THE ULTRASTRUCTURE OF BLOOD VESSELS IN THE PERIODONTAL LIGAMENT OF HUMAN MAXILLARY FIRST PREMOLAR TEETH

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SUMMARY

Many different observational techniques have been used to study the periodontal ligament in general and its vascular system in particular. Transmission electron microscope studies of the ligament vascular system are, however, scarce. It was felt that examination of the full width of the ligament from the dento-periodontal interface to the alveolo-periodontal interface would provide useful information as to the types of vessels, their characteristics, frequency and general distribution. Comparisons could then be made with other connective tissue studies and vascular characteristics peculiar to the periodontal ligament could be identified and described.

Specimens were taken surgically from adolescents who required extraction of teeth to facilitate their orthodontic treatment. Each specimen consisted of the crown, coronal half of the root, and a segment of the buccal alveolar plate together with the interposed periodontal ligament.

The specimens were fixed in 4% glutaraldehyde in Clark's buffer at 4^{o} Centigrade, and post-fixed in 1% OsO_4 in Clark's at 20^{o} Centigrade. Material was demineralised in 10% EDTA in Clark's at 4^{o} Centigrade. After demineralisation, the block was further reduced, embedded in Spurr's resin, and then sectioned in the silver range, that is 75 ± 5nm, for the transmission electron microscope. A mixture of diamond and glass knife ultramicrotomy was used, and sections were stained with uranyl acetate and or lead

citrate.

In about 5% of capillary-type blood vessels, intra-endothelial fenestrae were seen. This agreed with studies on non-specific connective tissue such as Rhodin (1967, 1968). The fenestrae were in all respects typical in that they occurred in areas of the thinnest endothelium, and were closed by a diaphragm.

In intermediate sized blood vessels, bundles and sheets of organised collagen were identified running longitudinally in between pericyte cell bodies, larger pericyte processes, and endothelial cells. Rhodin (1967, 1968) reported occasional fibrils in this region, but no organised sheets or bundles.

Where the vessels were almost totally collapsed, the endothelial cells and pericytes collapsed differentially. The fibro-collagenous lamina was then seen as a series of longitudinal bundles within a cell process bound tube. This ultrastructural feature will be the subject of further investigation.

Other extracellular fibres that were seen generally, and in close association with blood vessels were oxytalan fibres. Collagen was seen with its characteristic cross banding. In contrast, oxytalan appeared as a much finer, reticular pattern.

On many occasions abluminal endothelial inclusions were seen. They closely resembled fine non-myelinated nerves and could be seen in intimate relationship with the endothelium and clearly within the endothelial basement membrane.

There were many close approximations between endothelial cells and pericytes. These approximations could be quite complex. The ends of pericyte processes penetrated quite deeply into endothelial invaginations, and, at the penetration pericytes and endothelium seemed to share the same basement membrane. The deep invaginations appeared to be of a very specific type.

It must be added that no smooth muscle cells were found in the tunica media of any vessels at all. On this basis, all vessels seen in the specimens examined in this study were classified as non-arterial.

Technical impediments encountered were considered sequentially and possible solutions proposed where applicable.

The multiplicity of classifications and their basis in ultrastructure was considered. The question of consistency of terminology in such classifications was discussed. The question of conceptual validity of conventional classifications was examined.

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My supervisor Dr. M.R. Sims and Dr. W.J. Sampson have provided indispensable guidance in the preparation of this report. I would also like to thank Mrs. L. Zaleski and Mrs. L. McMahon for advice regarding photographic matters.

STATEMENT

This report contains no material which has been accepted for the award of any other degree or diploma in any university.

To the best of my knowledge and belief this report contains no material previously published or written by another person except when due reference is made in the text of the report.

D.R. GILCHRIST

SECTION 1

INTRODUCTION

The periodontal ligament is a unique structure. It stands at the interface between teeth and the supporting skeleton. This interface is critical to many processes ranging from frankly pathological to highly physiological. In periodontal disease potential aetiological factors have been cited but one thing is certain; the vessels that conduct the cellular nutrients and chemicals that are necessary to combat disease must be involved in the pathogenesis and prognosis. In orthodontic tooth movement the mechanisms of dissolution of bone may be obscure but the vitality of the cells that are involved in it is dependent to a certain extent on the integrity of the vascular system. Upon the structure and integrity of the vascular system rests the continuance of life whether at a biochemical, cellular, organ or organism level when dealing with mammals and many other species.

In its normal functional state the periodontium is subject to extreme physical loads under the influence of masticatory forces. One of the fundamentals of the viability of structures and processes in their environment is their capacity for repair, function and indeed merely to live. As Majno (1965) has stated, the vascular membrane is the anatomical and conceptual culmination of the vascular system. Thus, it is not unreasonable to consider that some of the keys of specialised function in this anatomical locality may be found in its vascular system.

The development of the transmission electron microscope has provided a unique opportunity to examine the ultrastructural components of tissues, including blood vessels, in various parts of the body. There have been many technical impediments to the application of this technique to the periodontal ligament due to:

- the need for improved embedding techniques to overcome the relatively impervious nature of the tissue specimens and, in particular, the development of resins with very low viscosity to allow full penetration of tissue blocks.
- the nature of tissue specimens has required development of new techniques in ultramicrotomy in order to cut suitably thin sections of variable hardness structures, for example the use of diamond knife ultramicrotomy.

The periodontal ligament has been the subject of a great deal of study and many references are found that mention the vascular structures observed. Relatively few studies, however, have made the vascular system their prime concern and even fewer have been histological.

Information on the ultrastructure of blood vessels in the periodontal ligament, as seen with the transmission electron microscope, is very scarce.

SECTION 2

PROJECT AIMS

This project aimed to examine the morphology of vessels found in the coronal half of the periodontal ligament of human maxillary first premolar teeth.

The specimens were studied using a transmission electron microscope. Using the observations an attempt was made to extrapolate structure to function in the light of other ultrastructural vascular research.

Typing of vessels was attempted using established classifications in order to develop a feasible topographical model of periodontal ligament vasculature.

SECTION 3

REVIEW OF THE LITERATURE

The review of the literature has been divided into two parts.

1. The literature as it relates to the ultrastructure of the microvascular bed in non-dental tissues.
2. The literature as it relates to the periodontal vascular bed in particular.

The review of the literature as presented in this study is based largely on microanatomical features. There are many individual studies that deal with similar vascular structures. Rather than discuss all of the findings presented in a publication at one time, it is proposed to discuss each vessel type and make back reference, where appropriate, to the studies that have considered this type of vessel.

MICROVASCULATURE IN NON-DENTAL TISSUES

CAPILLARIES

The classification of capillaries is clouded since opinions vary as to what exactly is a capillary, or for that matter any other microvessel (Baez 1977). Rhodin (1968) largely accepted Majno's (1965) criterion of any vessel less than 8 μm in diameter. Cliff (1976) took a pragmatic view and considered that the term 'capillary' had become part of the conventional vascular jargon and that it was "by now far too late in the day" to expect to change. He went on to explain that the "so called 'capillaries' of physiology" were best construed as exchange vessels in a particular location in the vascular tree rather than some equivalent to a pure dimensional concept.

Rhodin (1967, 1968) only used the noun 'capillary' in conjunction with some qualifying terminology. Rhodin's sequence of vessels through the terminal microvascular bed was: precapillary sphincter, venous capillary and post-capillary venule.

Laguens and Gomez-Dumm (1969) alluded to the possibility of a capillary that consisted of an endothelial tube only and other vessel types that had perivascular cells. It is possible however, that in their material the pericytes were too branched to show in electron microscopic sections.

Rhodin (1967, 1968) would have classified as capillary types, vessels described by Wiedeman (1963)

using the terms: arterioles, capillaries and post capillary venules.

Herdson (1967) considered that there were three distinct types of capillary:

1. muscle type - many perivascular cells
2. fenestrated visceral type
3. sinusoidal type

Wolff (1977) considered that, because of their great structural variability, it was impossible to define in generally applicable terms the capillary location in the microvascular bed. This lack of definition also applied to the structure of the capillary wall. Different arrangements considered did not, overall, bear any relation to structural features central to the classification of Bennet, Luft and Hampton (1959).

The definitive classification of capillaries remains the work of Bennet, Luft and Hampton (1959). Their classification was based purely on morphology. While difficult in conversational usage it is the most accurate morphologically and, where applicable in this project, the Bennet et al. (1959) classification will be adhered to. The classification is a three digit unit based upon:

1. basement membrane,
2. endothelial cell type,
3. pericapillary cellular investment.

BASEMENT MEMBRANE

Type A - complete continuous basement membrane

Type B - without a complete investment of basement membrane.

ENDOTHELIAL CELLS

Type 1 - capillaries without fenestrae or perforations

Type 2 - capillaries with intracellular fenestrations

Type 3 - capillaries with intercellar perforations

PERICAPILLARY CELLULAR INVESTMENT

Type α - capillaries without a complete pericapillary cellular investment

Type β - capillaries with a complete pericapillary cellular investment

The classification is expressed as a sequence of three units.

A or B; 1,2,or 3; α or β.

At one stage of our knowledge, it was thought that fenestrae could be either open or closed depending on the functional state of the endothelium at a particular moment. Individual fenestrae, it was thought, could remove or reconstitute their own fenestral diaphragms as circumstances dictated. Rhodin (1962b) considered that, due to deficiencies in the embedding process and limitations of the embedding media themselves, the presence or absence of diaphragms was artefactual in almost all circumstances with the possible exception of capillaries in the renal glomerulus. Developments in embedding technology paralleled a proportional diminution of the numbers of fenestrae observed that had no diaphragm. Therefore, Rhodin postulated that, aside from exceptional circumstances such as the

renal glomerulus, all fenestrae were normally closed by a diaphragm possessing a central knob. This, however is questioned by Casley-Smith (1977a) who refers to "diaphragm-less fenestrae".

VEINS

The terminology is no less varied for the venous side of the microvascular bed. Rhodin (1968) divided the venous side of the microvascular bed into segments based on diameter and structural differences in the walls.

Venous capillaries - less than 8 μm diameter

Postcapillary venules - 8-30 μm diameter

Collecting venules - 30-50 μm diameter

Muscular venules - 50-100 μm diameter

Small collecting veins - 100-300 μm diameter

Movat and Fernando (1964) maintained that venules could only be separated from capillaries by their size, so obviously their idea of venules was more restricted than Rhodin's several categories of venules. Laguens and Gomez-Dumm (1969) considered that venules had a single layer of smooth muscle cells in their tunica media and had walls that were thin. But they also included post-capillary venules as a separate entity. Wiedeman (1963) based her categorization on diameter and she presented diameter ranges which matched Rhodin's fairly well but an entirely different system of nomenclature was used. Rhodin (1967, 1968) provides the most comprehensive morphological classification to date and is used, where appropriate, throughout this report. Bennet, Luft and Hampton's (1959) subclassification of capillaries will be

used in conjunction with Rhodin (1967, 1968). A schematic representation of the sequence of vessel types described by Rhodin (1967, 1968) is presented in Section 3, (3.7).

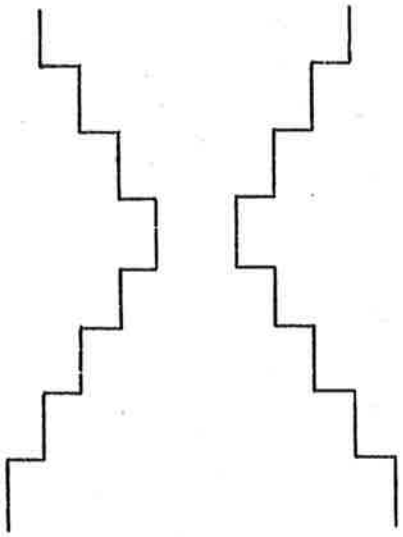
CLASSIFICATION AND ULTRASTRUCTURAL FEATURES OF THE MICROVASCULAR BED

The named sequence of vessels from the arterial to the venous end of the microvascular bed, including their approximate functional diameter as reported by Rhodin (1967, 1968) was as follows.

Arterioles,	100-50 μm diameter;
Terminal arterioles,	50-10 μm diameter;
Precapillary sphincters,	10 μm diameter;
Venous capillaries,	up to 8 μm diameter;
Postcapillary venules,	8-30 μm diameter;
Collecting venules,	30-50 μm diameter;
Muscular venules,	50-100 μm diameter;
Small collecting veins,	100-300 μm diameter;
Lymphatics,	(Diameter variable)

SCHEMATIC REPRESENTATION OF NAMED MICROVASCULAR TYPES

Representation of the sequence of terminology, afferent to efferent based on Rhodin (1967, 1968). Not to scale.



1. Arterioles
2. Terminal arterioles
3. Precapillary sphincters
4. Venous capillaries
5. Postcapillary venules
6. Collecting venules
7. Muscular venules
8. Small collecting veins

Note: a 1:1 ratio between types does not exist, Wiedeman (1963) published the most complete figures to date related to length, mean diameter and numbers of branches of individual named types.

SUMMARY OF CAPILLARY CLASSIFICATIONS

At this point it is appropriate to summarize the classification that has been used in this report.

Rhodin (1967, 1968) did not describe a specific vessel type that was appropriately served by the term "capillary" used in isolation. When dealing with capillaries Luft's (1973) concept has been used. Luft considered that any vessel possessing capillary type function should be called a capillary and that vessels having these functions were generally less than 20 μm in diameter. Therefore, superimposing Luft's concept over Rhodin's overall ultrastructural sequence leads to the conclusion that capillary function falls over the range of vessels described by Rhodin (1967, 1968) using the terms

a. terminal arteriole,
b. precapillary sphincter,
c. venous capillary,
d. postcapillary venule

This range of vessels fills the dual role of both conceptual and anatomical culmination of the vascular system alluded to by Majno (1965). Moreover, this conceptual 'capillary' may have an ultrastructure that varies from tissue to tissue as well as changing along the microvascular bed in the same tissue. Using Luft's (1973) concept of a 'capillary', the Bennet et al. (1959) subclassification has been used in conjunction with Rhodin's (1967, 1968) purely ultrastructural vascular sequence to

describe more adequately those vessels to which capillary status could be ascribed.

