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TO THE QUESTION OF ANGIOPATHY IN RHEUMATOID ARTHRITIS

An Electron Microscopic Study

By

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INTRODUCTION

Over the recent years an increasing interest has been focussed on the vascular changes in synovial tissues in rheumatoid arthritis. Various phenomena have been recorded all of which point to the presence of angiopathy as a characteristic feature in the pathomorphology. Opinions differ as to which part of the vascular bed becomes most involved. The type of lesion is also of some concern. Some investigators emphasize the engagement of arterioles as the crucial problem (*Cruickshank* 1954 (1), *Sokoloff & Bunim* 1957 (2), *Schmid et al.* 1961 (3)). Others present strong evidence for the reaction of the venules to be a prime factor (*Kulka et al.* 1955 (4), *Kulka* 1959 (5), *Brånemark et al.* 1963 (6)). *Kulka* 1966 (7) includes the capillary system in his general concept of the venular derangement and dysfunction in synovial tissues in rheumatoid arthritis.

VASCULAR CHANGES IN LIGHT MICROSCOPY

In rheumatoid arthritis the vascular involvement is regarded as a primary factor. Definite signs of disseminated arteritis have been observed in locations far from the obvious tissue lesion (*Cruickshank* 1954 (1), *Sokoloff & Bunim* 1957 (2)). *Cruickshank* 1954 (1) found at autopsy signs of active or healed arteritis in 25 per cent of cases with rheumatoid arthritis. This figure may, however, be much larger as the

localisation of vascular changes is most irregular (*Brånemark et al.* 1963 (6)). Difficulties may also arise in identifying healed lesions.

The irregularity of vascular changes has been studied by *Virtama* (1959). In an arteriographic study at autopsy of ten cases of rheumatoid arthritis he found in the digital arteries of the hand local obliterations of the arterial trunks especially in the vicinity of the affected joint spaces; local poststenotic shuttle-like dilations of the arteries; hypervascularisation and dilation of the arterioles close to erosions of bone.

The changes encountered are proliferative with a marked cellular infiltration in and around the vessel wall of lymphocytes and small mononuclear cells (Figure 1). Deposition of fibrinoid substance in the vessel wall is common (*Kulka* 1959 (5)). Destructive changes may also be seen such as necrosis, thrombosis, aneurysm formation and invasion of neutrophils. These features are indicative of the fulminant, malignant form of rheumatoid arthritis.

In extensive investigations on the microvascular derangement in synovial tissues in rheumatoid arthritis, *Kulka* (1959 (5), 1966(7)) concludes that there is a segmental angiopathy which particularly involves the venules and capillaries. Larger vessels may nevertheless become engaged and the angiopathy can appear without relation to other lesions. The most pronounced effect following this angiopathy is exudation and ischaemia. According to *Kulka* (1959 (5)) the angiopathy in slowly progressing cases is indistinguishable from secondary forms of vascular involvement occurring in any other chronic inflammatory process. The angiopathy is regarded as a primary manifestation in rheumatoid arthritis with a particular predilection for venules, which become obliterated by necrosis and fibrin impregnation or by endothelial proliferation.

Observations have been made by *Kulka* (1959 (5)) in a rheumatoid nodule of one day's duration. The venules in this exhibited an intense inflammatory reaction and an escape of eosinophilic material could be shown which had a delicate reticular structure typical of inflammatory fibrin. In this region of fibrin deposition, localised to the periendothelial zones, necrosis of leukocytes was observed.

Besides the above mentioned changes including fibrinoid necrosis of the vessel wall and vascular obliteration, *Kulka* (1966 (7)) has also described varying degrees of venular and capillary dilation as well as exudative leakage.

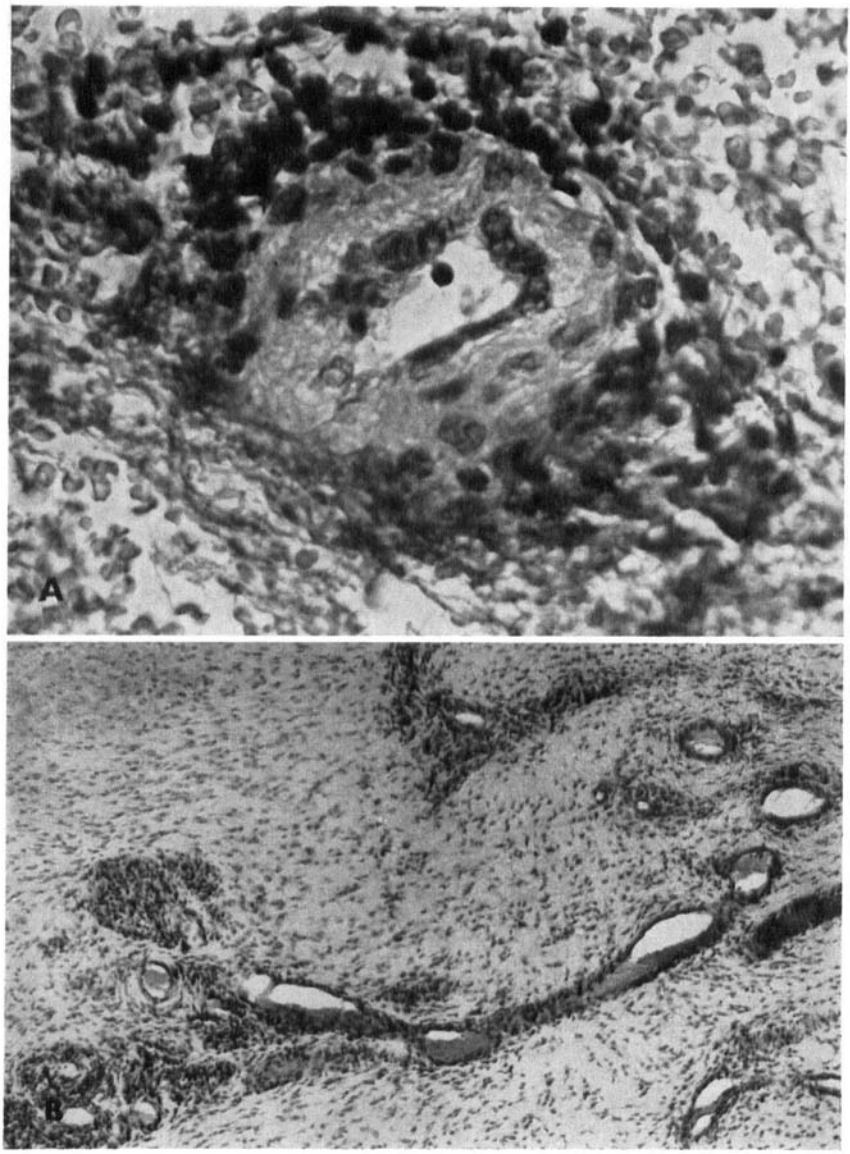

Figure 1 a. Synovial tissue from knee-joint in rheumatoid arthritis. Proliferative changes with marked lymphocytic and mononuclear infiltration around arteriolar wall. × *640.*

Figure 1 b. Same as in 1 b but venules, which are slightly dilated. × *640.*

VASCULAR CHANGES IN VITAL MICROSCOPY

Using vital microscopic techniques *Brånemark et al.* (1963 (6)) carried out *in vivo* investigations of synovial tissues in human knee joint exposed at operation. They found that tissue adjacent to a synovial tissue with old rheumatic changes may have a capillary system which appears quite normal in structure and function.

The venules of the rheumatic tissue exhibited dilation and varying caliber which resulted in an uneven outline and tortuosity and slow, almost stagnated blood flow. Arteriolar-venular shunts at the basis of synovial capillary loops were observed.

