflammatory and normal synovial membranes. However these junctions were found essentially on the cytoplasmic digitations of the lining cells of the inflammatory synovial membranes, most often of type A (Fig. 1), but occasionally of B or intermediate type. The gap between the outer leaflets of adjacent membranes, filled with the homogenous electron dense material following tannic acid, measured about 5 nm in width. *Desmosomes and hemidesosomes* were found in 5 inflammatory synovial membranes and in 4 normal controls. These junctions are found in the superficial layer of the synovial membrane. Desmosomes are generally grouped in a series of junctions spaced along the same intercellular spaces (Fig. 2). A cells are the ones most often joined by these junctions, but B and intermediate cells are occasionally concerned. The desmosomes consist of two portions of the adjacent plasma membranes which lie parallel to one another, separated by a 40 to 60 nm interspace, and are connected to the subjacent cytoplasm by an attachment plaque. The intercellular space is occupied by a moderately electron opaque disc about 20 nm in width. Hemidesmosomes are to be found in the 3 types of synovial lining cells but with a predilection for A cells. They are most often found in an intercellular space occupied by a series of desmosomes but are occasionally located on the plasma membrane of synovial cells, without cellular contact. Hemidesmosomes consist of a well differentiated reinforcement of the inner leaflet of the plasma membrane connected to intracellular filaments. One light and one dense layer corresponding to the interspace elements of desmosomes are found just outside the attachment plaque.

Tight junctions (Fig. 3) are very rarely found on synovial cells: these junctions were found in 5 cases of inflammatory synovitis and in 1 control, but only one tight junction was found in each case. All types of synovial cells of the intermediate layer were involved.

Microvasculature. Gap junctions (Fig. 4) were not found frequently in the synovial microvasculature. The mean number of gap junctions per vessel was higher in controls (0.023) than in inflammatory synovitis (0.012). Gap junctions were found in arterioles in 80% of cases and in venules in 20% of cases. Gap junctions were not found in true capillaries. In about 30% of cases, tight junctions were found in the same inter-endothelial space as gap junctions.

Desmosomes were very rare on vascular walls. We only observed one desmosome between two endothelial cells of a true capillary in an inflammatory synovitis and one desmosome between 2 pericytes of a venule in a normal control.

Tight junctions (Fig. 5) were the most frequent junctions between endothelial cells. The mean number of tight junctions per vessel was similar in inflammatory synovitis (0.35) and in controls (0.36). Tight junctions were found on arterioles, venules and true capillaries. The number of punctate cytoplasmic contacts ap-