ULTRASTRUCTURE OF VARIOUS VESSEL TYPES (Rhodin 1967, 1968)

This project is essentially an ultrastructural study of periodontal blood vessels. Thus it is necessary that a thorough examination of current ultrastructural knowledge be undertaken. Even though in his introduction Rhodin (1967) considered his papers to be of a preliminary type a more complete work has not yet been published by him. Rhodin (1967, 1968) remains the authoritative tabulation of vascular ultrastructural features of microvessels in mammals. He considered a range of vessels from arterioles, through the microvascular bed, to small collecting veins. A schematic representation and approximate diameters was presented earlier in the literature review.

ARTERIOLES: approximate functional diameter 100-50 μm.

Tunica media:

There were approximately three layers of smooth muscle cells. There was a circular arrangement on the inner layer but there was a possibility of a helical arrangement in the outer layer of about 18^{o} pitch. All smooth muscle cells had a basement membrane approximately 800 Å thick. There were many perforations in the basement membrane with close contact between the plasma membrane of individual cells. The total thickness of the tunica media was approximately 5 μm.

Tunica intima:

The endothelium was very flat varying from about

2.0 μm at the nucleus and diminishing to a minimum thickness of about 0.15 μm away from the nucleus. There were no endothelial fenestrae. Individual endothelial cells were seen to overlap at the cellular junctions with the luminal overlap pointing downstream. There was a continuous basement membrane and internal elastic lamina. Both the basement membrane and internal elastic lamina were approximately 500 Å thick when the overall vascular diameter was near 100 μm. As the diameter decreased the internal elastic lamina became more patchy. The maximum space between the tunica intima and tunica media was approximately 0.3 to 0.5 μm. The tunica adventitia showed occasional fibroblasts and loose collagen. Nerves were often seen with all sizes of arterioles at a distance of approximately 5 μm from the media, they were not myelinated and had Schwann cells. As the diameter of the arterial vessels decreased toward precapillary sphincter size the number of nerves seen increased.

TERMINAL ARTERIOLES:

The functional diameter ranged from 50-10 μm.

Tunica media:

Usually only one layer of smooth muscle cells was seen and the average thickness of the tunica media was 0.5 to 1.0 μm. Each smooth muscle cell had a basement membrane and basement membrane contact between adjacent cells was common. Collagen in the tunica adventitia could be seen in close contact with the basement membrane of smooth muscle cells, sometimes enclosed by the tunica media

in a tunnel-like arrangement.

Tunica intima:

Endothelial junctions were seen to overlap, as in arterioles, with the luminal overlap pointing downstream. Many filaments were seen within the cytoplasm of endothelial cells. Rhodin considered that they were probably tonofibrils which may be the endo 'skeleton' of the cell. There were areas where the basement membranes of endothelium and the cells of the tunica media were deficient and processes of endothelial cells came into close contact with the smooth muscle cells; these were termed myoendothelial junctions. The basement membrane was intact but the internal elastic lamina was largely absent at this level. When the diameter of the vessel was below 30 μm myoendothelial junctions became more common. They were seen as membrane to membrane contacts approximately 0.5 to 0.1 μm in diameter (or long). There was usually a tight junction seen as part of the myoendothelial junction. The intermembrane gap at myoendothelial junctions was approximately 45 Å.

Tunica adventitia:

Occasional fibroblasts could be seen together with collagen fibres. These fibroblasts, or veil cells, had no basement membrane. Nerves were seen in the tunica adventitia. They were nonmyelinated and close to the tunica media, being about 1 μm from the smooth muscle cells. The axons were about 500 Å in diameter and there were occasional contacts between axons and smooth muscle cells.

PRECAPILLARY SPHINCTERS:

The functional diameter was about 10 μm. These vessels branched at right angles to the terminal arterioles and had a diameter of 10 to 15 μm : 50-100 μm down stream they had tapered to about 7 μm. There were no components of the elastic lamina remaining at this level.

Tunica media:

Bundles of circular smooth muscle cells were seen in a characteristic pattern, hence the term 'sphincter'. When the diameter diminished to about 7 μm the muscle coat was no longer apparent and this point was described as the arterial capillary.

Tunica intima:

At this level the endothelial cells were shorter, fatter and nuclei bulged into the lumen in a more pronounced manner. Myoendothelial junctions were more common and plasma membrane to membrane contact was longer, approximately 1 μm. 10-20% of the abluminal side was modified into myoendothelial junctions. Nerves were more common at this level than at any other. The miminum distance between nerve axons and the sphincter like smooth muscle cells was 45 Å.

Tunica adventitia:

Many fibroblasts were seen but collagen was scant.

ARTERIAL SIDE SUMMARY

The work of Rhodin (1967) was mainly based on the number of cell layers in the tunica media. The internal elastic lamina was most obvious at the arteriole level while

myoendothelial junctions and nerves were more numerous at the precapillary sphincter level. The term "arterial capillary" seemed to have been used to denote the transition of a vessel from precapillary sphincter type to the next downstream type rather than a separate type on its own. The cellular features of the tunica media were characteristic; electron dense bodies were more common than in other types of smooth muscle. Endothelial modifications at the precapillary sphincter level were felt by Rhodin (1967) to allow passive blocking of the lumen under the influence of the sphincter-like contraction of smooth muscle cells. Rhodin felt that there was no evidence to support the hypothesis that the endothelium can block the lumen by swelling or by active endothelial contraction. Rhodin thought that the myoendothelial junctions were most interesting. He hypothesized that the junction may be mechanical and/or physiological to allow diffusion of molecules, e.g., monoamines. In this way some endothelial cells may act as receptors for chemicals like adrenalin and noradrenalin. Control of the contractile elements of the tunica media may therefore be via myoneural junctions and myoendothelial junctions as outlined above.

Wolff (1977) considered that there was no firm delineation to be made between arterioles, terminal arterioles, precapillary sphincters and metarterioles "as far as they exist". Furthermore he concluded that arterioles (being used as a pan-term for the above structures) varied considerably from place to place. In

some areas elastic laminae were lacking whereas in others the muscle cell layer was incomplete. He considered the existence of metarterioles to be a matter of continuing doubt as adequate topographical control of specimens was lacking in most publications related to various aspects of metarterioles. Wolff (1977) cited Westergaard and Brightman (1973) as having demonstrated that in aterioles the endothelial layer was permeable to even large tracer molecules. Wolff found that perfusion with markers 20 minutes after the onset of perfusion fixation demonstrated that pinocytosis or vesiculation was not the mechanism involved. Therefore, he postulated, there must exist an open extracellular communication between the vessel lumen and the tunica media. This hypothesis would seem to be consistent with the results of Luft (1973) and his work with ruthenium red.

VENOUS CAPILLARIES:

Rhodin (1968) considered that the venous side of the circulation began with the venous capillaries. These structures basically consisted of an endothelial tube with a few highly branched pericytes and had a functional diameter of up to 8 µm.

Tunica intima:

Junctions, thought to be equivalent to myoendothelial junctions, were seen between pericytes and the abluminal surface of endothelial cells. The endothelium, as described, was non-specific and very flattened, except in the area of the nucleus. The nuclear bulge was directed abluminally and not into the lumen. The average

thickness of the cytoplasm was 0.3 μm down to a minimum of 0.04 μm. In Rhodin's specimen of rabbit thigh fascia occasional fenestrae were seen and all were closed with a diaphragm which had a central knob. Electron density of cytoplasm was increased due to rough endoplasmic reticulum and many longitudinal fine filaments. Membrane-bound granules were observed within the cytoplasm, these were mainly spheroidal but elongated forms were seen. The Golgi apparatus was limited to a small volume of cytoplasm near the nucleus.

PERIENDOTHELIAL CELLS:

The tunica media and tunica adventitia had no structural delineation at this level. Two main types of periendothelial cell were seen.

1. Pericytes: these cells had a complete basement membrane and many basement membrane to basement membrane contacts between pericytes and endothelial cells were seen. Pericytes were highly branched and it was proposed that in the smallest vessels pericyte processes might be difficult to detect.
2. Veil Cells: these cells were fibroblasts and as such did not have a complete basement membrane.

There was an even distribution of intracellular organelles in the cytoplasm of pericytes. The number of mitochondria was moderate. The Golgi apparatus was prominent as were large numbers of free ribosomes. Membrane-bound granules and micropinocytotic vesicles were more apparent near the

adventitial plasma membrane than elsewhere in the cell. Fine filaments were widely scattered and small electron dense areas were seen to occur in the cytoplasm near the plasma membrane on the surface adjacent to the endothelial cells.

POSTCAPILLARY VENULES:

These vessels had a diameter ranging from 8 to 30 μm and were a direct continuation of the venous capillary. The degree of completion of the periendothelial envelope of pericytes and the number of veil cells were seen to increase.

Tunica intima:

The endothelial cells were larger than in venous capillaries and somewhat thicker, the average thickness being more than 0.4 μm. Adjoining endothelial cells overlapped and there were tight junctions between them. Endothelial processes were seen on the abluminal side but it was thought possible that they merely contacted pericyte processes out of field. As the diameter of the vessel increased toward 30 μm the pericyte processes overlapped and therefore provided a complete periendothelial cellular layer. Where there were no periendothelial cells there was contact between the adventitia and abluminal endothelial basement membrane. There were frequent contacts between the plasma membranes of pericytes and endothelial cells but not between pericytes themselves.

Tunica adventitia:

The number of veil cells was relatively low when

the diameter was small but as the diameter increased so did the number of veil cells. Many collagen fibrils were present. They were never collected in bundles but occasionally were seen between pericytes and endothelial cells. Together with pericytes they formed a sort of collar or sphincter arrangement around the postcapillary venules.

COLLECTING VENULES:

The functional diameter of these vessels varied between 30 and 50 μm.

At this level the pericyte layer was complete as was the veil cell layer. A new type of periendothelial cell occurred. Rhodin (1968) termed it a primitive smooth muscle cell. As the diameter of the venule approached 50 μm it was apparent that they were nearly fully differentiated smooth muscle cells. It seemed that a progressive transition from pericytes to smooth muscle cells had occurred. Primitive smooth muscle cells had a nearly complete basement membrane compared with pericytes which seemed to share a basement membrane with endothelial cells. It was thought that the basement membrane may be important in the differentiation of primitive smooth muscle cells. Contacts between primitive smooth muscle cells were common, perforating their respective basement membranes. Rhodin felt that probably the primitive smooth muscle cells were differentiated from the outer layer of pericytes. At this stage, veil cells formed a thin, single layer with associated collagen fibrils.

MUSCULAR VENULES:

Tunica media:

At a diameter of 50 μm it was obvious that the tunica media comprised well differentiated smooth muscle cells with a continuous layer of at least one cell. As the diameter increased, the number of cell layers in the tunica media increased. The tunica intima had a thickness of about 0.4 μm and there were no particular distinguishing characteristics.

The smooth muscle cells were in a spiral arrangement of which the pitch varied. Smooth muscle cells had a modest number of myofilaments. Electron dense bodies were seen free in the cytoplasm. Organelle distribution varied between pericytes and smooth muscle cells. Rhodin thought that this might indicate a stage of transition between the two cell types. There were many scattered micropinocytotic vesicles. Myoendothelial junctions were also present.

Tunica adventitia:

Veil cells and collagen were seen. The cells were quite thin, about 0.2 μm thick and very wide. The Golgi apparatus was very pronounced as was the number of mitochondria and amount of rough endoplasmic reticulum. There was no basement membrane on the adventitial aspect of the veil cell.

SMALL COLLECTING VEINS:

These vessels ranged in diameter between 100 and 300 μm.

Tunica media:

When the diameter of the vessel was between 100

and 200 μm, two or three layers of well differentiated smooth muscle cells were seen. As the diameter increased to between 200 and 300 μm more than three layers of muscle cells were seen. No valves analogous to those known in larger, named veins were seen. The smooth muscle was of the standard type with a large number of myofilaments. There was a continuous basement membrane, although several cells might have been in the same basement membrane envelope. At the junction between muscular venules and small collecting veins a sphincter-like arrangement of smooth muscle cells occurred. Myoendothelial junctions occurred but they were of a different type; long and flat, parallel to endothelium with many individual membrane to membrane contacts.

Tunica intima:

Many membrane-bound granules were seen in the endothelial cytoplasm. The average diameter of these granules was 0.35 μm. The matrix within the granules varied in density.

Tunica adventitia:

Two layers of veil cells were seen and the collagen became more organised. Two layers were demonstrated, one parallel to the vessel and the other at right angles to it. Collagen was abundant between the smooth muscle cells both as individual fibrils and continuous layers. As the diameter approached 300 μm an elastic lamina appeared. Nerves were seen which followed the vein at the level of veil cells although they were seen to approach no closer than 5-10 μm.

ULTRASTRUCTURAL SUMMARY

Rhodin (1962a) summarized in anticipation some of his later findings.

Tunica intima:

There was general uniformity at the noncapillary level but at the capillary level differences were apparent. Endothelial cells were cuboidal in the contracted state and elongated when the vessel was observed in a distended state. Fine filaments were seen. It was considered that these may be contractile or elastic organelles. Mitochondria and rough endoplasmic reticulum were also seen but were not abundant. This might indicate that energy requirements were fairly low for this cell type and that the limited protein production was not for extracellular use. The surface of endothelial cells was usually smooth with some microvilli.

Tunica media:

Muscle cells were seen to be arranged helically and the pitch of the helix varied. There was a clearly defined plasma membrane and basement membrane. Micropinocytotic vesicles were common but less so than in other types of smooth muscle, e.g., visceral. Smooth endoplasmic reticulum was scarce but rough endoplasmic reticulum was common. Rhodin (1962a) concluded that vascular smooth muscle had more rough endoplasmic reticulum than did visceral smooth muscle.

Tunica adventitia:

Elastic fibres were rare in the tunica adventitia but abundant in the tunica media. Fibroblasts had abundant

rough endoplasmic reticulum and many free ribosomes, thus it was concluded that protein production was essentially for extracellular use e.g., collagen precursors.