In summary it then appears that light microscopic investigations have yielded information about the vascular pathology of synovial tissues in rheumatoid arthritis that indicate an angiopathy of varying intensity. Arterioles, venules and capillaries become involved to varying degrees.

The information thus obtained has of late become further expanded on and also scrutinized with the development of more refined methods such as electron microscopy.

VASCULAR CHANGES IN ELECTRON MICROSCOPY

In 1964 *Hirohata & Kobayashi* carried out an electron microscopic study on biopsies from 41 joints with rheumatoid arthritis. They found that many factors are involved in the vascular changes of rheumatoid arthritis. In vessels less than 10 μ in caliber there is an increase in the height of the endothelial cells and several cytoplasmic processes extend into the vascular lumen occasionally causing an obstruction. In the exudative phase of rheumatoid arthritis the endothelial cells of arterioles and venules become flattened and their cytoplasm becomes bright. A swelling of mitochondria is noted and small vacuoles appear in the endothelial cells. The intercellular space between the endothelial cells becomes widened and the basement membrane turns thicker though in places disruptions are noted. There is an atrophy of the muscle cells in the arterioles, fibroblasts appear in the tunica media and in the adventitia there is a marked hyperplasia of the fibers. In the exudative phase leukocytes, lymphocytes, monocytes and plasma cells are found at the basal surface of the endothelial cells and in the adventitia perivascular cells and collagen fibers are noted.

In chronic cases *Hirohata & Kobayashi* describe numerous cytoplasmic processes extending into the lumen from the endothelial cells and

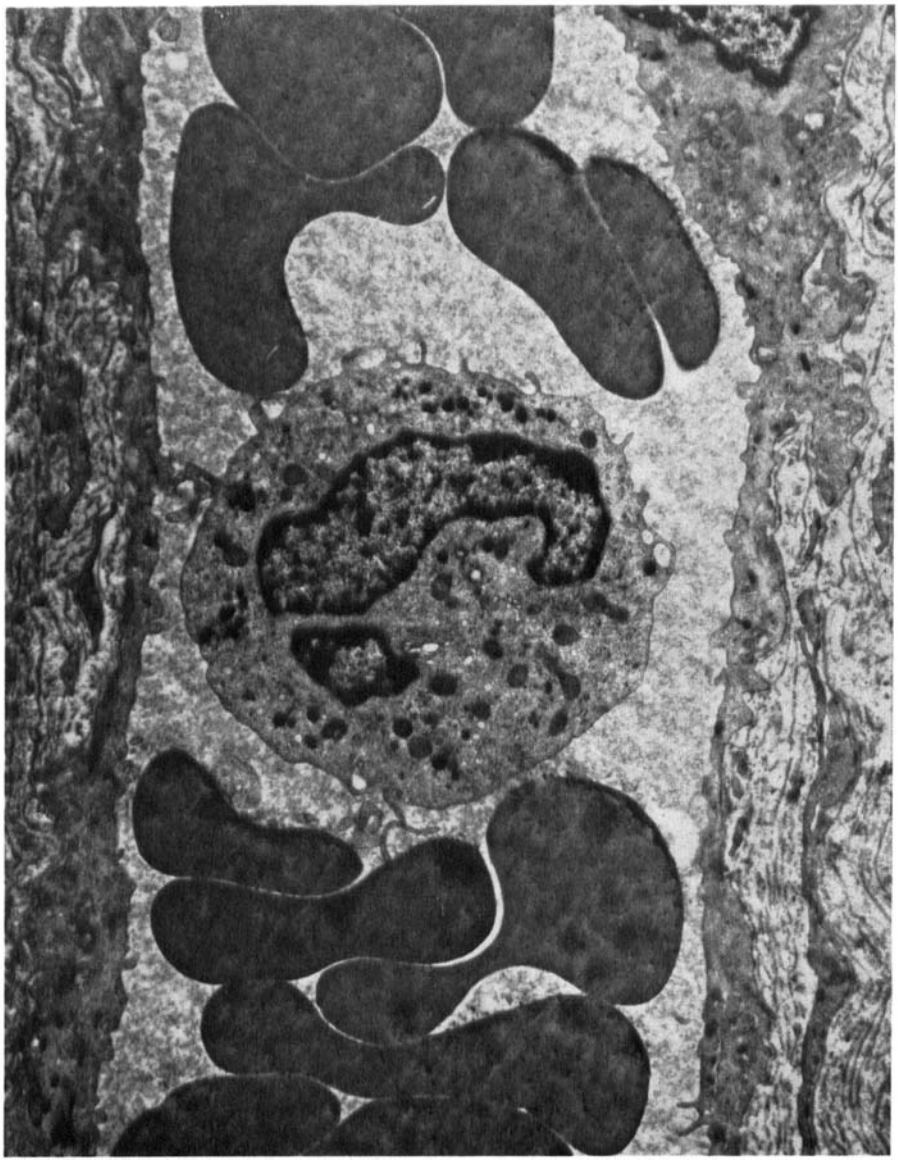

Figure 2. Electron micrograph from normal synovial tissue showing a longitudinally cut venule containing a granulocyte and several erythrocytes. The endothelium is generally rather thin but thickens in the nuclear region (upper right corner). Outside the endothelium several layers of basement membranes and periendothelial cell processes are seen.—Magnification × 9,000.

