Light and electron microscopy study of capillaries in normal and inflammatory human synovial membrane

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SUMMARY Synovium aspirated from the knee joint by trochar was studied by light and electron microscopy in 40 cases of inflammatory arthritis and in 10 controls. The morphology of synovial capillaries, extravascular plasma diffusion, interendothelial vascular gaps, extracapillary blood cell migration, vascular congestion, endothelial hyperplasia, and obliteration of the capillary lumen by endothelial cells were compared in normal and inflammatory synovia. Inflammatory synovitis was characterised by the number and diversity of blood cells migrating through the interendothelial pathway out of the capillary lumen. Polymorphonuclear leucocytes were the blood cells most often seen at interendothelial junctions. No other capillary changes that might be related to synovial inflammation were found.

Despite their important role in inflammatory processes the capillaries of human synovial membrane have so far received little attention. In a light microscopy study of rheumatoid synovitis Kulka (1966) described a segmental angiopathy affecting the venules and capillaries. In vital microscopy of rheumatoid synovial membrane Brånenmark et al. (1963) found a venular dilatation with arteriovenular shunts. In an electron microscopy study of rheumatoid synovitis Bierther and Wegner (1971) found a progressive transformation of endothelial cells to fibroblasts. On the other hand, Brånenmark et al. (1969) did not find ultrastructural alterations of the capillaries in rheumatoid synovial membrane.

In our present study we therefore aimed to define by light and electron microscopy what changes in synovial capillaries might be caused by inflammation.

Material and methods

A trochar synovial biopsy of the knee was performed in 50 patients, 40 of whom had inflammatory arthritis and 10 (who were used as controls) had mechanical disorders of the knee. The 40 cases of arthritis included 24 of rheumatoid arthritis according to the criteria of the American Rheumatism Association (Ropes et al., 1958)—12 cases being classical and 12 being definite rheumatoid arthritis—13 cases of monoarthritis without rheumatoid factor and with a clinical course of at least 3 months; and one case each of gout, systemic lupus erythematosus, and filarial arthritis.

Histopathological examination of the synovial membrane by light microscopy in the 40 cases of inflammatory arthritis showed three types of inflammation (Table): (1) rheumatoid type with at least three of the characteristics of rheumatoid synovitis according to the ARA criteria (Ropes et al., 1958); (2) subacute type characterised by moderate villous hypertrophy, proliferation of cells in the marginal layer without palisading, and moderate diffuse cell infiltration particularly with lymphocytes; and (3)

Table Histopathological type of inflammatory synovitis according to clinical diagnosis

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<tr>
<th>Clinical diagnosis</th>
<th>Histopathological type</th>
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<tr>
<td></td>
<td>Rheumatoid*</td>
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<tr>
<td>Classic rheumatoid arthritis*</td>
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<tr>
<td>Definite rheumatoid arthritis*</td>
<td>6</td>
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<td>Monoarthritis of knee</td>
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<td>Miscellaneous</td>
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*According to the ARA criteria (Ropes et al., 1958).
Capillaries in synovial membrane

sclerous type characterised by discrete or no villous hypertrophy, marginal layer reduced to one or two layers of cells, discrete diffuse cell infiltration of monohistiocytic type, and striking development of collagen fibres in the deep layer.

The histological appearance of the synovial membrane was normal in the 10 cases of mechanical disorder of the knee used as controls.

Biopsy specimens sliced into fragments of 1 mm² were immediately fixed in a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer pH 7.3 at 4°C for two hours. The fragments were rinsed for one night in 0.1 M phosphate buffer pH 7.3 at 4°C. After post-fixation in a 2% osmium tetroxide solution in phosphate buffer for one hour the fragments were dehydrated in ethanol and embedded in a mixture of Epon and Araldite. Sections 1 µm thick were stained with toluidine blue and examined with the light microscope. Ultrathin sections were cut with an ultramicrotome (Reichert OmU 2), stained with a 5% aqueous uranyl solution, and then with lead citrate. The sections were examined with a Philips EM 300 electron microscope at 40 kV. A minimum of six blocks containing capillary-rich areas were examined for each of the 50 biopsies. In all, we examined 7500 capillaries with a minimum of 100, a maximum of 300, and an average of 150 for each biopsy.