BASEMENT MEMBRANES

Rhodin (1967, 1968) summarised the relationship between basement membranes and various cell types. Basement membranes were separated from cell plasma membranes by an electron-lucent layer. Basement membranes were always present with endothelial cells and smooth muscle. However several muscle cells could be within the same envelope of basement membrane. Fibroblasts and veil cells, which were modified fibroblasts, had no continuous basement membranes.

On a developmental basis Cliff (1976) considered that basement membranes were formed as a result of some interaction between endothelial cells and elements of the adventitia rather than a product of the endothelium alone.

Pericytes had a complete basement membrane and seemed to share the basement membrane of endothelial cells at endothelial - pericyte interfaces.

VASCULAR INNERVATION

The relationship of nerves to the microvasculature has been covered in reasonable depth by Rhodin (1967, 1968). There appears to be general agreement regarding the innervation of smooth muscle cells in the media and the relationship of nerves in the adventitia of vessels. Rhodin made no mention of innervation of the intima but Cliff (1976) citing Abraham (1969) stated

categorically that no nerves were found in the intima of vessels.

In summary then, the classification of vessels is most commonly based on the following criteria:

1. vascular diameter,
2. perivascular elements,
3. relation to the vascular system as a whole.

CONTENTS OF ASSOCIATED CELLS

Rhodin (1967, 1968) used cellular contents and form to augment identification of various cell types. This is represented in Table 1, p3.23.

Rhodin (1968) proposed various potential functions of pericytes.

1. Synthesis: The production of its own basement membrane and perhaps the basement membrane of the endothelial cells as well.
2. Mechanical support: To provide support, for example, against the intramural pressure.
3. Protection: They might help to prevent extravasation of the streaming elements of blood into interstitial tissues.
4. Detection: They might act as a sensor of damage to the endothelium.
5. Differentiation: Pericytes may act as a cell pool from which various cell lines may

CELL TYPE	CYTOPLASMIC BRANCHING	BASEMENT MEMBRANE	ROUGH ENDO-PLASMIC RETICULUM	FILAMENTS	FUSIFORM DENSE BODIES	DENSE GRANULES	MICRO PINOCYTOTIC VESICLES	RIBOSOMES
ENDOTHELIUM	0	+	+	+	0	+	4+	2+
PERICYTE	4+	+	2+	+	+	2+	4+	2+
PRIMITIVE SMOOTH MUSCLE	+	+	+	2+	2+	+	2+	+
SMOOTH MUSCLE	+	+	0.5+	4+	4+	+	4+	0.5+
VEIL CELL	2+	0	4+	0	0	+	0	0.5+

Table 1

Frequency of occurrence of various ultrastructural features modified from Rhodin (1968).

Table legend: relative frequency of occurrence

0 - Zero
0.5+ - very few
\+ - few
2+ - moderate
4+ - abundant

differentiate as circumstances demand.

6. Contractile: Capillary contraction may be due to pericytes or intracellular endothelial filaments.

THE MICROVASCULAR MEMBRANE

The 'conceptual' and 'anatomical' culmination of the entire circulatory system is deserving of extremely close scrutiny. Majno (1965) and Luft (1973) have both published excellent reviews. Majno considered capillaries to be vessels with a diameter of 7 to 50 μm. Venules, having a diameter ranging from 10-100 μm were regarded as a separate system. Luft considered all vessels of diameter less than 20 μm to be functionally included. Majno differentiated capillaries from venules as follows.

1. Venules were more permeable,
2. more responsive to mediators,
3. more susceptible to injury.

However, all three factors have much in common and it is felt that the differentiation scheme used by Majno is unwieldy whereas Luft's simpler concept is more useful from a practical point of view. Luft considered that at this level the barriers were more important than the compartments.

The endothelium of continuous capillaries was about 0.2 μm thick with a bulge of about 2-3 μm at the nucleus.

Intracellular junctions had a gap of about

100-150 Å, and the contents of this gap have been the object of study. Luft (1973) has shown that the dye ruthenium red permeated and stained the contents of this gap. Ruthenium red is a stain which is specific for acidic proteoglycans and 'pectin' like substances. Also shown consistently were endocapillary sheaths or 'fuzz layers', the significance of these structures is yet to be explored. However, Majno and Luft both considered that the intercellular 'tight junction' is in fact one of the principal areas for rapid micromolecular exchange.

Basement membrane material was thought at one stage to be mucopolysaccharide but recent work has shown it to be predominantly collagen (Casley-Smith, 1977b). Luft (1973) considered that the basement membrane acted as a coarse filter, particles up to 200 Å in diameter being allowed relatively unimpeded progress, thus he considered it to be a functional component of the endothelial cell.

Luft (1973) considered that, when first described, the endothelial basement membrane was "almost certainly derived from pericytes" now it is thought, Luft reported, that the endothelial cells make their own.

Majno (1965) described intraluminal flaps and psuedopodia, commonly near 'tight junctions' and considered that they might have a role in pinocytosis.

Pericytes have undergone intensive study. Their presence is probably ubiquitous although perhaps not always seen due to their highly branched nature. Pericytes were first described by Zimmerman (1923) and not much has been

added to the description of their basic morphology since then. Majno's review showed that they are now considered to be phagocytic and probably act as 'support' cells for the endothelium. Luft (1973) considered that they were not active in capillary permeability. Majno highlighted the structural similarities between smooth muscle cells and endothelial cells; pericytes were described as structurally between the two. Majno (1965) has described how pericytes vary from place to place. Capillary pericytes for example are not exactly equivalent to venular pericytes. The relationship between perivascular cells described by Majno (1965) and Luft (1973) and the primitive smooth muscle cells described by Rhodin (1968) is, as yet, unclear.

Where intracellular fenestrae were seen the endothelium was thinner, sometimes as low as 20-40nm. In capillaries exhibiting fenestrae the diameter of fenestrae ranged up to 0.1 μm with a diaphragm and central knob (Majno, 1965). Rhodin (1967, 1968) considered that with the exception of capillaries in the renal glomerulus probably all fenestrae were closed by such a diaphragm. Majno (1965) considered that fenestrated capillaries were modified for the rapid exchange of fluids and solute. The nature of the diaphragm is unknown. However, it is NOT two adjacent trilaminar unit membranes. If it were its thickness would be of the order of 120 Å and in fact it is about 60 Å thick. The diaphragm was covered by the endocapillary sheath or 'fuzz layer' which was thought by Luft (1973) to be a macromolecular ultra-filter.

Fenestrae may or may not be regular in size, shape and distribution. Fenestrae are characteristic of specialised organs, for example endocrine glands and gut capillaries, but are found in non-specific areas (Rhodin, 1968 and Casley-Smith 1971). The high incidence in certain tissues was thought to be related to macromolecular movement across the endothelial wall. It has been shown that fenestrae are capable of differential permeability and (Casley-Smith 1977a) also showed that the possession or not of a diaphragm was not necessary for many proposed functional features of fenestrae. Casley-Smith (1977a) felt that fenestral features were not particularly critical to micromolecular transport across the endothelium. Micromolecular penetration extravascularly was thought to be more a feature of interstitial tissues than fenestrae.

Casley-Smith (1971) reported that the number of fenestrae at the venous end of the capillary was much greater than the arterial end and he considered it possible that fenestrae were the mechanically inferred large pore of the Starling hypothesis, although Luft (1973) considered that the distinction probably went to the intercellular 'tight' junction.

Wolff (1977) considered that fenestrae were not static structures as it was known that they could proliferate under the influence of inflammatory or hormonal stimuli and also by factors produced by the epithelial cells. Although usually closed by a diaphragm, Wolff proposed that fenestrae were permeable to large amounts of solute and fluids as well as larger molecules such as

ferritin. Casley-Smith (1977a) has proposed models of fenestral function in which the diaphragm becomes, conceptually at least, less than critical. Cliff (1976, citing Karrer 1960a) considered that fenestrae were probably features of vesicle function. There seems to be reasonable concurrence on the opinion that fenestrae are confined to "capillaries" and transitional forms between capillaries and venules, (Rhodin 1968, Cliff 1976).

Majno (1965) considered that there were four potential pathways across the vascular membrane:

1. direct - pinocytosis
   - fenestrae
   - intercellular 'tight' junctions,
2. basement membrane,
3. pericytes,
4. perivascular structures.

Wolff (1977) might consider this list inadequate. He mentioned the existence of a fourth direct path. He called this endothelial modification a "transendothelial hole" as a short term example and more long lasting types "transendothelial channels". He thought that these features along with some types of fenestrae were due to vesicularisation effects.

LYMPHATICS

The material related to lymphatics is not abundant. Majno (1965) described them as non-fenestrated with a diameter of up to 14 μm, having very thin endothelium with a pronounced nuclear bulge. Endothelial flaps (microvilli or psuedopodia) were long and thin and located

mainly near interendothelial junctions. The basement membrane was less conspicuous and more irregular. Casley-Smith (1973) described them as similar to the blood vascular system from the venous capillaries onward. They were seen as usually collapsed and having no fenestrae, however vesicles were seen. Intercellular tight junctions were seen and proportionally more were wider open than in the blood vascular system. With the transmission electron microscope they were seen to have fewer erythrocytes, less plasma protein and to be generally hard to tell apart from blood vessels unless lymphatic markers had been used and even these were regrettably fallible (Casley-Smith, 1973).

PERIODONTAL VASCULATURE

TECHNIQUES

As stated previously, the periodontal ligament is studied for a variety of reasons and many passing observations of vascular structures have been made. A variety of investigational techniques have been used in studies where vascular structures have been alluded to. The techniques used include:

1. infusion,
2. perfusion,
3. numerical,
4. microscopy, a) light
   b) electron i) scanning
      ii) transmission.

1. INFUSION

Various infusion techniques have been used. Usually, arterial or venous infusion is followed by a corrosive cast technique to leave the vascular bed as a three dimensional cast of the lumina.

Most productive in this field have been Kindlova and Matena (1959 and 1962), Castelli (1963), Kinlova (1965, 1968 and 1970), Castelli and Dempster (1965).

This work has been done on various laboratory animals including rats, hamsters and monkeys.

Microsphere infusion has been used both as a marker of vascular networks and in an attempt to produce ischaemia. Folke and Stallard (1967) used plastic microspheres of 15 ± 5 μm in diameter. Vandersall and Zander (1967), used 35 ± 5 μm spheres. The aim in both of these

studies was evaluation of tissue sections with light microscopy.

2. PERFUSION

Boyer and Neptune (1962) perfused with potassium dichromate and lead acetate producing a precipitate of lead dichromate. This technique did not involve large intraluminal pressures because of the low viscosity of the solutions and thus overextension and extravasation were kept to a minimum. Rather than producing a corrosion cast, the material was sectioned for light microscopic examination, the precipitate acting as a vascular marker.

3. NUMERICAL

Birn (1966) produced an impression of the wall of the bony socket and used the number of perforations as an index of vascularity to compare different sockets and different areas within the same socket.

4. MICROSCOPY

a) Light: Very brief references to vascular structures using light microscopy are legion. Cohen (1960) is typical and very limited with respect to the microvascular bed. Other studies have attempted to correlate light microscopic with electron microscopic findings such as Rygh (1972 and 1976) and Sheetz, Fullmer and Narkates (1973).

Sims (1975 and 1976) has used light microscopy together with relatively specific staining techniques to show oxytalan fibres. The relationship shown between 'vascular elements' and the termination and intraligamentous mesh of these fibres in the periodontium will be

referred to when dealing with hypotheses relating structure to function.

b) Electron: Two basic electron microscope techniques have yielded material and observations on the vascular elements of the periodontium.

i) Scanning Electron Microscopy (S.E.M.)

The S.E.M. allows observation of the pattern of reflected electrons when striking the heavy-metal plated surface of the object to the considered. Therefore, it is purely a topographical observation. S.E.M., does however have the advantage that the specimen is entire rather than sectioned and its orientation to the beam can be changed. Both of these features can be reflected in the image produced. Svejda and Skach (1973) and Roberts and Chamberlain (1976) both refer to vascular findings with the S.E.M.

ii) Transmission Electron Microscopy (T.E.M.)

The T.E.M. allows detailed intracellular and extracellular observations to be made. References to vascular elements observed with the T.E.M. have arisen in studies where the specimens were of scrapings of periodontal ligament thus the true anatomical relations were not seen in full context. Griffin and Harris (1974) and Harris and Griffin (1974) observed such material while investigating the innervation of human periodontium. Rygh (1972, 1973 and 1976) recorded observations of vascular structures when considering ultrastructural changes in the periodontium due to orthodontic forces transmitted to it by the teeth. Corpron, Avery, Morawa and Lee (1976) recorded very brief observations of fenestrated capillaries seen in mouse

periodontium. However, very few details of materials and methods were recorded so exactly what they were looking at and how it was processed is unclear.

Avery, Corpron, Lee and Morawa (1975) recorded observations and some details of microvascular ultrastructure of periodontal ligament of mice.

Sheetz, Fullmer and Narkates (1973) in an attempt to correlate the light microscope and electron microscopic picture of oxytalan fibres recorded relationships between those fibres and unspecified blood vessels.

Carranza, Itoiz, Cabrini and Doho (1966) marked periodontal blood vessels using an adenosinetriphosphatase labelling procedure. The results were analysed with light microscopy, demonstrating only limited anastomosis between the gingival and ligamentous circulation.

GENERAL ANATOMY

Saunders and Rockert (1967), in their excellent review summed up knowledge thoroughly and comments on their material will precede some more specific observations reported subsequently. They observed that very few changes had been made to Hayashi's (1932) report. The general pattern remained as follows.

Alveolar branches of the named dental arteries gave off branches that passed intraosseously toward the alveolar crest. At various levels, from apical to coronal, lateral branches were given off. These 'perforating' arteries then passed through the bony wall of the socket and gave rise to a network of intraligamentous vessels orientated approximately parallel with the long axis of the

tooth.