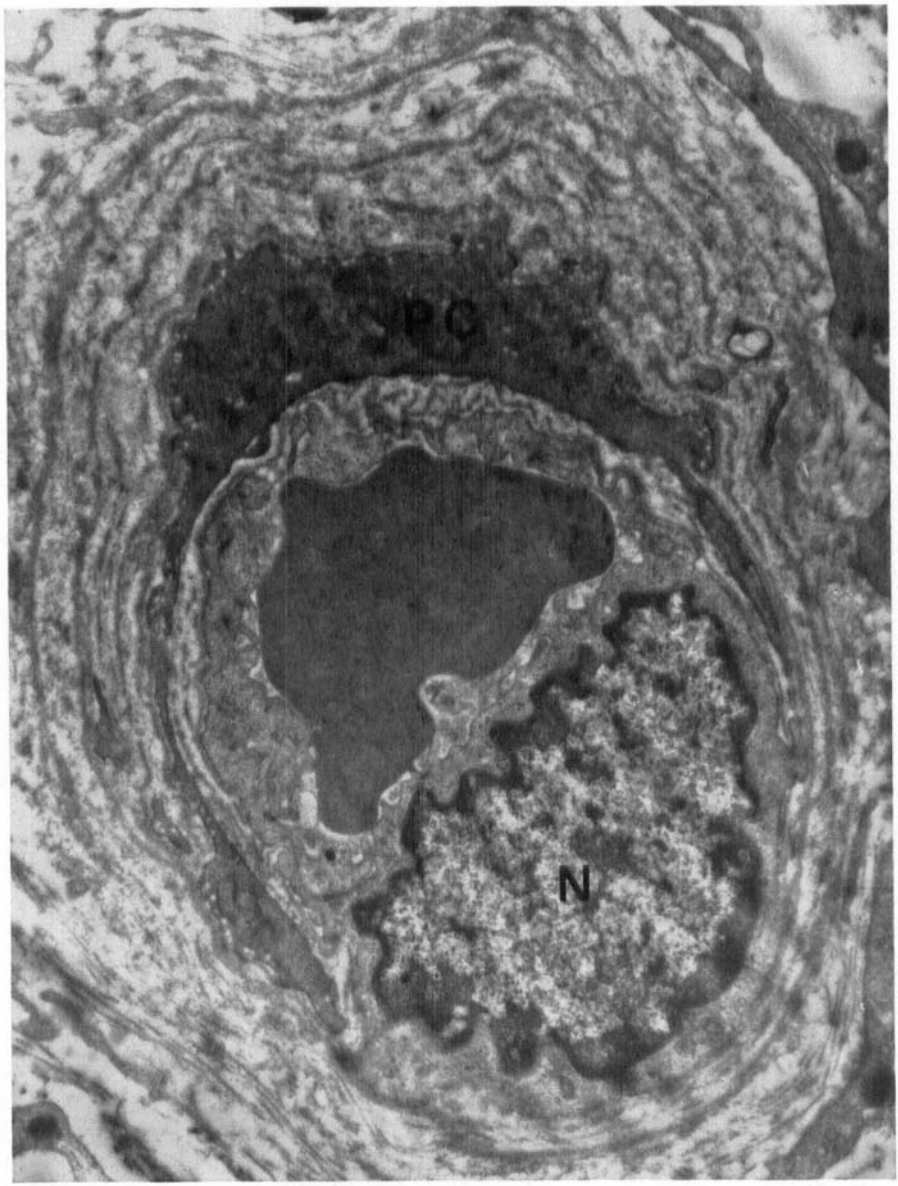

Figure 3. Electron micrograph from normal synovial tissue showing a transversely cut capillary. The lumen is very narrow and almost filled by a red blood cell. The endothelial lining of the capillary is thin except for the region occupied by the endothelial cell nucleus (N). Outside the endothelium a periendothelial cell (PC) and several basement membranes are seen.—Magnification × 13,000.

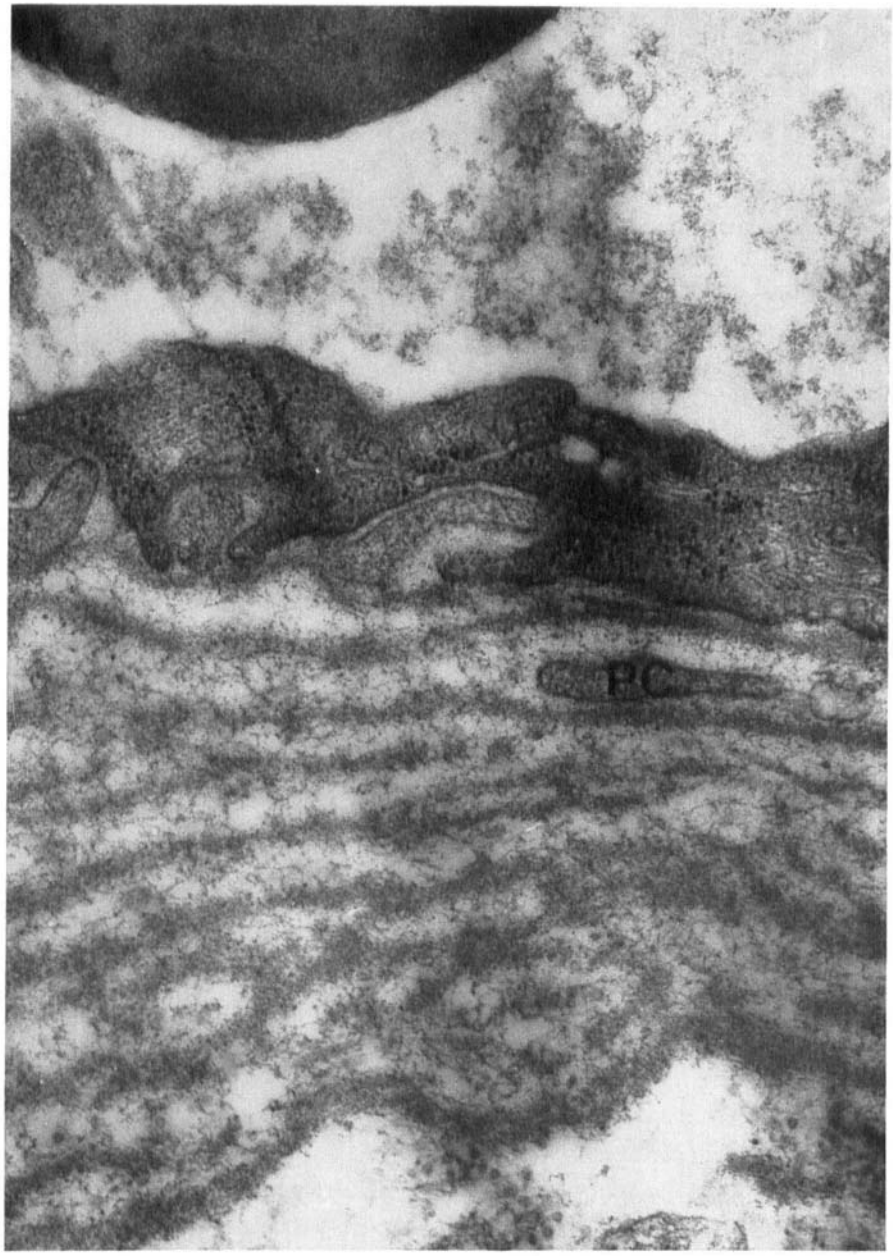

Figure 4. Electron micrograph from normal synovial tissue showing a detail of a capillary wall. Outside the thin endothelium there are several concentrically arranged basement membranes of somewhat varying thickness. Only a small portion of periendothelial cell process (PC) is found in this section. Magnification × 48,000.

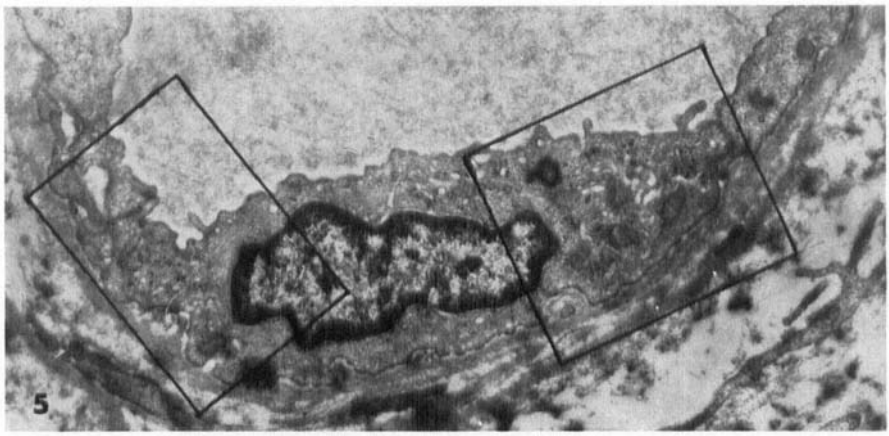

Figure 5. Survey electron micrograph from normal synovial tissue showing a part of a venule wall. Note the thick, partly split endothelial basement membrane. Magnification × 14,000.

pinocytosis is seen. The mitochondria of the endothelial cells decrease in number and become swollen and filaments appear in the cytoplasm.

According to these authors the inflammation commences in the endothelial cell and is then spread to the tunica media, and tunica externa.

In an electron microscopic investigation on synovial tissues in rheumatoid arthritis *Norton & Ziff* (1966 (9)) devoted their main interest to the cellular components but some mention is made of the vascular appearance. They conclude that, apart from a certain hypertrophy of connective tissue elements about blood vessels, there is little evidence that the vascular bed is characteristically changed in rheumatoid arthritis.

At a recently held symposium on early synovectomy in rheumatoid arthritis *Ball* (1967 (10)) in summary of the works of *Barland, Novikoff & Hamerman* (1964 (11)), *Wyllie, Haust & More* (1966 (12)), and *Norton & Ziff* (1966 (9)) stated that electron microscopic studies (mainly based on the relatively late stages?) leave the question of a specific structural target within the synovium unanswered.

At the same symposium *Paul* (1967 (13)) maintained that in rheumatoid disease vasculitis, segmental or focal, is a hallmark of the disease. Arteritis and arteriolitis occur most often in advanced or highly active disease. The most significant type of vascular involvement, however, is the venulitis and capillaritis. The endothelial cells of the minute vessels display hypertrophy and proliferation with numerous filopodia.