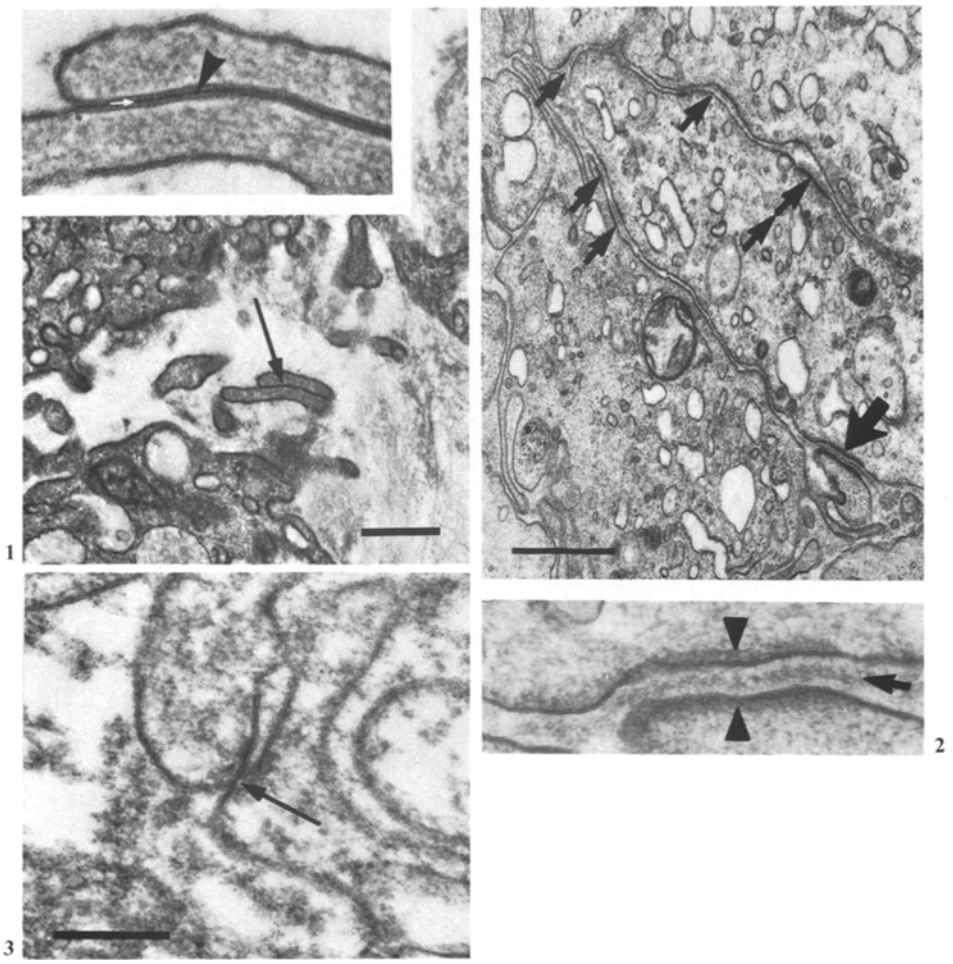


Fig. 1. Gap junction (*arrow*) on cytoplasmic digitations of lining cells of A type in inflammatory synovitis. Magnification: ×20,400. Bar: 0.5 μm. *Inset:* tannic acid (white arrow) fills the gap (*arrow head*) between outer leaflets of adjacent membranes. Magnification: × 105,000

Fig. 2. Desmosomes (*arrows*) and hemidesmosome (*double arrow*) between A type cells of superficial layer of normal synovial membrane. Magnification: ×13,500. Bar: 1 μm. *Inset:* Higher magnification of desmosome marked by large arrow: attachment plaques (*arrow heads*); intercellular opaque disc (*arrow*). Magnification: × 63,000

Fig. 3. Tight junction (*arrow*) on cytoplasmic digitations of A type cells in intermediate layer of inflammatory synovitis. Magnification: ×60,000. Bar: 0.25 μm

pearing in an inter-endothelial space varies from one to six. The incidence of tight junctions with few (1 or 2) or numerous (3 or more) cytoplasmic punctate contacts was similar in inflammatory synovitis and in controls.

Freeze Fracture Replicas

Gap junctions (Fig. 6) can be identified on the P face by the aggregate of closely packed particles 8 nm in diameter and on the E face by geometrical arrays