Prior to entering the apical foramina of the teeth, the pulpal vessels gave rise to branches which arranged themselves similarly to, and anastomosed with, the networks of the 'perforating' arteries. Probably there was no functional differentiation to be made between 'pulpal' and 'perforating' arteries. Anastomosis between the vascular beds of crestal periodontal vessels was a matter of continuing contention and apart from the extreme inter-dental area the vascular beds appeared to be functionally distinct and largely unconnected. On the more microscopic aspects Saunders and Rockert (1967) highlighted our general ignorance of ultrastructural aspects of the terminal periodontal vasculature. Vessels were described as lying in areas of loose connective tissue between the principal fibre bundles.

The glomerulus-like 'complex vascular cells' were first described by Wedl (1881) and have been mentioned regularly since then. These entities were thought by Ishimitsu (1960) to be arteriovenous anastomoses whose prime role was the regulation of periodontal blood flow. Emphasis was placed upon the specialised function of the periodontal ligament and the possible role of vascular elements with respect to that specialised function. Saunders and Rockert (1967) cited the problems of trans-mission electron microscope work in this area as:

1. tissue destruction during decalcification,
2. specimen embedding,
3. section cutting.

The relationship between these problems and this project will be considered in the section dealing with materials and methods and also in discussion.

The more recent observations on the subject will be considered under the following headings:

1. Embryology.
2. Intraosseous arteries.
3. Perforating arteries.
4. Apical arteries.
5. Intraligamentous arteries.
6. Microvascular bed of the periodontal ligament.
7. Venous drainage.

EMBRYOLOGY

Kindlova (1968) has determined that the vascular bed of the periodontium, which began to form with the roots of the developing tooth, was complete before eruption of the tooth and erupted with it. Blood supply to the epithelial attachment area developed de novo with the enamel organ and also erupted with the tooth.

INTRAOSSEOUS ARTERIES

Boyer and Neptune (1962) described the principal periodontal ligament supply as arising from the medullary intraosseous branches of named dentoalveolar arteries.

Carranza et al. (1966) described these medullary vessels as particularly important in the mid and apical thirds of the alveolar dental socket. Castelli (1963) described the medullary course of the afferent vessels as 'sinuous' and mentioned that they were most important in the apical two thirds of the root. Svejda and Skach (1973)

recorded that the bony channels, in which the medullary vessels ran, were lined with compact bone.

PERFORATING ARTERIES

The conclusion by Carranza et al. (1966) and Castelli (1963) that the perforating arterial branches of the intraosseous vessels are most numerous in the mid and apical thirds and thus by inference least important in the coronal third was not supported by Hayashi (1932) or Birn (1966). Equating the number of perforations found with the number of perforating vessels using an impression technique, Birn found that the greatest number of perforations occurred, in descending order, in the:

1. coronal third,
2. apical third,
3. middle third.

Birn found no significant differences between the various faces of the socket. Generally the blood supply increased from anterior to posterior. The diameter of the perforations ranged about 150 μm. Perforations with a diameter less than 150 μm were more numerous than perforations with a diameter greater than 150 μm. Carranza et al. (1966) found that the perforating arteries branched off medullary arteries at about right angles and passed into the ligament space where they divided and rearranged in a plexus, again, approximately parallel to the long axis of the tooth.

Castelli (1963) found that about 12 alveolar arteries branched from the named arteries each with a diameter of approximately 280 μm. These arteries supplied

the alveolar bone, dental pulp and periodontal ligament. Folke and Stallard (1967) considered that perforating arteries which were seen to run parallel to Sharpey's fibres were a 'universal' finding.

Vandersall and Zander (1967) attempted experimental obstruction using plastic microspheres the diameter of which ranged from 35±5 μm. The smallest number of spheres was found in the periodontal ligament. They felt that 35±5 μm might therefore be regarded as close to the upper limit of lumen diameter of perforating vessels entering the ligament space.

APICAL ARTERIES

It is probable that dividing the blood supply into perforating and apical arteries is unnecessary. Kindlova (1965) suggested that apical and perforating arteries anastomosed freely in the intraligamentous portion and therefore apical arteries were best considered apical perforating arteries.

INTRALIGAMENTOUS ARTERIES

There is general agreement that once in the ligament space the perforating arteries form a plexus of vessels parallelling the long axis of the tooth. Folke and Stallard (1967) were unable to verify the existence of intraligamentous apico-gingival arteries although this may be due to the diameter of the microspheres that they used, i.e. 15±5 μm.

The orientation of this plexus is an area of some controversy. Sims (1976) described in the coronal aspect of human premolar periodontiums an essentially central

location of the larger vessels. Castelli and Dempster (1965) recorded that the capillary plexus was close to cement and the mean diameter of vessels was 9-10 μm. The bulk of workers, Kindlova (1965), Carranza et al. (1966), Roberts and Chamberlain (1976), Folke and Stallard (1967) and Rygh (1972, 1973), observed that the plexiform arrangement was closer to bone than to tooth. Cohen (1960) described vessels that lay in grooves in the wall of the socket. This issue is not necessarily contentious as it may be that the afferent plexus is orientated toward the bone and the microvascular bed is projected toward the cement from it.

Sims (1975, 1976) described the orientation of oxytalan fibres with respect to this vascular plexiform arrangement. The anatomical relationship suggests a functional relationship between teeth and blood vessels via oxytalan fibres.

MICROVASCULAR BED OF THE PERIODONTAL LIGAMENT

At this level the material becomes very patchy. Most workers agree that the microvascular bed is more tooth orientated than the arteriolar plexus. At the extreme crestal margin of the bony socket however, contention exists. Kindlova and Matena (1962) and Kindlova (1965, 1970) described a 'circulus' arrangement of the terminal crestal portions of the arteriolar network as against the longitudinally orientated arterioles more apically situated. From this circulus, capillary loops were given off to supply all marginal gingiva except for the interdental 'Col' region. The interdental 'Col' drew its supply from

the crestal arteries which were themselves terminal branches of the medullary or intraosseous arteries.

Folke and Stallard (1967) however, described the capillary loops as rising in periosteal vessels and from arteries in attached gingiva. Gavin and Trotter (1968) described the capillaries in marginal gingiva. According to Bennet et al. (1959) these vessels would be described as follows:

90% type A - -

10% type B - -

and:

97% type - 1 -

3% type - 2 -

This matter will not be dwelt upon as no marginal material is included in this study.

Avery et al. (1975) and Corpron et al. (1976) described some features of periodontal 'capillaries'. Both reported finger-like projections into the lumen. Bennet et al. (1959) type A - - capillaries were reported and also Bennet et al. (1959) type - - α capillaries. Avery et al. (1975) reported finding no fenestrae in their material. Corpron et al. (1976) reported central capillaries, Bennet et al. (1959) type - 1 - and peripheral capillaries, Bennet et al. (1959) type - 2 - . Both studies reported occasional myoendothelial junctions although Avery et al. (1975) referred to them as myoepithelial junctions, but it is presumed that they are the same entity. All fenestrae reported by Corpron et al. (1976) were closed by a diaphragm. 'Precapillary' vessels

were described in both studies as having an incomplete tunica media enclosed in its own basement membrane and separated from the endothelial cells by a basement membrane. These may be equivalent to the primitive smooth muscle cells described by Rhodin (1967 and 1968). Harris and Griffin (1974) described an encapsulated metarteriole giving rise to a capillary plexus which was then drained by small collecting venules at the periphery of the 'compound mechanoreceptor' described by Griffin and Harris (1974). Castelli (1963) concluded that the venous drainage did not parallel the arterial supply. Castelli and Dempster (1965) described two principal avenues of egress from the ligament space.

1. Through the alveolar wall where they joined the medullary plexus (central septal venous plexus). This observation was supported by Kindlova and Matena (1962).
2. Toward the apex of the tooth linking up with pulpal veins. Kindlova and Matena (1962) described an intraligamentous apical plexus of veins. Presumably then the named veins were joined to complete the arteriovenous circuit.

HYPOTHESES

Many hypotheses relating structure to function have been proposed.

The existence of a fluid phase within the periodontal ligament has given many workers the idea that some of the characteristics of the periodontium are those of a hydraulic damper. Bien (1966) postulated that tension in

the principal fibre bundles caused constriction of the periodontal vessels. These closed-off pools of blood would then be acted upon by the pressure exerted via the root of the tooth. Absorption of the pressure was theoretically possible by the constrictions acting as restriction valves. While it is uniformly agreed that the larger vessels in the periodontium occupy tissue spaces between the principal fibre bundles, Bien's hypothesis was offered largely as a model of function without any supporting anatomical evidence. Bien's simple model of function involving a single Maxwell unit was contradicted by the work of Wills, Picton and Davies (1972). Their experimental work in monkeys suggested that rather than the single Maxwell unit proposed by Bien (1966) a more complicated unit involving certainly 3 and possibly 5 Voigt units in series. Wills et al. found that the response was not linear. An early rapid change was followed by a later 'creep' change. They also suggested that at low forces e.g. 0.5N, the periodontal ligament accommodated the change by tooth movement. At higher forces, 5 N and above, an element of alveolar accommodation was observed.

(Note: Maxwell and Voigt units are mechanical models designed to explain variation in responses of systems to loads; the most elementary such unit is the Hookean spring).

Bien and Ayers (1965) subjected rat incisors to loads and observed the recovery of the teeth from the displaced positions adopted under the load. By timing intervals between loading the teeth and killing the experimental

animals it was shown that the functioning vascular system played a substantial part in the recovery of the teeth from the displaced positions adopted under load.

Cohen (1960) described vessels in the ligament space running longitudinally in 'grooves' in the socket wall and postulated that the bony alcove would protect blood vessels from physical impingement. Such a system would not necessarily protect the vessel from hydraulic pressures and indeed could make vessels more subject to hydraulic forces.

Gavin and Trotter (1968) postulated that due to the low numbers of fenestrae observed, fenestrae were not responsible for changes in periodontal capillary permeability in the case of the appearance of plasma proteins in the crevicular fluid. Kindlova and Matena (1962) felt that the complex marginal periodontal blood flow was not solely nutritive in function.

Griffin and Harris (1974) and Harris and Griffin (1974) postulated that the relationship between vascular and neural elements in compound mechanoreceptors allowed a function analogous to a periodontal baroreceptor. This allows some inference of a reflex central connection feeding into the neuromuscular reflex control of muscles of mastication.

Provenza et al. (1960) felt that 'glomerulus'-like structures were purely a compensatory development to offset a diminished vascular supply in some pathological conditions, for example periodontosis. Saunders and Rockert (1967) considered that these structures may be

arteriovenous shunts providing for functional variation in periodontal blood flow under central or peripheral neurological control.

Rygh (1976) felt that vascular changes seen in orthodontically moved teeth were compensatory mechanisms. Some very characteristic changes to erythrocyte anatomy were shown to occur within the lumen of the vessels on the compression side.

Bien (1966) proposed that in function periodontal blood vessels are strangulated by the principal fibre bundles. Sims (1976) considers that with the exception of a small area near the apex, since vascular occlusion does not occur under heavy occlusal loads even in lathyritic animals, the oxytalan-vascular relations seen may not be involved in maintaining vascular patency. Vascular-oxytalan function may be to allow for rapid topographic differentiation of vascular flow according to the physiological requirements under varying functional circumstances. A highly specific relationship between oxytalan fibres and vascular elements in the periodontal membrane has been proposed.

SECTION 4

MATERIALS AND METHODS

SAMPLE: Human subjects and site selection

The patients from whom the specimens were taken were white Caucasian adolescents aged from 11 years to 15 years.

Because of the necessity for extractions to facilitate orthodontic treatment the specimens, which consisted of tooth, periodontal ligament and bone, were taken in conjunction with these planned extractions. All teeth were fully erupted and functionally in occlusion.

No specimens had been involved with orthodontic tooth movement prior to the taking of the specimen.

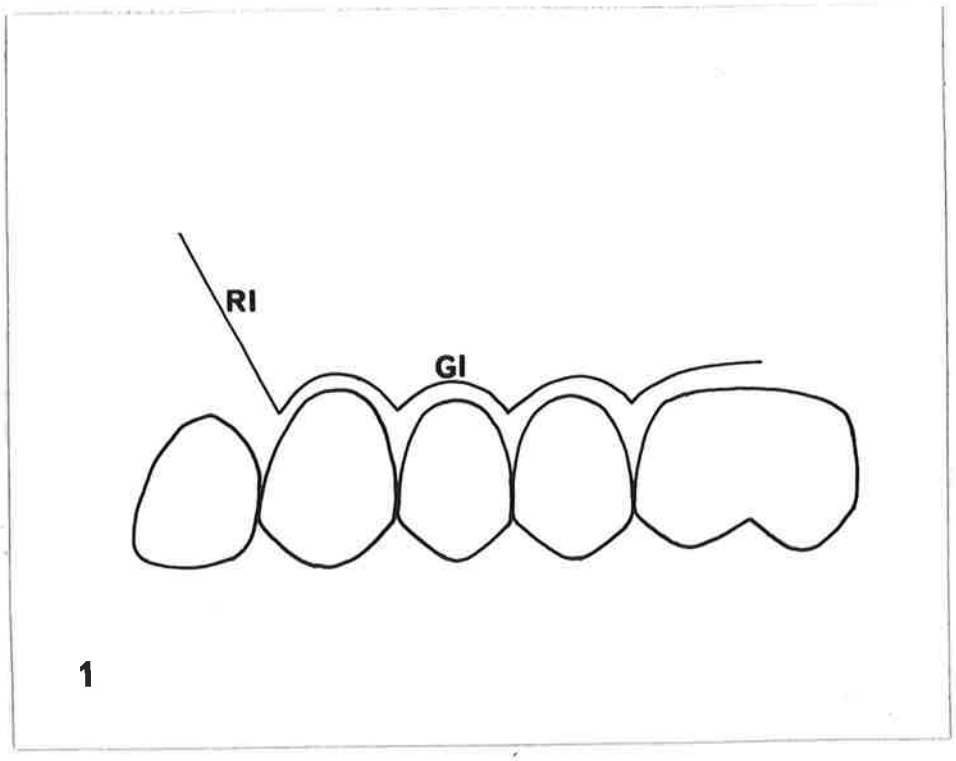
SURGICAL TECHNIQUE

The specimens were removed under local anaesthesia using 4.4 mls of 2% lignocaine hydrochloride incorporating a vasoconstrictor, 1:80.10^3 adrenalin (xlyocaine, Astra). This was administered in three 1 ml increments, extending from the lateral incisor to the first permanent molar, in the appropriate dental quadrant. A further increment was added to the palatal mucosa adjacent to the site of operation. An incision, using a Swann-Morton number 15 scalpel blade in a Bard-Parker number 3 handle, was made through the gingival crevice to the crestal bone of the alveolar process from the mid-buccal aspect of the first permanent molar around the necks of all teeth to the mesial aspect of the canine tooth. A relieving incision was then made

obliquely into the vestibular mucosa (Fig.1). A full thickness two-sided mucoperiosteal flap was then raised exposing the buccal aspect of the maxillary alveolus (Fig.2).