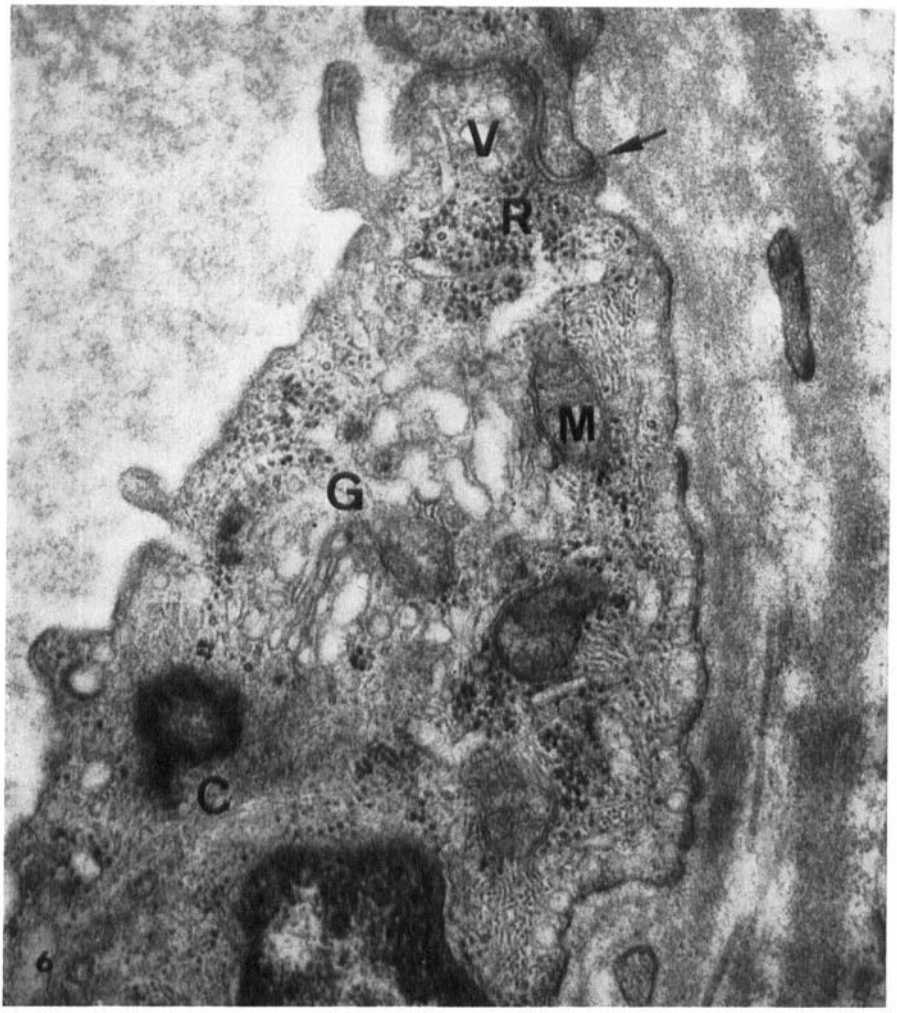

Figure 6. Electron micrograph showing a detail of the endothelial cell in Figure 4. The cytoplasm contains several mitochondria (M), a well developed Golgi apparatus (G), a few pinocytotic vesicles and numerous ribosomes (R). C denotes a centriole. The arrow marks a cell junction.—Magnification × 56,000.

The subject of vascular pathology in rheumatoid arthritis thus becomes most controversial in view of which method is used for investigation. To further elucidate this problem we have pursued an electron microscopic study on the vascular appearance in normal synovial tissues and in cases of rheumatoid arthritis.

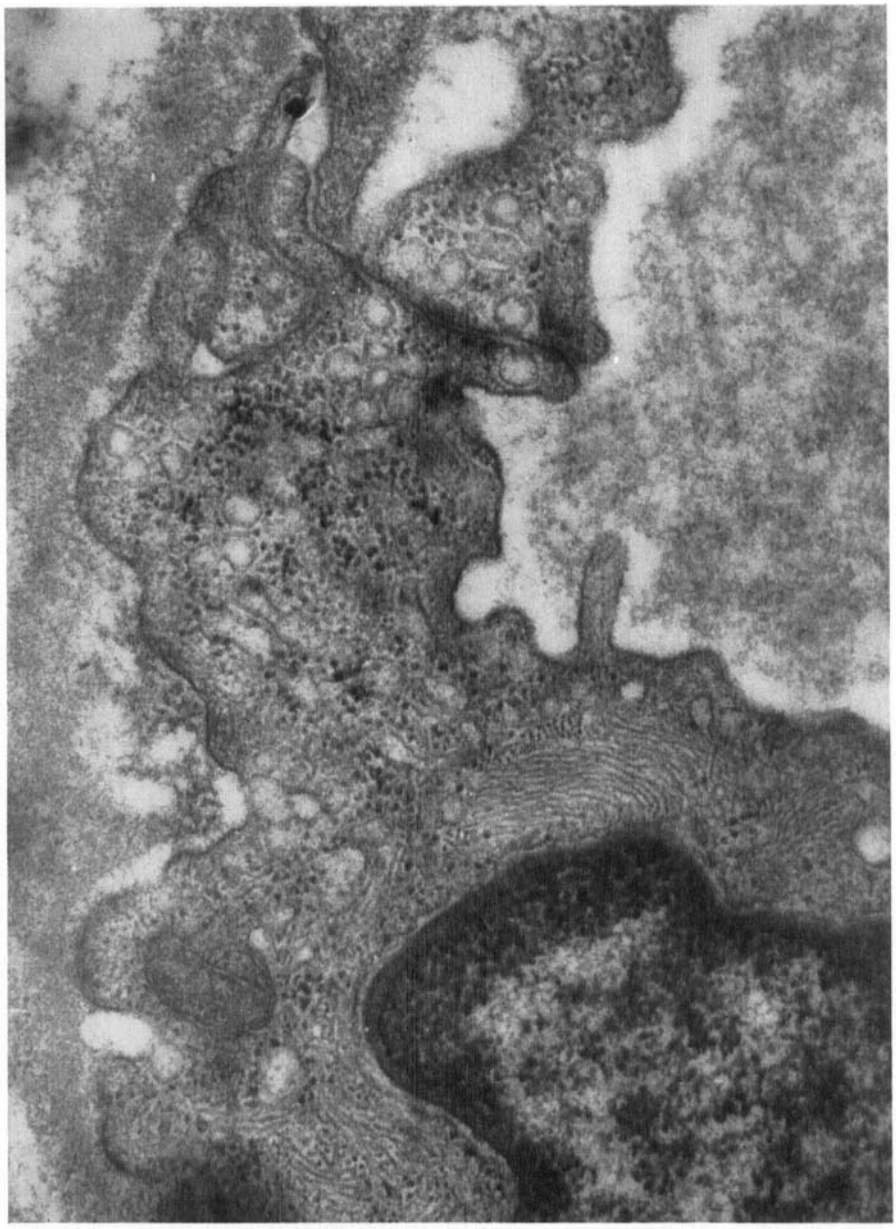

Figure 7. Electron micrograph showing another detail of the endothelial cell in Figure 4. The most characteristic components of the cytoplasm in this section are bundles of thin filaments. This part of the cell also contains numerous pinocytotic vesicles.—Magnification × *58,000.*