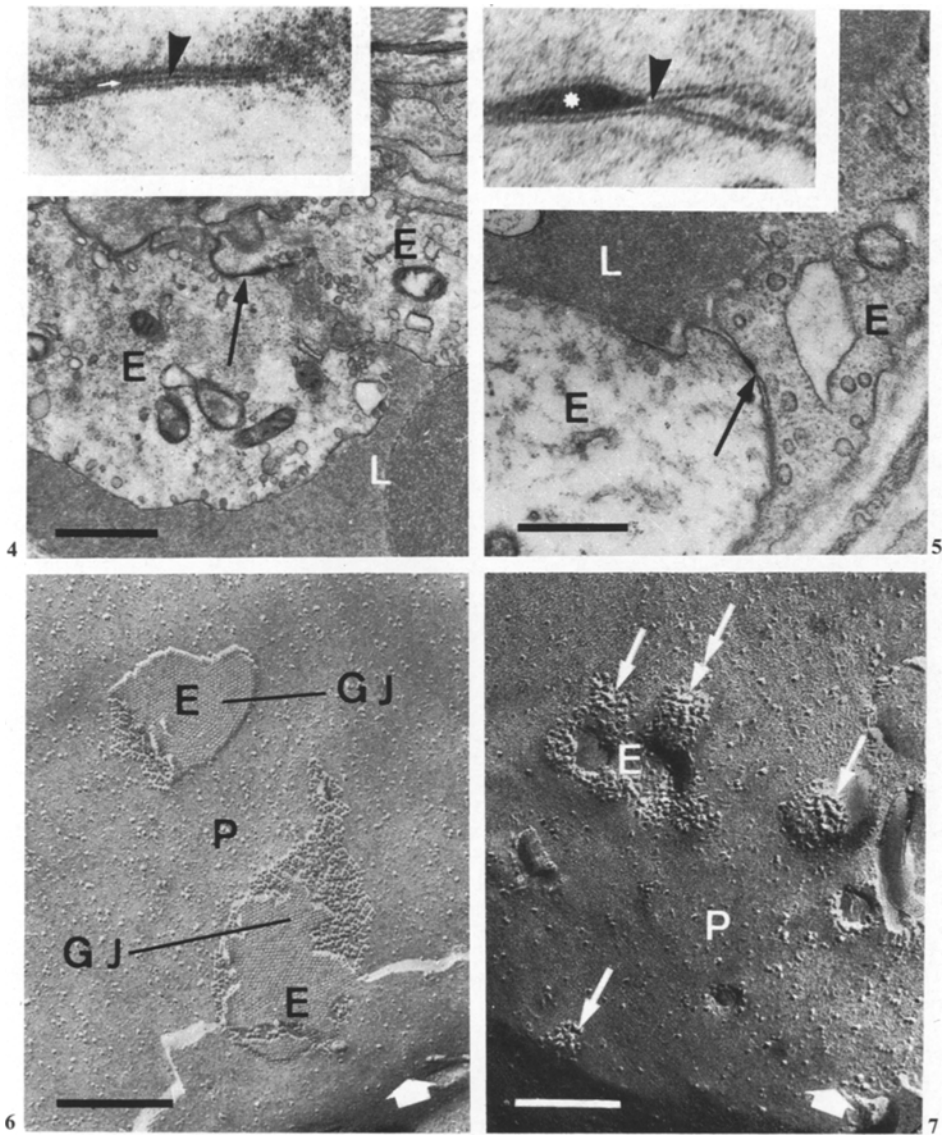
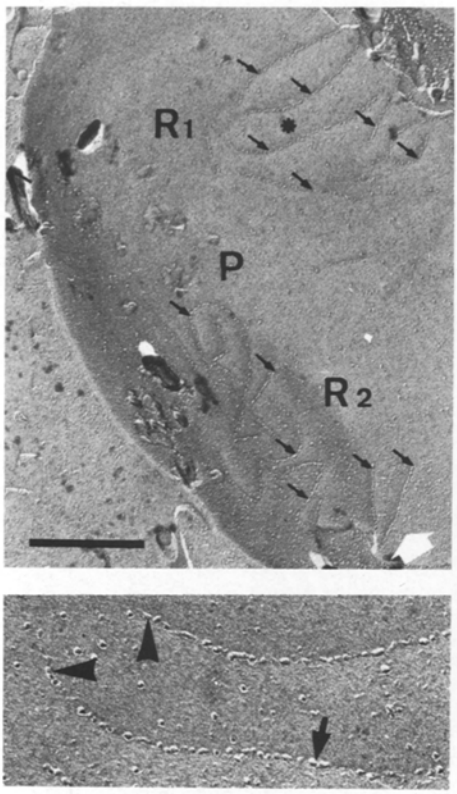