Under a normal saline irrigation the piece of buccal plate and periodontal ligament to be incorporated into the specimen was outlined with a small rosehead bur in a straight handpiece. Care was taken that the specimen did not dry out and that the cuts went fully through the buccal plate into the substance of the root of the tooth.

The root(s) were then sectioned in a buccolingual direction, approximately parallel with the occlusal plane, also under copious saline irrigation (Fig.2). Using a Warwick James elevator the crown, coronal two-thirds of the root(s) and attached buccal plate of bone were elevated swiftly from the alveolar process and placed in the primary fixative (Fig.3). The total elapsed time from the severing of all vessels to the portion of the periodontal ligament retained in the specimen until fixation was in no case longer than four minutes. The time from elevation until immersed in the primary fixative was no more than 20 seconds. A minor surgical procedure was then performed to remove the retained apical segment(s) and thus render the extraction complete. The bony surfaces were smoothed at the junction of the cut surface and the anatomical periosteal surface and the mucoperiosteal flap replaced. Flaps were sutured with 000 braided black silk on swaged atraumatic needles. In all patients healing was prompt and very satisfactory. A residual bony deficiency was not



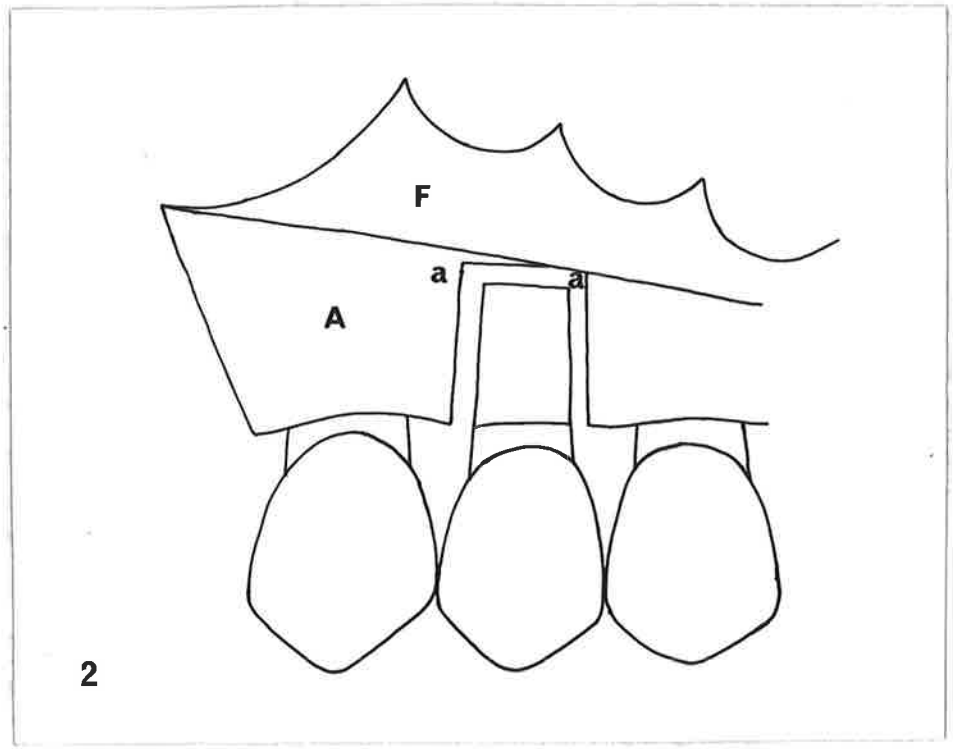


Fig. 1. Surgical technique: Relieving incision (RI), gingival incision (GI).

Fig. 2. Surgical technique: Sectioning plane (a-a), mucoperiosteal flap (F), alveolar bone (A).

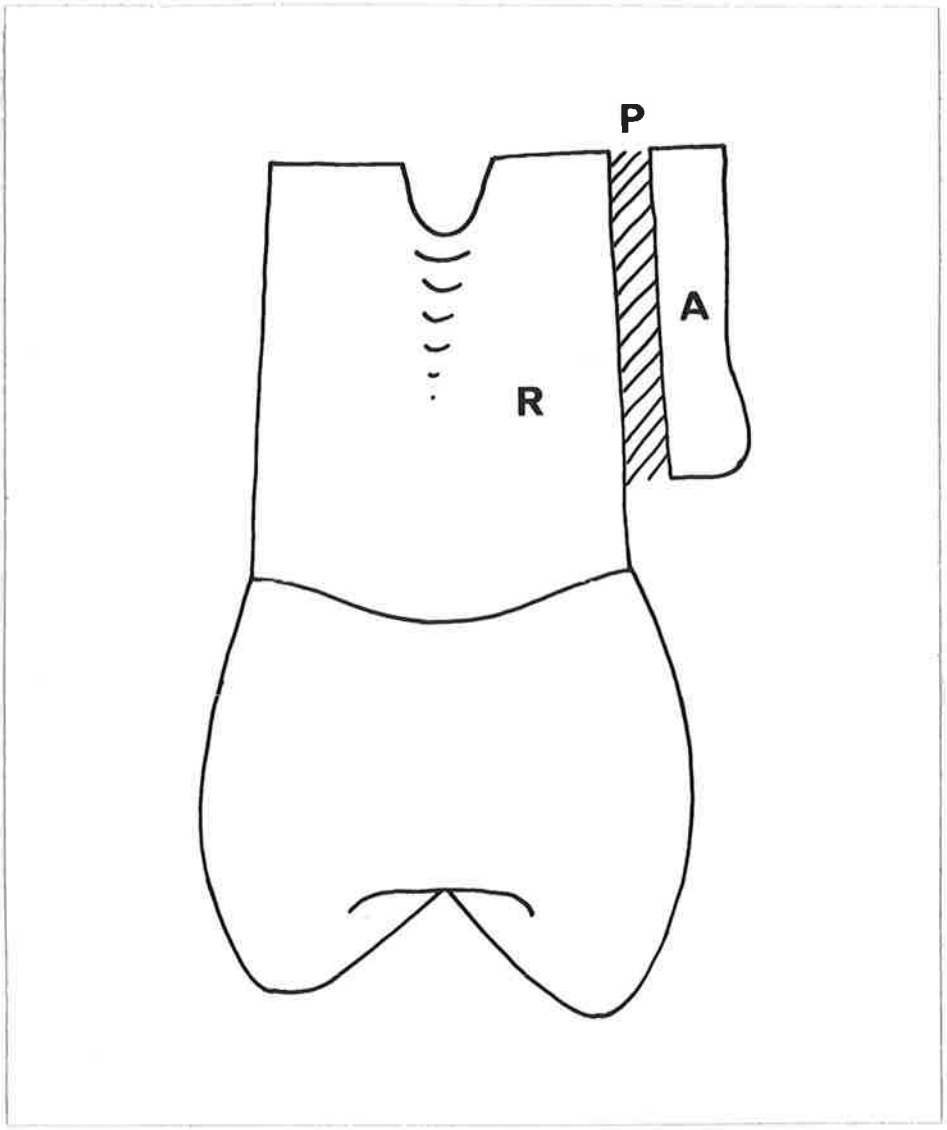


Fig. 3.

Specimen after elevation: Alveolar bone (A), periodontal ligament (P), tooth root (R).

observed in any patient other than that which would have been expected from a simple forceps extraction.

FIXATION STORAGE AND DEMINERALISATION

The basic solution for fixation, storage and demineralisation was a nutrient phosphate buffer (Clark's) at 4°C prepared as follows :

Stock Solution A

NaCl	14.00gm
KCl	0.75gm
$MgSo_4$ anhydrous	0.55gm
$Ca(NO_3)_2.4H_2O$	1.50gm

Mixed with deionized distilled water to 100ml.

Stock Solution B

D.Glucose	17.00gm
$NaHCO_3$	1.10gm
$Na_2HPO_4.7H_2O$	0.22gm
(in lieu of $Na_2HPO_4.12H_2O$	0.30gm)
KH_2PO_4 anhydrous	0.525gm
Phenol Red	0.01gm

Deionized distilled water to 100ml.

The definitive solution was prepared as follows: 50ml solution A and 50ml solution B added to 1900ml deionized water to give 20 volumes dilution and a pH of 7.4

FIXATIVES

The primary fixative into which specimens were placed within 20 sec of elevation was 4% glutaraldehyde in Clark's buffer at 4°C for 60 min pH 7.4.

The secondary fixative was 1% osmium tetroxide in

Clark's buffer at 20°C for 60 min pH 7.4.

DEMINERALISATION

The solution employed was 10% Ethylene Diamine Tetraacetic Acid (E.D.T.A.) in Clark's Buffer pH 7.4 at 4°C (Fejorskov, 1971; Charman and Reid, 1972). Two techniques were used to complete demineralisation.

1. Specimens were suspended from a stainless steel hook (through a hole drilled in the residual root) into a beaker containing demineralising solution at 4°C agitated continuously with a magnetic stirrer. By this method the time taken to reach Demineralisation End Point (D.E.P.) was 17 days.

2. To reduce this time a second technique was substituted. The teeth were sectioned with an edge cutting, flexible diamond disc at 6000 rpm (Fig.4). Under a copious stream of normal saline at 4°C the crown was removed transversely and a cut made down the middle of the buccal root in a mesio-distal direction. These specimens were placed in a stainless steel mesh cage in demineralising solution at 4°C agitated continuously in a similar way. The cut down specimens demineralised in 10 days.

DEMINERALISATION END POINT DETERMINATION

D.E.P. was determined using radiographic means, (Fejorskov, 1971), using the following technique :

1. Standard dental occlusal radiograph.
2. Type - long cone.
3. Line Voltage - 50 kV.
4. Current - 15 mA.s.
5. Exposure - 0.5 sec

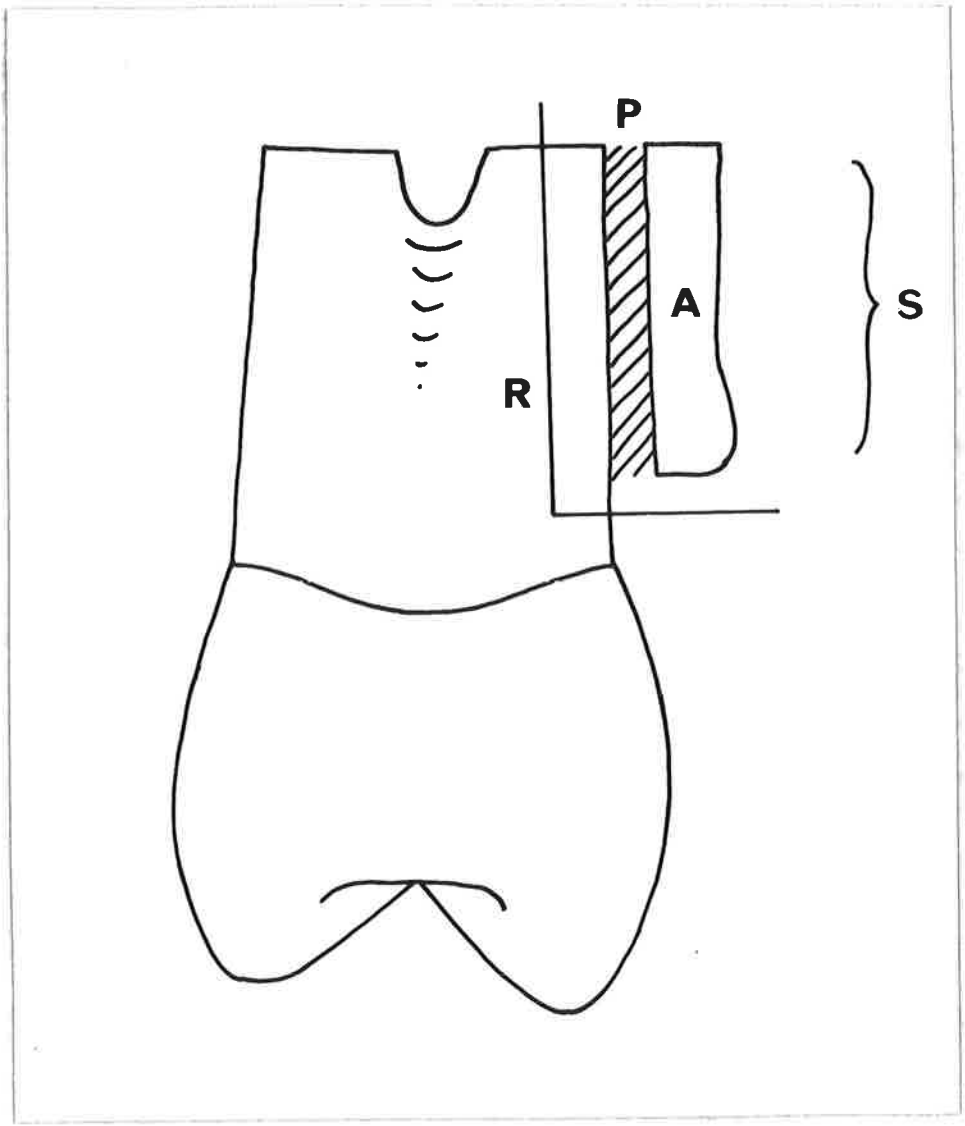


Fig. 4.