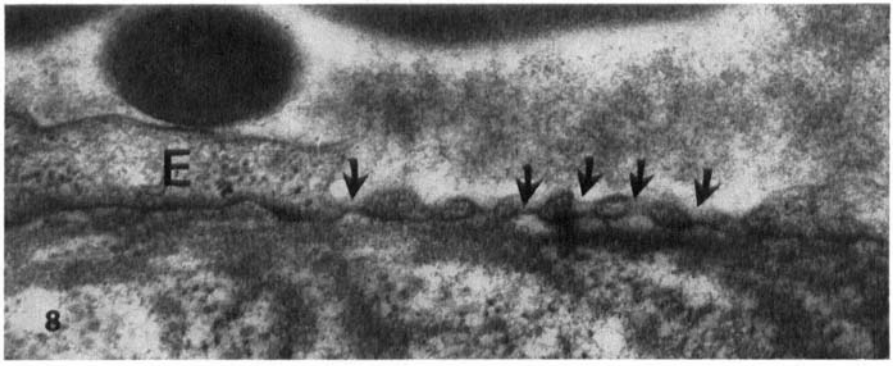

Figure 8. Electron micrograph from normal synovial tissue showing a part of capillary wall. The endothelium (E) is extremely thin in some areas (arrows) but there are no true discontinuities.—Magnification × *47,000.*

MATERIAL AND METHODS

Synovial tissue was obtained at synovectomies for rheumatoid arthritis in knee-joints in 15 cases. All of these patients had suffered from their disease for more than five years and been subjected to various conservative treatments without any obvious improvement. On macroscopic examination at synovectomy the synovium was glossy and congested, hyperemic and coated with fibrin, studded with hypertrophic villi often with necrotic tips. The articular cartilage was in all cases destroyed in a patchy way and numerous erosions filled with granulation tissue were present along the bone cartilage borders. In most cases only remnants of the menisci remained and the cruciate as well as the collateral ligaments were lax. In all cases an excessive exudate was present. Arthrotomy for investigative exploration was done in 3 cases. As nothing abnormal was noted in either macroscopic or microscopic appearance of the synovial tissue this served as normal material. Specimens were prepared for electron microscopy as described below. The specimens from rheumatoid arthritis were subjected to light microscopic study in order to ascertain that changes were present in this tissue which in general are accepted as being compatible with the described pathomorphology in rheumatoid arthritis.

For electron microscopy small pieces of synovial tissue were excised and immediately immersed into a fixative consisting of 3 per cent glutaraldehyde buffered at pH 7.2 by sodium cacodylate. Two hours later the tissue pieces were transferred to a second fixative containing buffered 1 per cent osmic acid. After postfixation for 1.5–2 hours the tissue was dehydrated in ethanol and embedded in Epon. The sectioning was performed on an LKB Ultrotome and the electron microscopical examination in a Siemens Elmiskop I.

OBSERVATIONS AND DISCUSSION

The tissues obtained from the cases of rheumatoid arthritis were studied in the light microscope with special attention to the appearance

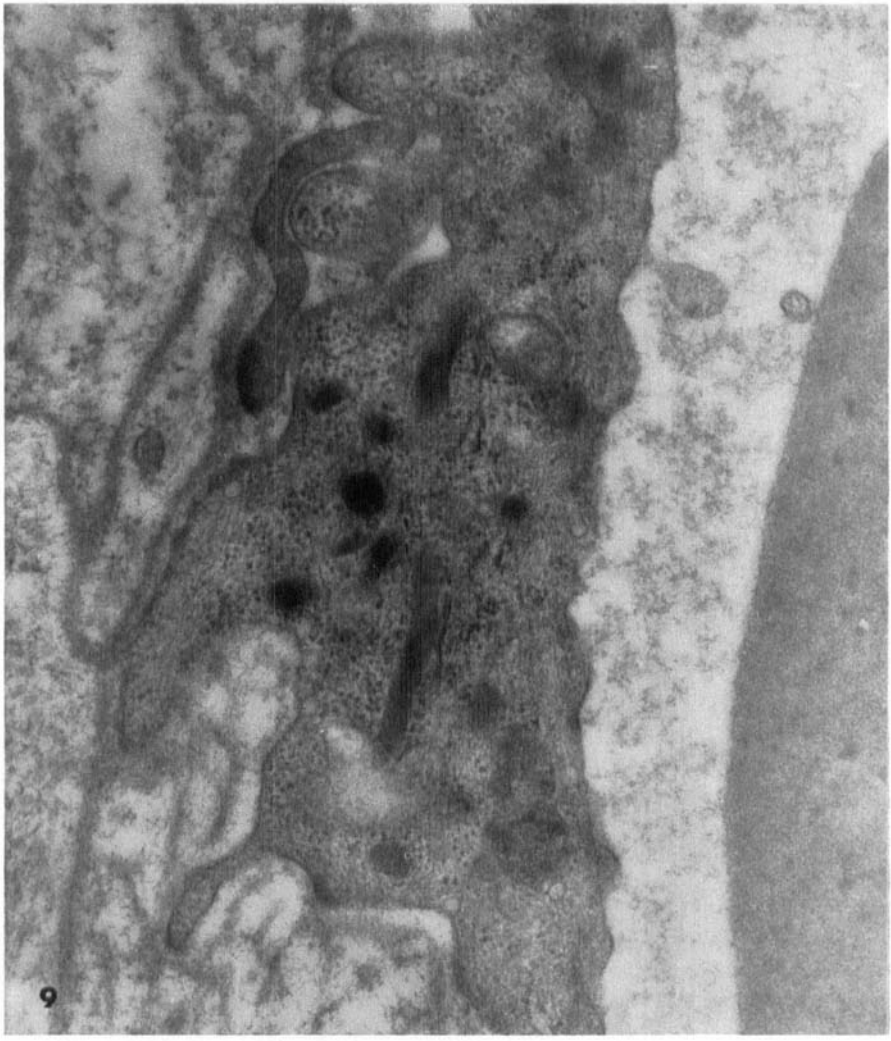

Figure 9. Electron micrograph from normal synovial tissue showing part of venule wall. The endothelial cell contains several dense bodies which might be lysosomes. Magnification × *42,000.*

of the vessels. The changes observed conformed with those described by *Kulka* (1959). No certain abnormalities were seen in the arterioles. The venules were dilated and congested. Many venules were surrounded by small clouds of extravasated erythrocytes. There was marked cellular infiltration around many venules and in some of them the infiltration was intramural. Fibrin deposition was noted as irregularly scat-