Results

LIGHT MICROSCOPY

In light microscopy attention was paid to capillary congestion, endothelial hyperplasia, and obliteration of the capillary lumen by endothelial cells. The incidence of capillary congestion was identical in proportion in inflammatory synovitis (21/40) and in controls (5/10). Endothelial hyperplasia was estimated by the count of endothelial nuclei in synovial capillaries. The mean number of endothelial nuclei per capillary was similar in controls (2.1) in rheumatoid arthritis (2), and in other inflammatory synovitis (1.8). The incidence of obliteration of the capillary lumen by endothelial cells was proportionately similar in inflammatory synovitis (34/40 with an average of 2.5 obliterations for 100 capillaries) and in controls (7/10 with an average of 2.3 obliterations for 100 capillaries).

ELECTRON MICROSCOPY

Morphology of synovial capillaries In both the inflammatory synovitis and control cases the following types of capillaries, according to Simon's (1965) classification, were found with identical frequency: (1) Continuous capillaries with flat (Fig. 1) or high (Fig. 2) endothelium. Neither in inflammatory

![Fig. 1 Continuous capillary with flat endothelium in normal synovial membrane. L = lumen, E = endothelial cell, B = basement membrane. Original magnification × 3300](image-url)
Fig. 2  Continuous capillary with high endothelium in rheumatoid synovitis. Cytoplasmic organelles and microfibrils and pinocytotic vesicles have the same appearance as in flat endothelium. L = lumen, E = endothelial cell, B = basement membrane, P = pericyte. Original magnification × 3300

Fig. 3  Continuous capillary with flat endothelium in normal synovial membrane, multilayered pattern of basement membrane. L = lumen, E = endothelial cell, B = basement membrane, P = pericyte. Original magnification × 10 000

Fig. 4  Fenestrated capillary with pores closed by a membrane in normal synovial membrane. RBC = red blood cell, L = lumen, B = basement membrane, arrow = pore closed by a membrane. Original magnification × 10 000
synovitis nor in controls did high endothelia appear to have greater cell activity than flat endothelia—indeed, cytoplasmic organelles and microfibrils and pinocytotic vesicles had a similar appearance in both. In these continuous capillaries the basement membrane sometimes showed a multilayered pattern (Fig. 3), which was found as often in inflammatory synovitis as in controls. (2) Fenestrated capillaries of various types—capillaries with pores closed by a membrane (Fig. 4), capillaries with pores without membrane (Fig. 5), and transitional forms between continuous and fenestrated capillaries. All types were seen equally often in inflammatory synovitis and in controls.

Extravascular plasma diffusion, interendothelial gaps
A pattern suggesting a plasma impregnation of basement membrane was seen in all types of synovial capillaries (Fig. 6). In some capillaries with continuous endothelium plasma appeared to penetrate into the basement membrane through large interendothelial gaps (Fig. 7). Extravascular plasma diffusion and interendothelial gaps were seen with an identical frequency in all 50 biopsies.

Extracapillary blood cell migration
In the controls extracapillary blood cell migration was rare and was restricted to red blood cells. In inflammatory synovitis extracapillary blood cell migration was much more frequent, mostly of polymorphonuclear leucocytes and only occasionally of red blood cells, platelets, lymphocytes, and blast cells. All these blood cells migrate out of the capillary lumen following an interendothelial pathway (Fig. 8).

Vascular congestion and obliteration of capillary lumen by endothelial cells
No ultrastructural change in the capillary wall was seen when the capillary lumen was obstructed by packed red blood cells. In cellular obliteration of the capillary lumen the endothelial cells appeared to coalesce by a junction of their intraluminal plasmatic membrane. Important ultrastructural changes in the endothelial cells were often seen in both inflammatory synovitis and controls, with expansion (Fig. 9) or retraction (Fig. 10) of the cytoplasm.

Discussion

Light microscopy
In his extensive study of vascular derangement in rheumatoid arthritis Kulka (1966) stated that endothelial hypertrophy and hyperplasia with varying degrees of encroachment on the vascular lumen were typical of the proliferative lesions as exemplified by the synovitis. In a semiquantitative approach to these capillary modifications no characteristic changes related to synovial inflammation were found in our light microscopy study.

Electron microscopy
Morphology of synovial capillaries
The repartition of the different types of capillaries (continuous, fenestrated) was identical in all 50 biopsies. Thus in

![Fig. 5] Fenestrated capillary with pores without membrane in normal synovial membrane. RBC = red blood cell, B = basement membrane, P = pericyte, arrow = pore without membrane. Original magnification × 10 000

![Fig. 6] Continuous capillary with flat endothelium in normal synovial membrane, pattern suggesting plasma impregnation of basement membrane (arrow). L = lumen, E = endothelial cell, B = basement membrane, P = pericyte. Original magnification × 10 000
Fig. 7  Continuous capillary with flat endothelium in normal synovial membrane, interendothelial gap (arrow).  
L = lumen, E = endothelial cell, B = basement membrane. Original magnification × 10000