Specimen trimming prior to demineralisation:

Alveolar bone (A), tooth root (R), periodontal ligament (P), specimen after trimming (S).

When the specimen was uniformly homogeneous according to its radiographic image complete demineralisation was assumed.

After demineralisation further trimming was performed in order to :

1. reduce specimen size,
2. remove any residual tissue that might have been heat damaged during the surgical procedure.

Trimming was done using a Swann-Morton number 23 scalpel blade at room temperature. From the buccal aspect the specimen was divided into thirds, the mesial and distal thirds being discarded. The prominent bulge at the alveolar crest on the buccal plate was trimmed, (Fig.5). The dentine was trimmed to an equal thickness with the alveolar bone and at the apical end an oblique cut was made to allow orientation of the specimens in the moulds. The approximate size of each block when fully trimmed was 8x1x1mm, (Fig.5).

SPECIMEN PREPARATION SEQUENCE

4% Glutaraldehyde in Clark's	4°C	60 mins
Rinse with Clark's 4°C pH 7.4		
10% E.D.T.A. in Clark's	4°C	10-17 days
Rinse (Clark's)		
1% Osmium tetroxide	20°C	60 mins
Rinse (Clark's)		
70% Acetone	20°C	10 mins
70% Acetone	20°C	10 mins
70% Acetone	20°C	10 mins

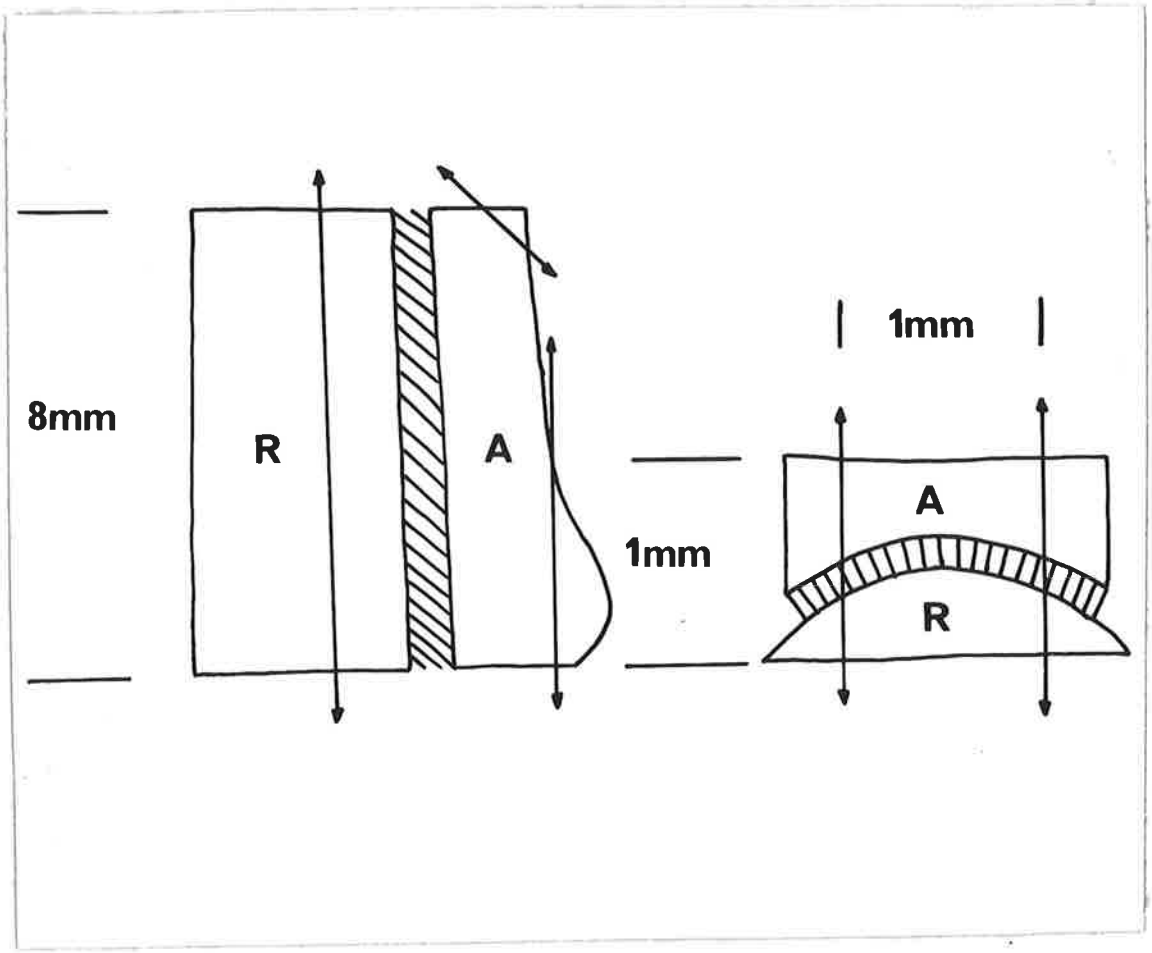


Fig. 5.

Specimen trimming after demineralisation:

Alveolar bone (A), tooth root (R), periodontal ligament is shaded. Approximate dimensions as marked. Arrows indicate cuts.

90% Acetone	20°C	10 mins
100% Acetone	20°C	10 mins
100% Acetone anhydrous $CuSO_4$	20°C	10 mins
Acetone (anhydrous)	20°C	10 mins
Acetone : Spurr's Resin 1:1	20°C	4 hrs
Spurr's Resin	20°C	14 hrs
Spurr's Resin in Moulds	70°C	6 days

The specimens were then stored until required.

SPECIMEN EMBEDDING

Specimens were orientated in the moulds so that cutting would commence from the apical aspect in half of the specimens and from the coronal end in the other half. In this way a control was established so that early results could be compared with later results to eliminate, as far as possible, the variable of operator skill and increasing experience with the machines involved.

SECTION CUTTING

Both thick and thin sections were cut on a Reichert model O.M.U. 3 ultramicrotome. Preliminary sections, about 1 μm thick, were mounted on glass microscope slides, stained and processed in the following manner.

Aqueous solution of: 1% toluidine blue and
1% borax.

Sections stained at 60°C for 30 secs

Mounted using pix, xylol and dibutyl phthalate medium (XAM) and glass cover slips.

Stored in the dark.

These sections were examined with a light microscope. In order to further reduce the block face for

cutting the definitive T.E.M. sections, the toluidine blue stained 1µm sections as seen with a light microscope (Fig.6) were used to :

1. select the most vascular areas,
2. reduce the component of dentine and bone on the block face to a minimum,
3. be able to concentrate on any particularly vascular structures seen,
4. mount the maximum number of sections on a single grid,
5. present the most favourable block-face outline to the knife in order to promote 'ribboning' of the sections as they were cut.

This trimming was performed on a special specimen stage adapted to the OMU 3 ultramicrotome. Viewed through a binocular zoom magnifying system the features representative of the structures under L.M. were easily identified on the block face. Fig. 6 is a typical light photomicrograph of a thick section and superimposed is a line diagram of how this block face might be further reduced prior to cutting definitive sections for the T.E.M.

Definitive sections were cut in the 'Silver' range, i.e. 75nm±5nm as determined by the interference pattern of reflected light technique which, according to Koeler (1973), is accurate provided the specimen thickness does not exceed 1 µm.

The definitive sections were cut, mounted and stained with a variety of techniques. Earlier sections, with a relatively large block face, were cut using a 46°

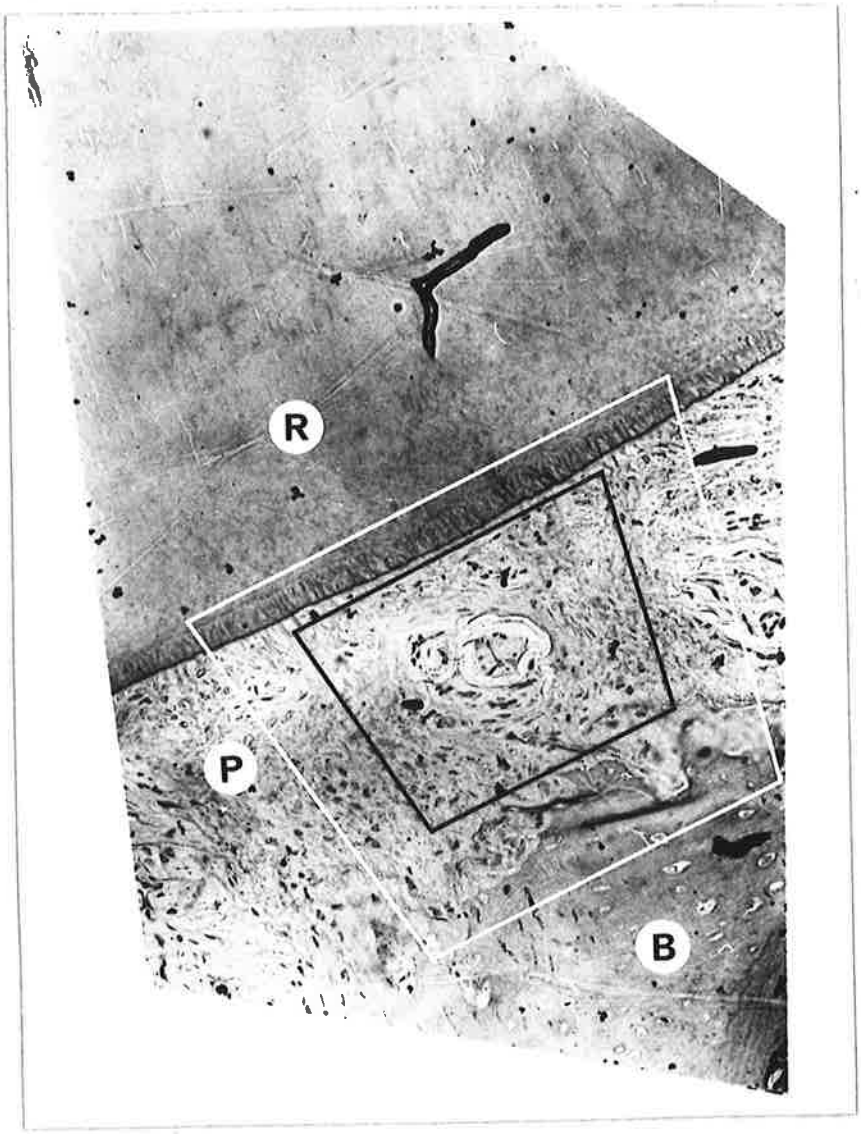


Fig. 6.

Section of gross specimen: Toluidine blue stained 1 μm section. Bone (B), tooth root (R), periodontal ligament (P). White outline represents block face for diamond knife ultramicrotomy. The black inner line represents the block face for glass knife ultramicrotomy.

Dupont diamond knife. This resulted in large sections suitable for scanning but with a variable degree of chatter. The specimens so cut were of only limited value for high quality intermediate-power electron micrography i.e. magnifications ranging from 2.10^3 up to 8.10^3. In accordance with the general principals laid down by Hayat (1970), ultramicrotomy variables were analysed and a technique using :

1. glass knives,
2. maximum reduction of specimen size; particular attention was paid to removal of demineralised tissue from the block face, (Fig.6).

This technique resulted in specimens that were of limited topographical value but much more suitable for high resolution low and intermediate magnification electron micrographs. Therefore, diamond cut large sections were used to assess general type, numbers and distribution of vessels in the periodontal ligament. Glass cut sections were used more frequently for the detailed electron micrographs of individual structures.

Mounting was always on 2.3mm copper grids, mesh values varying from 400 to 100 were used. 100 and 200 mesh grids were used with a supportive coating of polyvinyl formvar (TAAB laboratories). 400 mesh grids were used unsupported. Staining was by uranyl acetate and/or lead citrate. Specimens were examined using a Siemens Elminscop 1 electron microscope at 60kV.

SPECIMEN EXAMINATION

Once mounted in the T.E.M. the specimens (approximately three per grid) were examined under very low power to determine the best specimen to examine.

The magnification was increased to approximately 2.10^3 and this was the scanning magnification.

Dentine was identified by its characteristic tubules and these were used as the indicator of the direction of the periodontal ligament. This direction was followed until the characteristic Sharpey's fibres in cementum and periodontium identified the dento-periodontal interface. At this interface nuclei were seen and scanning proper commenced (Fig.7).

The general direction of the dento-periodontal interface was oriented to the bars of the supportive grids and this junction was then scanned from one side of the specimen to the other.

One side of the specimen was selected (arbitrarily) and the grid square transsected by the dento-periodontal junction was scanned at 2.10^3 magnifications, one field width at a time, changing direction at the grid bar. The scanning pattern is represented in Fig.8.

When a square was completed, the next grid square examined was the adjacent square away from the dento-periodontal junction and so on until the arrangement of collagen indicated that the alveolar bone-periodontal interface had been reached. Osteocytes in their lacunae with canaliculi were indicative of bone (Fig.9).