Fig. 4. Gap junction (*arrow*) on an arteriole of a normal synovial membrane. *E*, endothelial cell; *L*, lumen. Magnification: ×13,500. Bar: 1 µm. *Inset:* Tannic acid (*white arrow*) fills the gap (*arrow head*) between outer leaflets of adjacent membranes. Magnification: ×126,000

Fig. 5. Tight junction (*arrow*) on a venule of inflammatory synovial membrane. *E*, endothelial cell; *L*, lumen. Magnification: ×30,000. Bar: 0.5 µm. *Inset:* tannic acid in the luminal segment of interendothelial space (*asterisk*) does not pass beyond the punctate fusion of external leaflets of adjacent membranes (*arrow head*). Magnification: ×210,000

Fig. 6. Freeze fracture replica of 2 gap junctions (*GJ*) in inflammatory synovitis: with closely packed particles in P face (*P*) and geometrical array of complementary pits in E face (*E*). Magnification: ×63,000. Bar: 0.25 µm. In all freeze fracture replicas, large white arrow in the right lower corner of the view indicates direction of shadowing

Fig. 7. Freeze fracture replica of desmosomes (*arrows*) in normal synovial membrane, formed by grouping together of round, irregular particles similar in P face (*P*) and in E face (*E*). Double arrow: desmosome with both P and E face particles. Magnification: ×140,000. Bar: 0.1 µm



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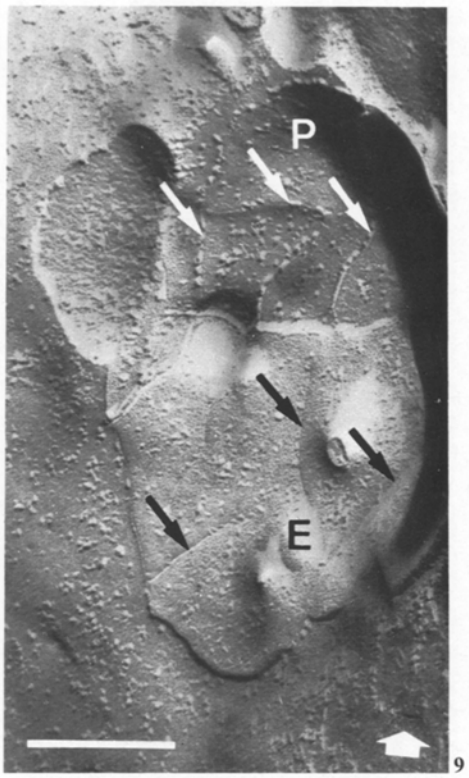

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Fig. 8. Freeze fracture replica of 2 vascular tight junctions in a normal synovial membrane. In the upper junction (*R1*) the ridges (*arrows*) show an approximately parallel course. In the lower junction (*R2*) the ridges (*arrows*) form branching and staggered lines. *P*: P face. Magnification: ×30,000. Bar: 0.5 μm. *Inset:* higher magnification of parallel ridges in R1 junction (*asterisk*). The ridges on the P face are marked by quasicontinuous protruding particles (*arrow*). The parallel ridges have free ends (*arrow heads*). Magnification: ×99,000

Fig. 9. Freeze fracture replica of a vascular tight junction in a normal synovial membrane. In the P face (*P*) ridges (*white arrows*) are marked by quasicontinuous protruding particles. In the E face (*E*) grooves (*black arrows*) are free of particles. Magnification: ×99,00. Bar: 0.2 μm

of complementary pits 4 nm in diameter. The diameter of gap junctions was similar in inflammatory synovitis (mean diameter for 84 gap junctions 290 nm, range 70 to 440 nm) and in controls (mean diameter for 31 gap junctions 235 nm, range 60 to 440 nm). Gap junctions are often disposed in groups of two or three junctions. Connection with tight junctions was not found except in two cases.

Desmosomes (Fig. 7) can be identified by the grouping together of round or elongated particles 8 to 10 nm in diameter with similar pattern in P and E faces. Clusters of such particles are often dispersed on the P face of the cellular membrane.