Fig. 8  Extracapillary polymorphonuclear leucocyte migration through interendothelial pathway in rheumatoid synovitis.  
L = lumen, PL = polymorphonuclear leucocyte, E = endothelial cell, B = basement membrane. Original magnification × 10000
inflammatory synovitis we did not find more capillaries with high endothelium and multilayered basement membrane, as described by Norton and Ziff (1966) in rheumatoid synovitis and by Schumacher and Kitridou (1972) in synovitis of recent onset. Norton et al. (1966) noted fenestrated capillaries more often in Reiter’s synovial membrane than in rheumatoid arthritis. We found only a preferential site in the superficial layer of all synovial membranes, whether normal or inflammatory. As with light microscopy, we did not find any specific ultrastructural changes in the capillary wall in inflammatory synovitis. The number of intraendothelial microfibrils, increased in rheumatoid arthritis according to Ghadially and Roy (1967), appeared to us identical in all synovial membranes. In the case of systemic lupus erythematosus we did not find any destruction of the capillary endothelium, as reported by Schumacher and Kitridou (1972). However, in our patient numerous intraergastoplasmic tubular formations, originally described by Győrkey et al. (1969) and first reported in synovial vascular endothelium by Schumacher (1970), were found in the synovial capillary endothelium.

**Extravascular plasma diffusion, interendothelial gaps**

A pattern suggesting extravascular plasma diffusion has not so far been mentioned. However, Brånemark et al. (1969) showed pictures of normal and rheumatoid synovial membranes which suggested to us the existence of extravascular plasma diffusion. Interendothelial gaps were not found by Norton and Ziff (1966) or by Brånemark et al. (1969) in normal and rheumatoid synovial membranes. However, in Reiter’s synovitis an opening in the endothelium was mentioned by Norton et al. (1966). Schumacher (1969) found gaps in capillaries of monkey synovial membrane more often after prolonged joint motion than at rest. In synovitis of recent onset these interendothelial gaps were often found by Schumacher and Kitridou (1972). The significance of and the mechanism inducing the appearance of these interendothelial gaps have not yet been completely elucidated. Majno and Palade (1961) described their
appearance in the venous capillaries of rats after local injection of histamine and serotonin. Since then these vascular gaps have been reproduced by numerous experimental methods. Cotran (1965) ascribed their origin to the possible role of a chemical mediator or to increased intracapillary hydrostatic pressure, or both. Although we cannot prejudge the mechanism responsible in our study the traumatism of the trochar biopsy may possibly be responsible for these interendothelial gaps by both releasing a chemical mediator and increasing intracapillary hydrostatic pressure.

**Extracapillary blood cell migration** The major difference between normal and inflammatory synovial capillaries consists in the number and diversity of the blood cells migrating out of the capillary lumen in rheumatoid and subacute synovitis. The pre-eminence of polymorphonuclear leucocyte migration is in accordance with the role of these cells in rheumatoid synovial inflammation. All blood cells migrate out of the capillary lumen by the interendothelial pathway. We did not observe, as did Kobayashi and Ziff (1973), lymphocytes passing through the endothelial cytoplasm of venular capillaries. On the other hand, Schoefl (1972) has shown in serial sections that this pattern of trans-endothelial migration corresponds in fact to the interendothelial passages.

**Vascular congestion and obliteration of capillary lumen by endothelial cells** Vascular congestion with packing of red blood cells in the synovial capillary lumen was not associated with ultrastructural changes in the capillary wall. We did not find the intracapillary aggregates of platelets, polymorphonuclear neutrophils, and red blood cells which Norton et al. (1966) described in Reiter's synovial membrane and Schumacher and Kitridou (1972) in synovitis of recent onset. Obliteration of the capillary lumen by endothelial cells was reported by Norton and Ziff (1966) in rheumatoid synovitis and by Schumacher and Kitridou (1972) in synovitis of recent onset. However, these authors did not describe ultrastructural changes in the endothelial cells. In our study this pattern, as common in controls as in inflammatory synovitis, might have been due to mechanical damage by the trochar to the loose tissue of the synovial membrane. We did not find, as did Bierther and Wegner (1971), a progressive transformation of the endothelial cells to fibroblasts in rheumatoid synovitis.

Our study shows, as reported by Bränenmark et al. (1969), that synovial inflammation does not change the structure of the capillaries, but the transcapillary migration of circulating blood cells is greatly increased in rheumatoid and subacute synovitis.

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**References**


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