The field was returned to the dento-periodontal

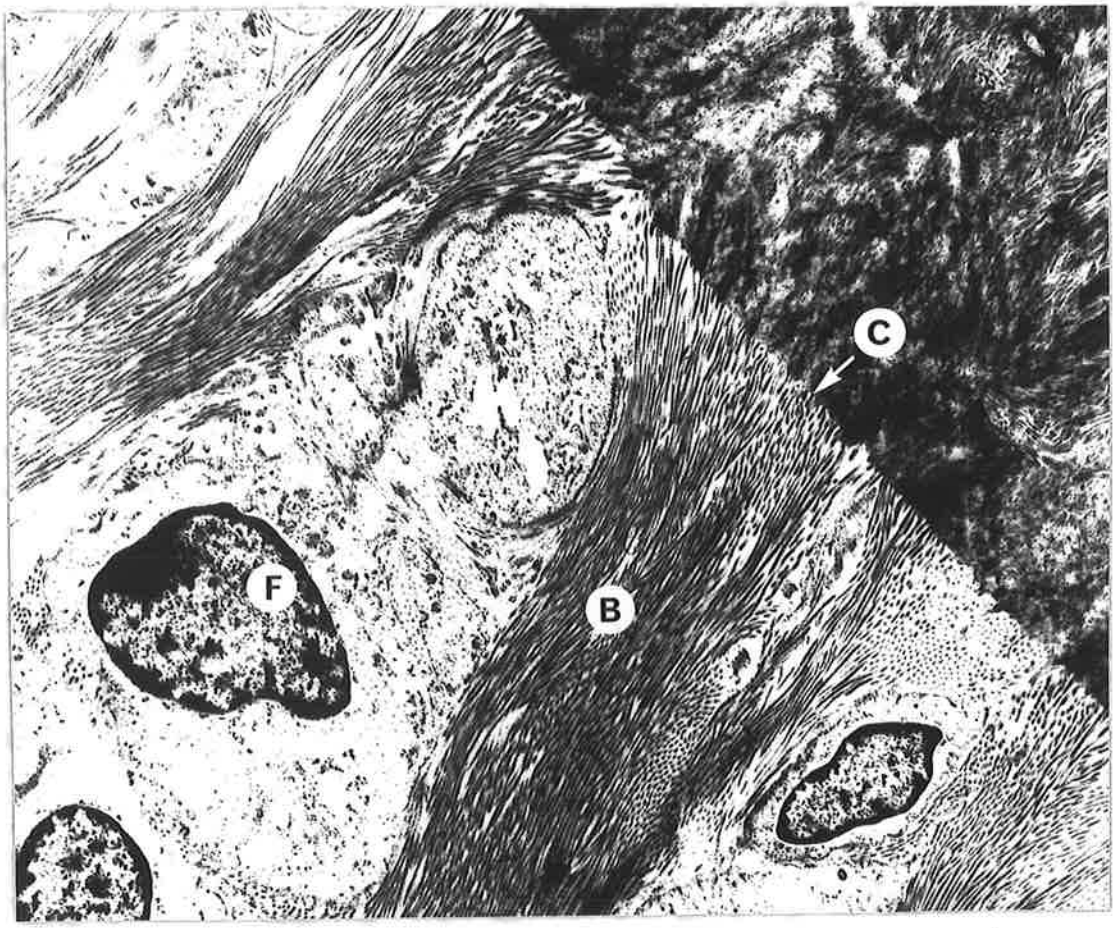
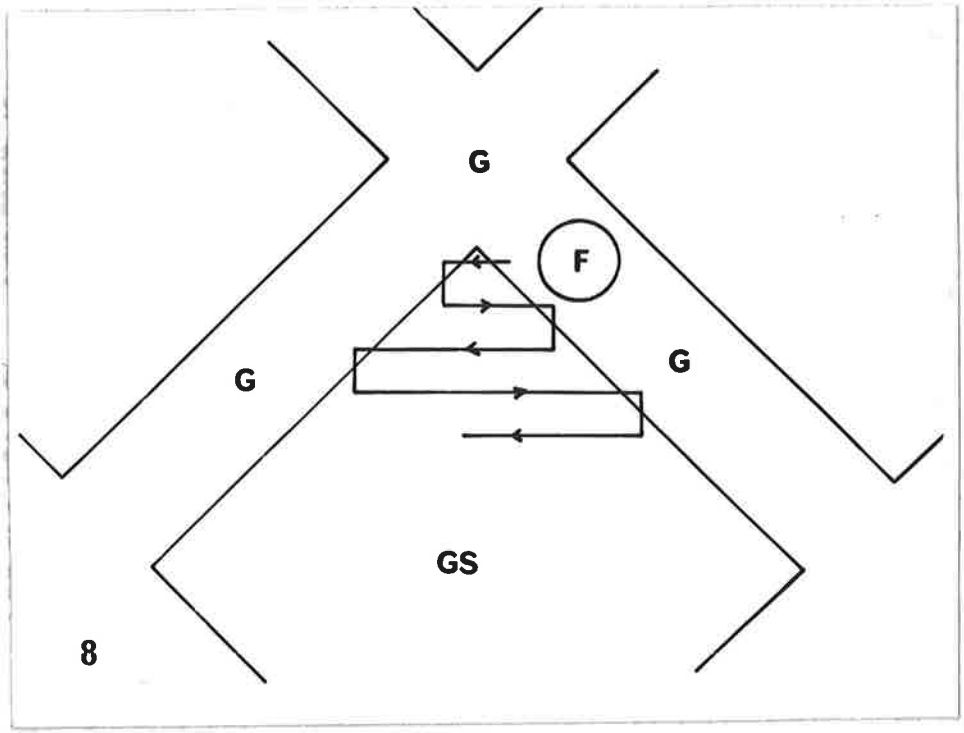


Fig. 7.

Dento-periodontal interface: Fibroblast (F), periodontal collagen bundles (B), mineralised front of cement (C) as used to orient scanning pattern (12.10^2).



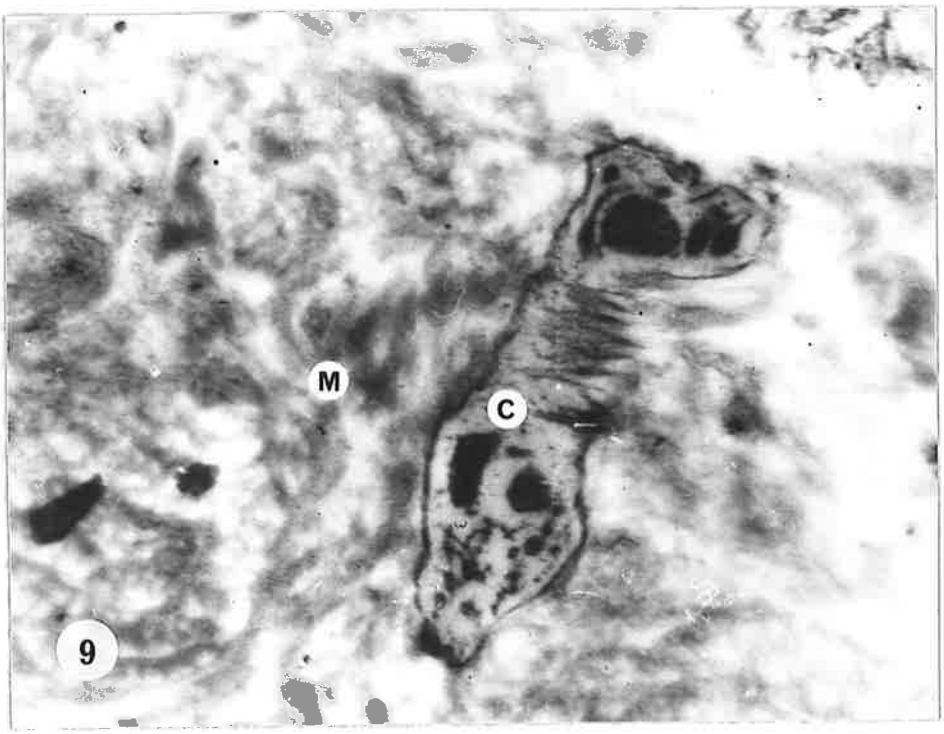


Fig. 8. Scanning pattern: Grid bars (G), grid square (GS). Arrows represent scanning pattern. Approximate field size at 2.10^3(F).

Fig. 9. Alveolar bone: Bone matrix (M), osteocyte process in its canaliculus (C). (11.10^3).

junction in the square first examined and then the second row as for the first row of squares, tooth to bone, etc. In this way, the whole specimen was scanned. Photographs were taken of all vascular structures and identifiable collections of cells and fibres involving blood vessels.

PHOTOGRAPHY

Photographs taken ranged in magnification from $0.5.10^3$ to 47.10^3. A magnification of 47.10^3 may be regarded as the upper limit of the particular electron source and focussing circuits used. Magnification from negative to print was a variable factor. For true magnification of the electron photomicrographs the magnification should be multiplied by the printing enlargement factor. This factor is of the order of twofold on an area basis. All enlargement figures referred to are machine enlargements. No printing enlargements are included in this report.

Electron micrographs presented in this report were not printed direct from glass T.E.M. negatives. Master prints were prepared on a 8" x 10" format. These prints were labelled comprehensively, photographed on 35mm negative film and then printed in a conventional manner. This technique conferred two benefits:

1. better labelling and
2. overall better contrast control.

There was one disadvantage: where the original negative was very high contrast the print obtained by the indirect method was also of very high contrast. This disadvantage is minor, however, as the more common T.E.M. negative is of relatively low contrast rather than high.

SECTION 5

FINDINGS

In this study there appeared to be four distinct blood vessel types which could be described as separate entities. Cellular terminology was based mainly on Rhodin (1967, 1968) but vascular classification per se was not applied in findings. Findings are presented under the following headings:

A. BLOOD VESSELS
- Type I. simplest type also most numerous.
- Type II. a) obvious yet incomplete periendothelial cellular investment.
- Type II. b) complete periendothelial cellular investment.
- Type III. complete, multilayered periendothelial cellular investment.
- Lymphatic type.

B. ASSOCIATED ADVENTITIAL STRUCTURES

In order to clarify the use of the terms pericyte, periendothelial and veil cell, the following standards have been applied:

Periendothelial: This term applies only to a locality. Usually it is used to localise cells that are on the

abluminal aspect of the endothelium but it may also apply to fibres. Depending on the vessel type those cells may be pericytes, veil cells or muscle cells of the smooth or primitive type. Periendothelial cells fully enclosed within a basement membrane have been considered to be cells of the tunica media, this includes pericytes and the muscle types. On the other hand, periendothelial cells without a complete basement membrane, i.e. veil cells, are considered to be cells of the tunica adventitia.

For the purposes of a broad localisation of vessels, the periodontium can be broken up into annular thirds called the dental third, middle third and alveolar third.

BLOOD VESSELS

TYPE I

Being the smallest type it was also the most numerous. Type I vessels were found in the middle and dental annular thirds. Their distribution was the most obiquitous. They were found in areas of dense collagen and also in the looser tissue between the principal periodontal fibre bundles. The vessels were seen to be mainly oriented parallel to the annular planes mentioned when vessels were seen cut in longitudinal section. They appeared not to course toward or away from the root surface but rather apico-gingivally or circumferentially.

The ultrastructural appearance was essentially that of an endothelial tube with a few highly branched

pericyte processes, (Figs.10 and 11).

Tunica intima:

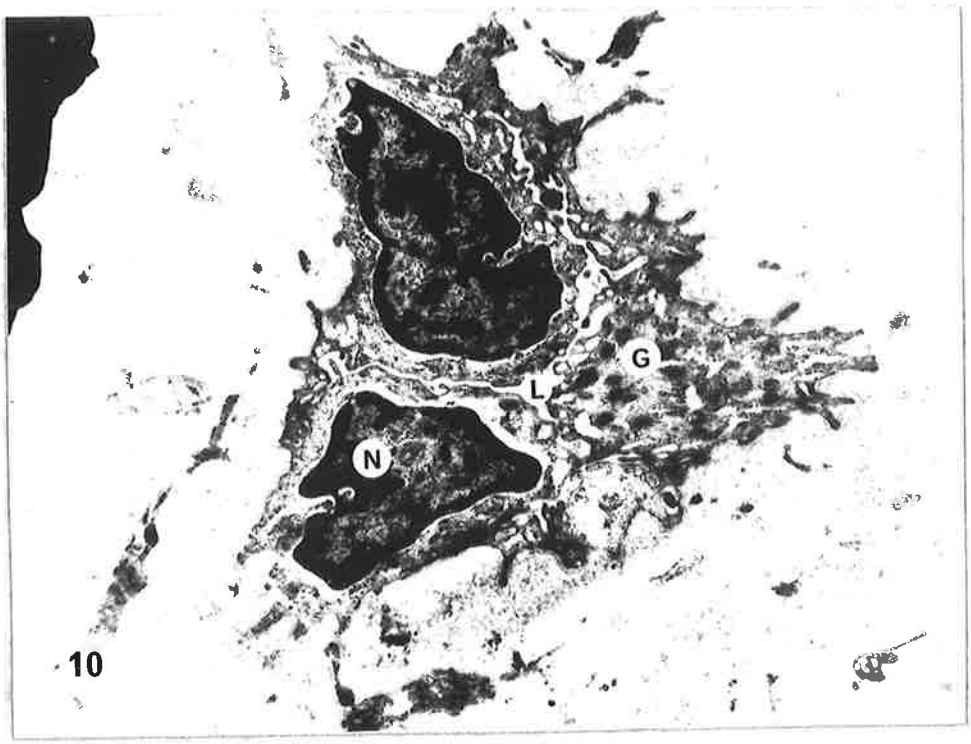
Endothelial cells were very flattened and irregular. The irregularity seemed to be consistent with the amount of luminal contents seen, particularly on the abluminal aspect. That is, very collapsed vessels had a very irregular abluminal endothelial surface (compare Figs.10 and 11). The thickness of the endothelium varied substantially; it was thickest at the nucleus and thinnest farther away from the nucleus.

Endothelial nuclei:

The nucleus itself appeared flattened, parallel to a luminal tangent, with prominent features. These features were peripheral chromatin condensation, nuclear membrane and multiple nuclear fenestrae. The endothelial nucleus usually demonstrated a prominent cleft. (Fig.10) Nuclear membranes appeared as a relatively amorphous membrane, well differentiated from the cellular cytoplasm and less well differentiated from the peripheral nuclear chromatin (Fig.12). Nuclear fenestrations were quite prominent. They consisted of an interruption to the peripheral chromatin condensation together with a thinning of the nuclear membrane. They occured on all nuclear peripheries including nuclear clefts.