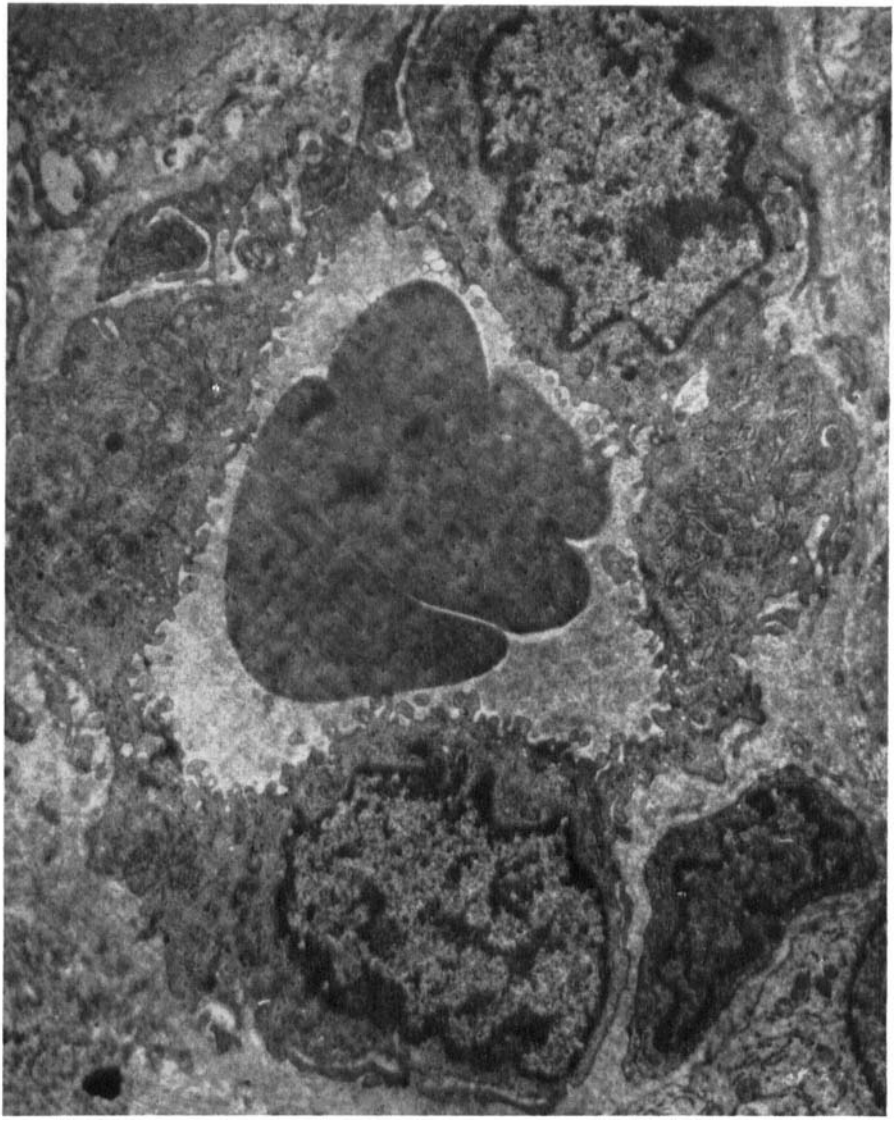

Figure 10. Electron micrograph of a capillary in synovial tissue from a case of rheumatoid arthritis. This survey picture does not disclose any abnormalities of capillary structure.—Magnification × *11,000.*

tered deposits both in vessel walls and extravascularly. Similar changes though more discrete were noted in capillaries.

A thorough knowledge of the normal ultrastructure of a tissue is a prerequisite for judging pathological changes in the same. Most reports on ultrastructural changes of synovial vessels in rheumatoid arthritis lack information about the normal ultrastructure of these vessels. Consequently long series of features have been described as pathological although they may in reality be normal. In this study the electron microscopical structure of capillaries and venules was assayed in normal synovial tissue as a background for evaluating the observations made in rheumatoid arthritis.

The ultrastructural appearance of the normal synovial capillaries and venules is greatly varying with respect to both the thickness and structure of the wall and the size and shape of the lumen. The height of the endothelial cells varies extensively from vessel to vessel and also between different portions of the same vessel. The thinnest parts of the endothelial lining measure only a few hundred Å, while the thickest portions, generally the nuclear region where the bulk of the cytoplasm is found, can be several microns thick. The luminal surface is sometimes rather smooth but as a rule it is furnished with a varying number of projections of various size and shape. The cell membranes of adjacent endothelial cells are always closely apposed and often equipped with desmosomes. The cytoplasm always contains pinocytotic vesicles but the number of these structures is extremely varying. Mitochondria and endoplasmic reticulum as well as free ribosomes exhibit no deviations from what is known and described about small vessels in other tissues. The most characteristic cytoplasmic component is a well developed system of thin filaments. These filaments which have a diameter of about 70 Å, are arranged in bundles. The bundles have a wavy course and occupy a considerable part of the cytoplasm. Some endothelial cells contain numerous dense, rounded or elongated bodies which could represent lysosomes. Such cells are sometimes found in large numbers in a portion of a vessel while in other portions the cells, almost or entirely, lack this type of cytoplasmic elements.

Figure 11. Electron micrograph of a venule wall in synovial tissue from a case of rheumatoid arthritis. The lumen is packed by red blood cells. Outside the endothelium (E) there are several layers of periendothelial cell processes (PC) and basement membranes, just as is found in normal synovial tissue.
Magnification × 11,000.

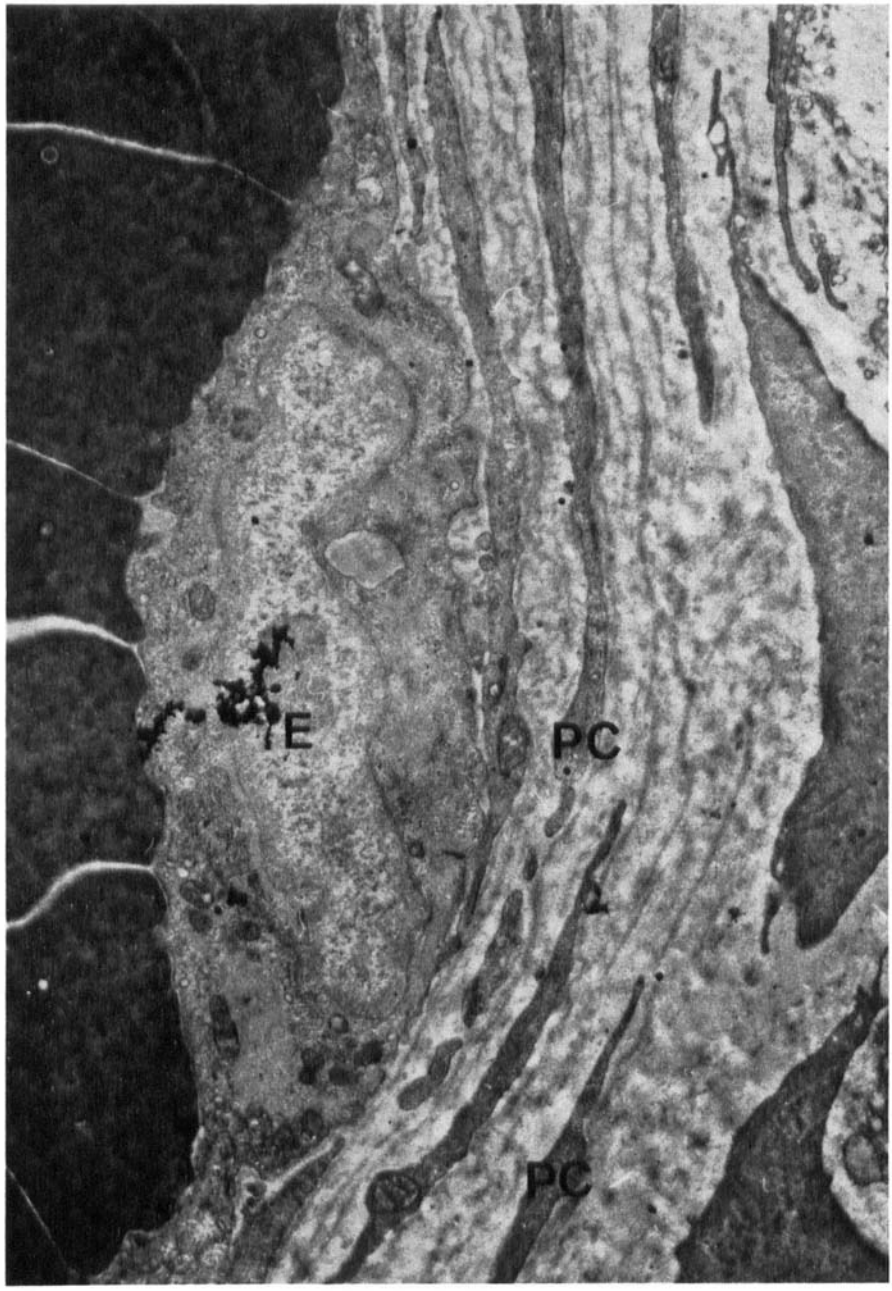

Figure 11.

Figure 12.