Tight Junctions. Vascular tight junctions consisted the most frequently of tight junctions found in inflammatory synovitis (20 junctions), and in controls (8 junc-

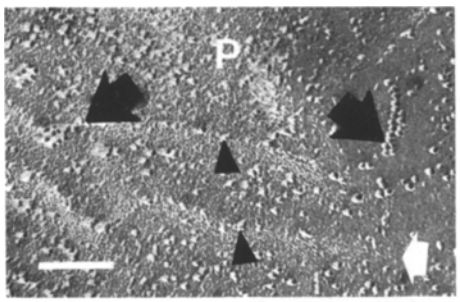

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Fig. 10. Freeze fracture replica of a vascular tight junction in an inflammatory synovial membrane. Small gap junctions (*large arrows*) are associated with low profile ridges of the tight junction marked by sparse protruding particles (*arrow heads*) *P*, P face. Magnification: ×99,000. Bar: 0.1 μm

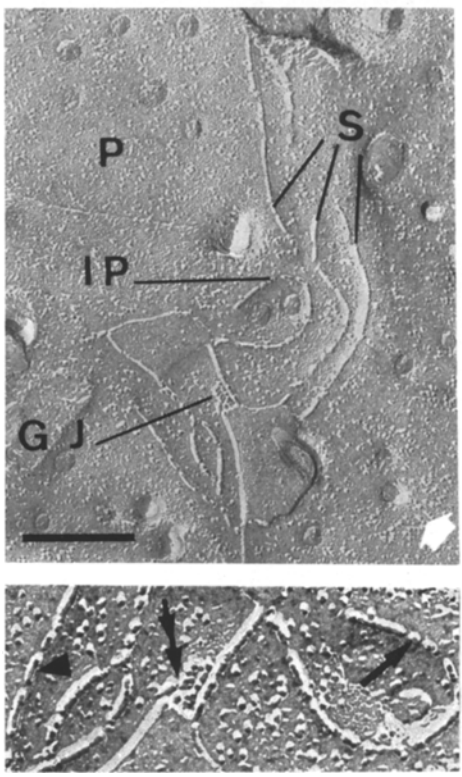

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Fig. 11. Freeze fracture replica of a tight junction of synovial cells in inflammatory synovitis. Discontinuous strands (*S*) are formed by bars 25 to 65 nm in length, occasionally separated by round intercalated particles (*IP*) 8 nm in diameter. Small gap junction (*GJ*) is associated with a strand. *P*, P face. Magnification: ×60,000. Bar: 0.25 μm. *Inset:* higher magnification of the central portion of the junction. *Arrow head:* straight bar on a strand. Arrow: intermediate round particle. *Double arrow:* small gap junction associated with a strand. Magnification: ×126,000

tions). These junctions (Fig. 8) appear as a system of 2 to 7 ridges marked on the P face by an alignment of protruding particles 8 to 10 nm in diameter. The frequency of these particles varies from quasi-continuous to absent. The grooves on the E face are free of particles (Fig. 9). The ridges or grooves may show an approximately parallel course with free ends, or else form a maze of branching and staggered lines. Association of a vascular tight junction with a gap junction (Fig. 10) was only found in one inflammatory synovial membrane. Small gap junctions are associated with, but not framed by, low profile ridges with few protruding particles of tight junction. The average number and spacing of junctional ridges were similar in inflammatory synovitis (mean number 3.5, range 2 to 7, mean spacing 116 nm, range 50 to 320 nm) and in controls (mean number 3.6, range 2 to 6, mean spacing 104 nm, range 70 to 250 nm).

Tight junctions of synovial cells: In two cases of inflammatory synovitis and in one control, tight junctions were found which appeared to be formed by association of bars 20 to 90 nm in length, straight in appearance, occasionally separated by round particles 8 nm in diameter (Fig. 11). Association with gap junction was found in one case.

Discussion

Ultrathin Sections

Three types of cell junctions have been found in human synovial membrane: gap junctions, tight junctions, desmosomes and hemidesmosomes. The frequency of these types of cell junctions depend on their location in the synovial tissue. On synovial cells, gap junctions are the type of junction most often found, and are preferentially located on cell process of A or intermediate cells. Desmosomes and hemidesmosomes are a less frequent type found on the three types of synovial cells. Tight junctions are very rarely found. In the microvasculature, gap junctions were only found on arterioles and venules, whereas tight junctions, the most frequent inter-endothelial junction, are found in arterioles, venules and true capillaries. Desmosomes were only found on two vascular walls.

Previous ultrastructural studies have paid very little attention to synovial intercellular junctions. In rat synovial membrane Roy and Ghadially (1967) have described indisputable desmosomes in the synovial cells. In cat and rabbit synovial membrane Groth (1975), using en bloc uranyl acetate staining, give a more extensive description of cellular junctions in synovial cells: gap junctions, desmosomes and hemidesmosomes are preferentially located on superficial layers of the synovia.

In rheumatoid human synovial membrane Grimley and Sokoloff (1967) described connection of synovial lining cells by desmosomes, a junction so far not found in human synovia. In the synovial microvasculature, interendothelial junctions have not been described to date. However, our results are in accordance with Simionescu's study (1975) on the rat microvasculature. Our present study demonstrates that in human synovial membrane, all types of junctions previously described in other mammalian or human tissues are regularly found on synovial cells and vessels.

Comparison between normal and inflammatory human synovial membrane shows that inflammation does not induce important modifications of intercellular junctions. In synovial cells gap junctions, located on cytoplasmic processes of A or intermediate type cells are more frequently found in rheumatoid synovitis than in controls. Hyperplasia of the lining cells with an increase of the number of cytoplasmic digitations in inflammatory synovitis might explain the higher frequency of communicating junctions in such synovial membranes. Desmosomes and hemidesmosomes have been found more often in normal controls than in inflammatory synovitis. This observation does not corroborate the hypothesis of Grimley and Sokoloff (1967) that desmosomes might represent a pathologic modification induced by rheumatoid inflammation, contributing to

an increase in the mechanical resistance of inflammatory synovitis. In the microvasculature, gap junctions have been found more often in normal controls than in inflammatory synovitis. Gap junctions are not present on true capillaries and an increase in the relative number of such vessels in inflammatory synovitis could explain this difference of frequency of the vascular gap junction in normal control and in inflammatory synovitis. Tight junctions are the junctions the most often found in synovial microvasculature. The frequency and pattern of vascular tight junctions were similar in controls and in inflammatory synovitis.

Thus, at the level of interendothelial junctions, no ultrastructural modifications of the synovial microvasculature were found that might be caused by inflammation. These results are in accordance with our previous study on synovial microvasculature (Dryll et al., 1977a).

Freeze Fracture Replicas

In freeze fracture replicas we found gap junctions, desmosomes, and tight junctions on synovial cells and vascular walls.

No previous freeze fracture study of the synovial membrane has been reported. In normal synovial membranes gap junctions and desmosomes were similar to those observed in other animal freeze fracture studies (Staehelin, 1974). Tight junctions on synovial cells were similar to those observed by Simionescu (1977) in rat mesothelium. Vascular tight junctions were similar to those described by Simionescu et al. (1975) in rat microvasculature.

In inflammatory synovial membranes the pattern of the junctions in freeze fracture replicas was similar to that observed in normal synovial membrane. In a semiquantitative study of the junctions, the diameter of the gap junctions the number and the spacing of the ridges of tight junctions were similar in inflammatory synovitis and in normal controls.

In conclusion our experience suggests that synovial inflammation does not induce any significant changes in cellular contacts in synovial membranes. At the level of synovial microvasculature, inter-endothelial junctions do not seem to contribute to the increased vascular permeability observed in inflammatory synovitis.

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