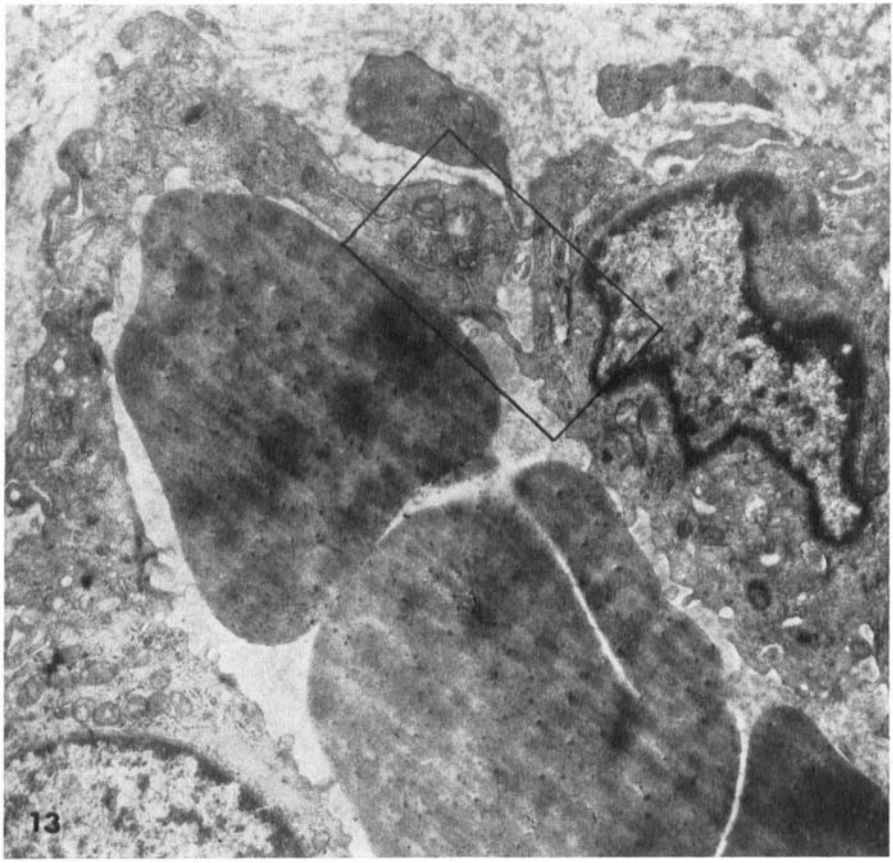

Figure 13. Electron micrograph of a synovial capillary from a case of rheumatoid arthritis. The endothelium is of varying thickness but without discontinuities. Magnification × 14,000.

The endothelial tube is surrounded by a continuous basement membrane the thickness of which varies from 400 to 2.000 Å. Outside the basement membrane periendothelial cells and slender projections from such cells are found. These cells which can form several layers are all furnished with basement membranes. In some areas where the cell pro-

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Figure 12. Electron micrograph of a venule wall in synovial tissue from a patient with rheumatoid arthritis. Outside the endothelium (E) two periendothelial cells (PC) are seen. On of them has a slender projection closely apposed to another similar cell process (arrow).—Magnification × 15,000.

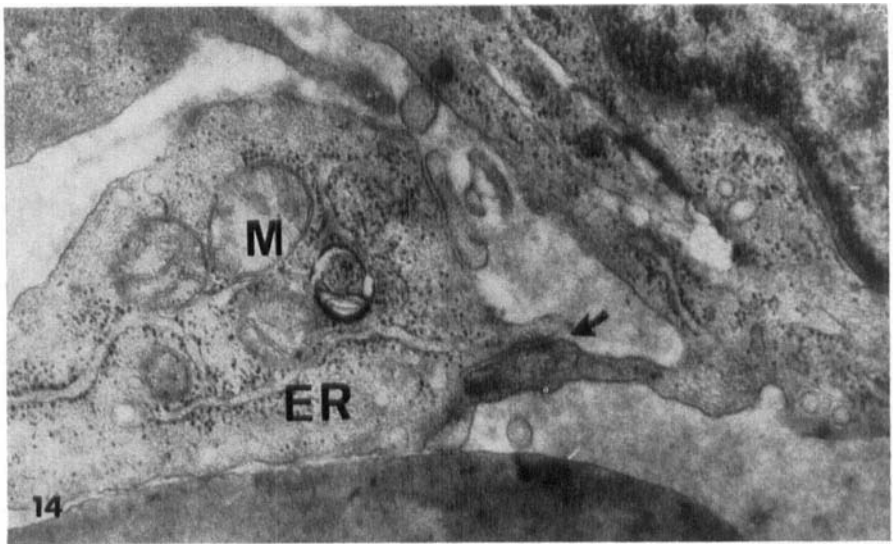

Figure 14. Electron micrograph of a detail of Figure 12 showing the junction of two endothelial cells (arrow). In the cytoplasm mitochondria (M) and endoplasmic reticulum (ER) are the most conspicuous components.—Magification × 37,000.

jections are lacking but the basement membranes retained a characteristic pattern is observed of multilayered basement membranes.

When studying the ultrastructure of capillaries and venules of synovial tissue in rheumatoid arthritis we found no significant deviation from this normal pattern. The endothelial cells are of varying heights, just as in the normal tissue, and their luminal surfaces are smooth or furnished with projections. There is no widening of the intercellular spaces and pores or discontinuities are not observed. The endothelial cell cytoplasm contains the same organelles of the same appearance as does the normal cytoplasm. The periendothelial structures, the basement membranes and periendothelial cells, do not exhibit any characteristic changes.

Figure 15. Electron micrograph of the wall of a synovial venule from a case of rheumatoid arthritis. The endothelial cell (E) contains a well developed system of 70 Å filaments and is covered by a basement membrane (BM). In the space between the endothelium and a periendothelial cells process (PC) and between this and another periendothelial cell (PC) are many cross-cut collagen fibrils. Magnification × 50,000.

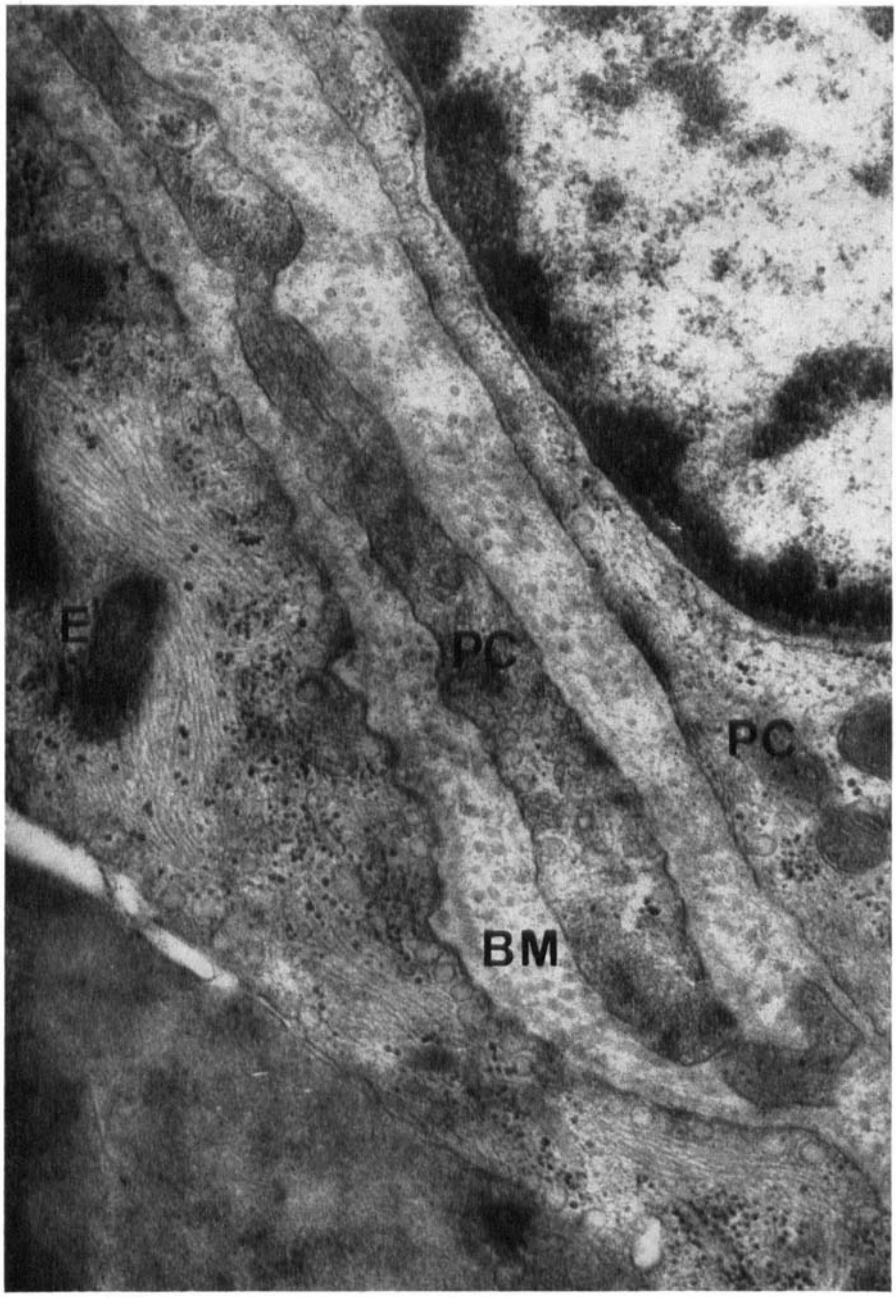

Figure 15.

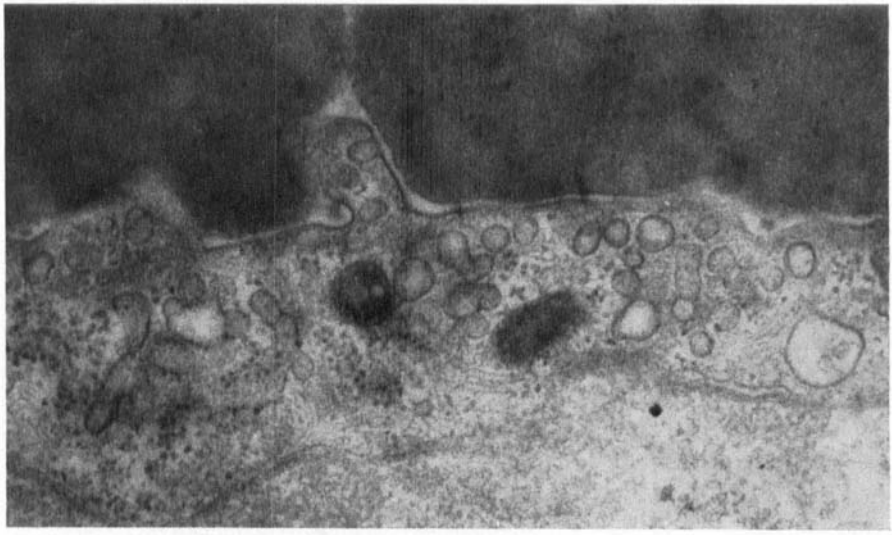

Figure 16. Electron micrograph of a venule wall in the synovial tissue from a rheumatoid joint. The endothelial cytoplasm contains a large number of pinocytotic vesicles. Magnification × 46,000.

In this context it is important to point out that the sections studied by electron microscopy were all the site of changes estimated as pathologic in light microscopy.

Again it must be emphasized that the irregularity with which the disease strikes the target organ may be of some significance in evaluating the observations made by electron microscopy. The vessel walls may be sites of characteristic changes which can only be detected by serial electron micrographs along the course of a vessel. On the other hand this investigation has revealed that vessels in rheumatoid arthritis, which in light microscopy disclose certain features regarded as pathologic, demonstrate a variation in structure which is entirely comparable to that we have found in the normal controls.

Some ultrastructural features reported by *Hirohata & Kobayashi* (1964 (14)) and *Paul* (1967 (13)) and regarded by them as pathological coincide, no doubt, with the structural appearance of small vessels of normal synovial tissue. The remaining observations, such as vacuolization, swelling of mitochondria and widening of intercellular spaces, may well be explained by an unsatisfactory technique used for the preservation of the tissue.

SUMMARY

Light microscopic investigations of the vascular bed in synovial tissues in rheumatoid arthritis have yielded the information that a vasculitis or angiopathy of varying intensity is present. Electron microscopic studies have indicated that in rheumatoid arthritis an inflammation commences in the endothelial cells of venules and capillaries and spreads to the tunica media and tunica externa.

The present study was carried out on synovial tissue from normal and rheumatoid knee joints. Light microscopy of the vascular bed in the rheumatoid synovial tissue revealed inflammatory changes largely corresponding to those described by earlier investigators. In order to be able to estimate and characterize these changes at the ultrastructural level we performed a thorough electron microscopical study on the venules and capillaries in normal synovial tissue. When comparing the ultrastructural pattern of these normal vessels with that of the corresponding vessels in the rheumatoid synovial tissue it turned out that no significant deviations could be established.

RESUME

Des recherches microscopiques de la couche vasculaire du tissu synovial dans des cas d'arthrite rhumatoïde ont révélé la présence d'une vasculite ou angiopathie d'une intensité variable. Des études microscopiques électroniques ont indiqué que dans l'arthrite rhumatoïde, l'inflammation débute dans les cellules endothéliales des vaisseaux et des capillaires pour s'étendre ensuite aux revêtements médian et externe.

La présente étude s'est basée sur le tissu synovial provenant d'articulations normales et rhumatoïdes du genou. La microscopie de la couche vasculaire du tissu synovial rhumatoïde a révélé des modifications inflammatoires correspondant largement à ecIles découvertes par des chercheurs précédents. Afin de pouvoir estimer et caractériser ces modifications au niveau ultrastructural, nous avons procédé à une étude microscopique électronique approfondie des vaisseaux et des capillaires du tissu synovial normal. Une comparaison entre le modèle ultrastructural de ces vaisseaux normaux et des vaisseaux correspondants du tissu synovial rhumatoïde a démontré qu'il ne pouvait pas être établi de déviations signifiantes.

ZUSAMMENFASSUNG

Mikroskopische Untersuchungen des Gefässbettes von Synovialgewebe in Fällen von rheumatischer Arthritis haben die Kenntnis ergeben, dass eine Vasculitis oder Angiopathie verschiedenen Grades vorhanden ist. Elektronmikroskopische Untersuchungen haben gezeigt, dass beim Gelenksrheumatismus eine Entzündung in den Endothelzellen der kleinen Venen und Kapillaren beginnt ud sich zur tunica media und tunica externa ausbreitet.

Die gegenwärtige Studie wurde an Synovialgewebe von normalen und rheumatischen Kniegelenken ausführt. Lichtmikroskopie des Gefässbettes von rheumatischem Synovialgewebe offenbarte entzündliche Veränderungen, die denen von früheren Untersuchern entsprachen. Um im Stande zu sein diese Veränderungen auf dem ultrastrukturellen Niveau zu beurteilen und zy charakterisieren, führten wir gründliche elektronmikroskopische Untersuchungen der Venülen und Kapillaren von normalen Synovialgewebe aus. Ween man die ultrastrukturelle Anordnung dieser normalen Gefösse mit der von entsprechenden Gefässen in rheumatischem Synovialgewebe verglich, stellte es sich heraus, dass keine bezeichnenden Abweichungen festgestellt werden konnten.

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