Endothelial cytoplasm:

The cytoplasm varied substantially from one cell to the next, both inter and intra-specimen (Fig.13). This change was thought to be due, at least in part, to cellular reactions to the various pathogenic agents in the specimen



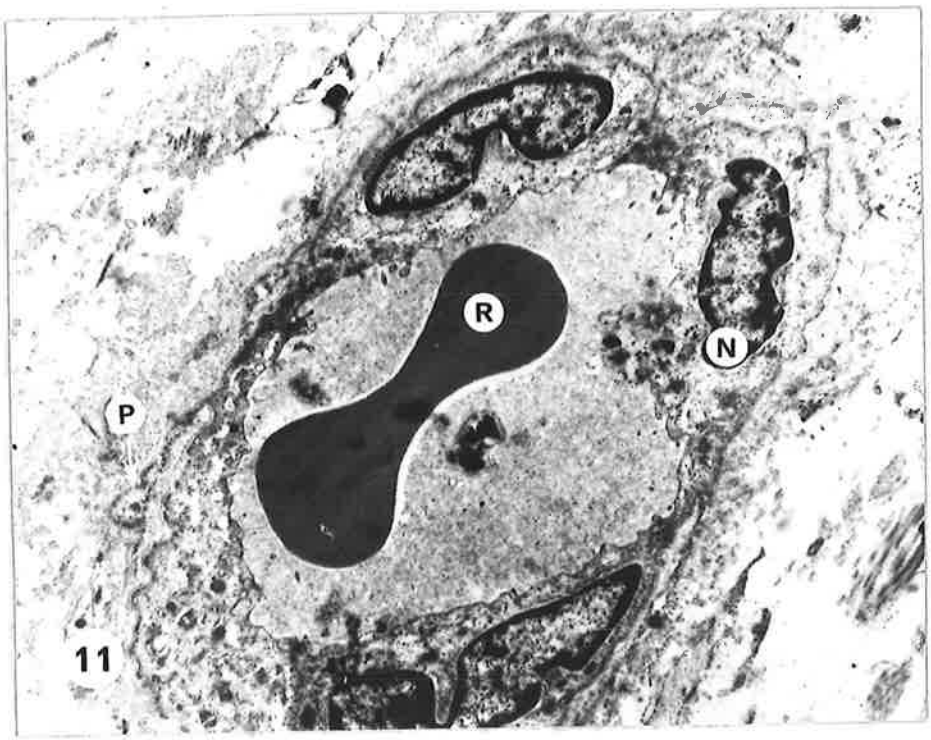
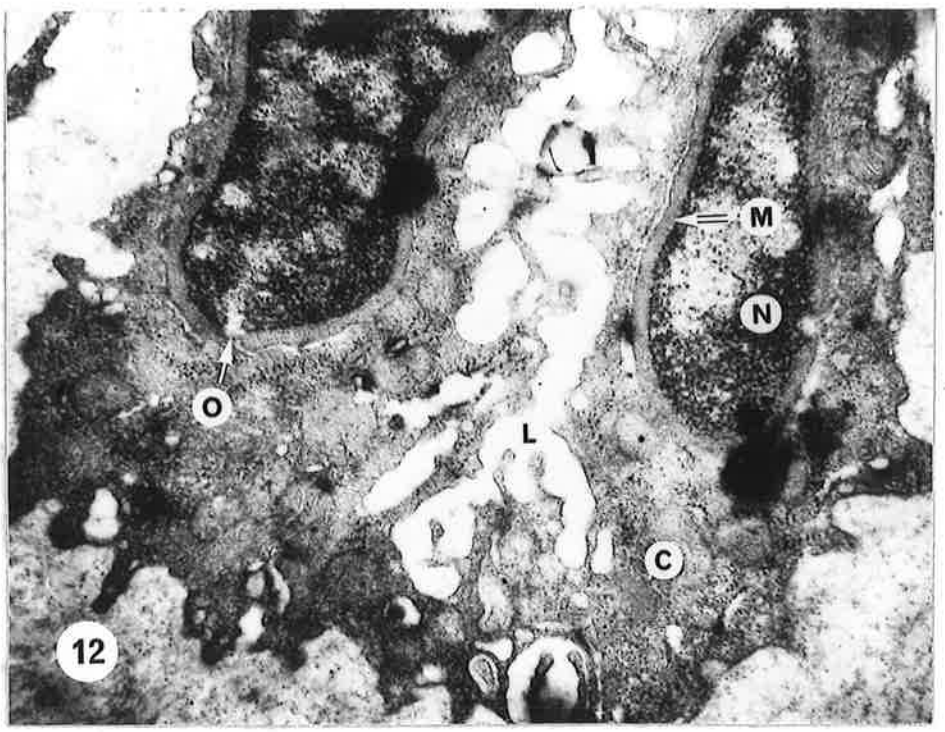


Fig. 10. Type I vessel: Showing collapsed lumen (L), endothelial nucleus (N), cytoplasm with dark granules (G). (5.10^3).

Fig. 11. Type I vessel: Showing a distended vessel with erythrocyte (R), pericyte process (P), endothelial cell and nucleus (N). (6.10^3).



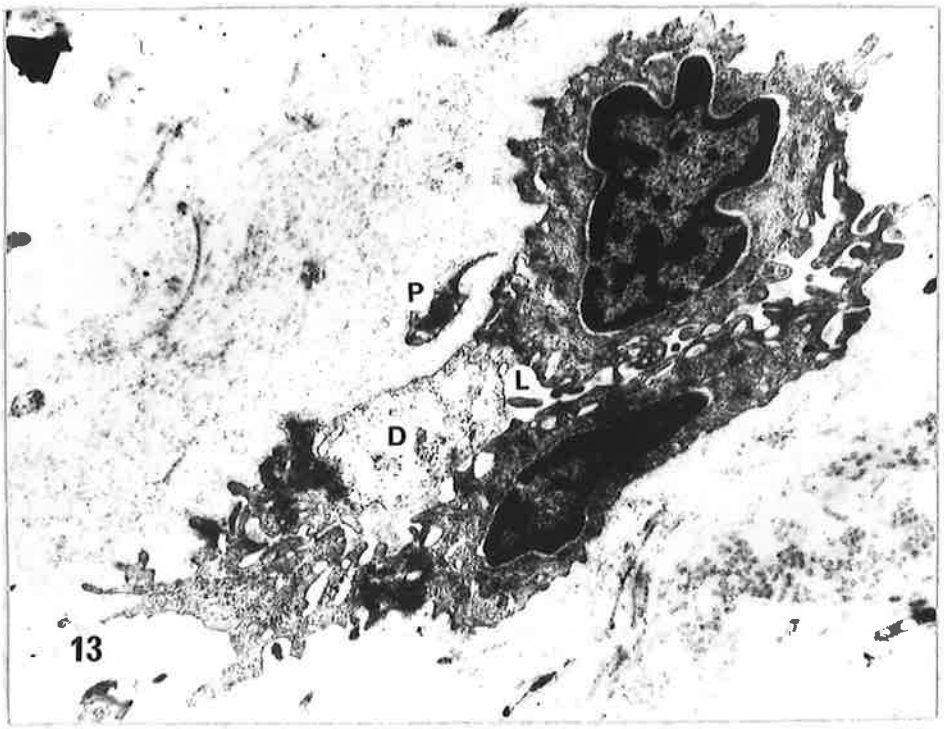


Fig. 12. Type I vessel: Demonstrating perinuclear membrane with fenestrae. Endothelial nucleus (N) and cytoplasm (C). Lumen (L), perinuclear membrane (M) and nuclear fenestration (O). (30.10^3)

Fig. 13. Type I vessel: Difference between 'normal' and 'damaged' endothelial cells. 'Damaged' cell (D), lumen (L) and pericyte process (P). (6.10^3).

preparation prior to fixation and perhaps even post-fixation. When the cytoplasm appeared swollen and damaged the endothelial cell appeared to bulge in a pronounced way in a luminal direction. Under pathological stimuli that could be considered 'normal', endothelial cells are known to swell and bulge in the manner described. It is thought that this picture could be due to factors introduced during preparation and no great issue will be made of this differential presentation. Generally speaking the cytoplasm was not markedly endowed with identifiable organelles.

Two features were promiment however, these were cytoplasmic fibrils (Figs.14 and 15) and circular electron dense bodies, or granules (Fig.10).

Dense bodies were probably spheroidal rather than circular and while not identical in size the range of sizes was not great. On occasions the fibrils were a prominent feature of endothelial cytoplasm. Other organelles were seen but were not abundant. Mitochondria with their characteristic cristae were occasionally seen and tended to be close to the nucleus.

The cytoplasm contained many vesicles and the size varied considerably as did the apparent contents. Abluminal vesicles tended to be larger and often had some electron-dense contents. These have been termed vacuoles to differentiate them from, more numerous, smaller and more uniform, luminally oriented vesicles. However, as it will be seen later in this section, the junctions between endothelial and periendothelial cells can be complex and

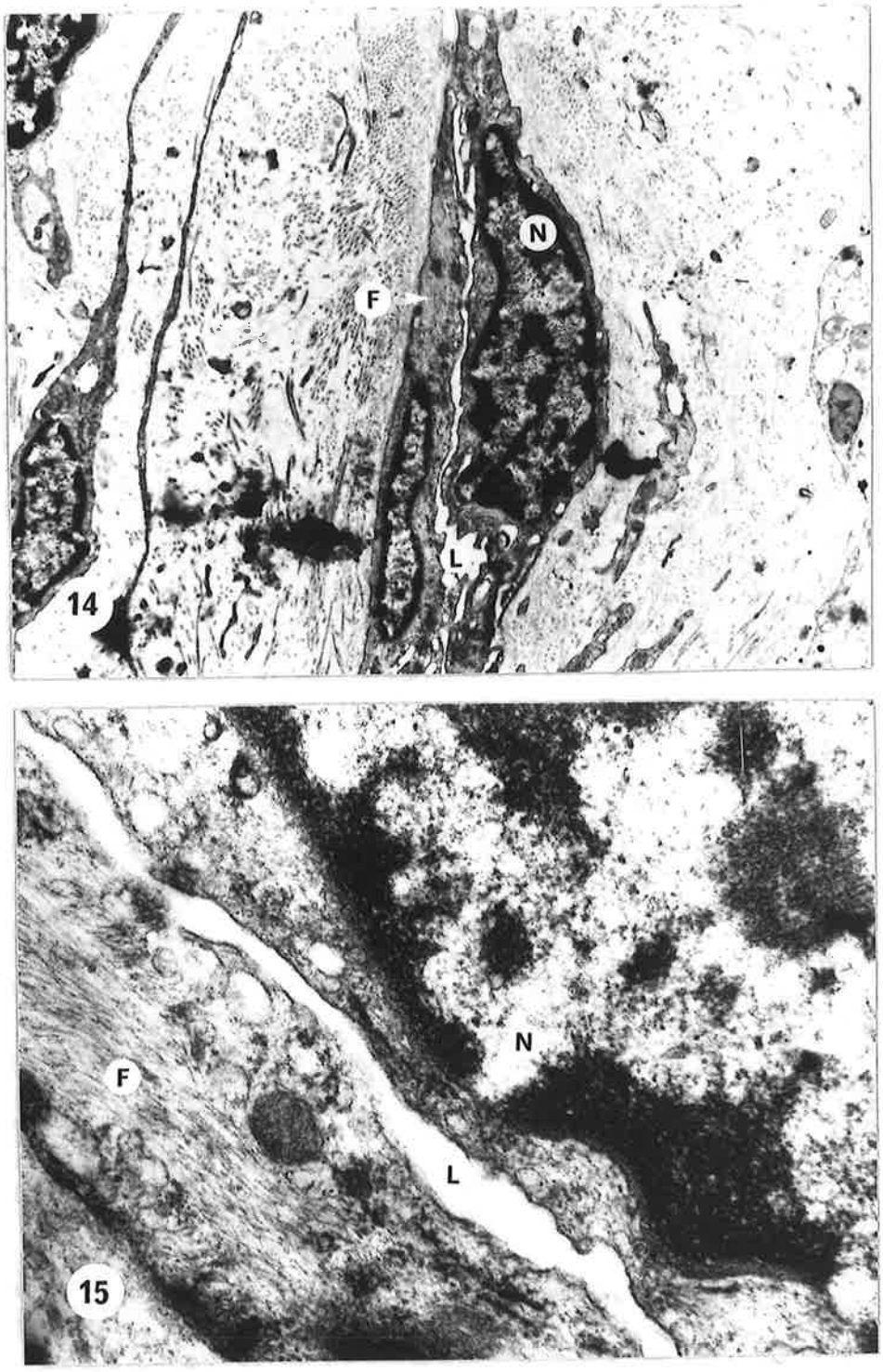


Fig. 14. Type I vessel: Demonstrating intraendothelial fibrils. Endothelial fibrils (F), lumen (L) and endothelial nucleus (N). (2.10^3).

Fig. 15. Type I vessel: "As above", also nuclear fenestration at (N). (30.10^3).

these 'vacuoles' may have been sectioning presentations rather than true vacuoles. On the luminal aspect the vesicles were much more regular in size, smaller and did not have electron-dense contents (Fig.16). Luminally oriented vesicles could be seen in all stages of vesicularisation with the luminal endothelial plasma membrane; even to the stage of transendothelial holes and channels alluded to by Wolff (1977), (Fig.17).

Depending on the degree of collapse of the blood vessel the luminal plasma membrane was thrown into varying degrees of convolution. That much of the convolution was due to collapse is incontrovertible, but there were luminal projections which seemed not to be passively dependent on collapse. This type of luminal flap was most common near interendothelial junctions (Fig.18). They appeared as simple membrane bound projections. Inter-endothelial junctions varied with the degree of collapse and within the same vessel. At times they appeared linear and direct, at others sinuous and indirect (Fig.19). Occasionally they did not show an equal intermembranous gap and at other times the gaps were quite consistent with the exception of contact areas that appeared to be of the zonula adherens or occludens type, (Fig.20). These junctions were characterised by an increased electron density at the junction which projected for a short distance back into the cytoplasm of the adjacent cells.

Intraendothelial fenestrae were seen in type I vessels (Fig. 21) and it was felt that despite the artefactual collapse such numbers of fenestrae as were present

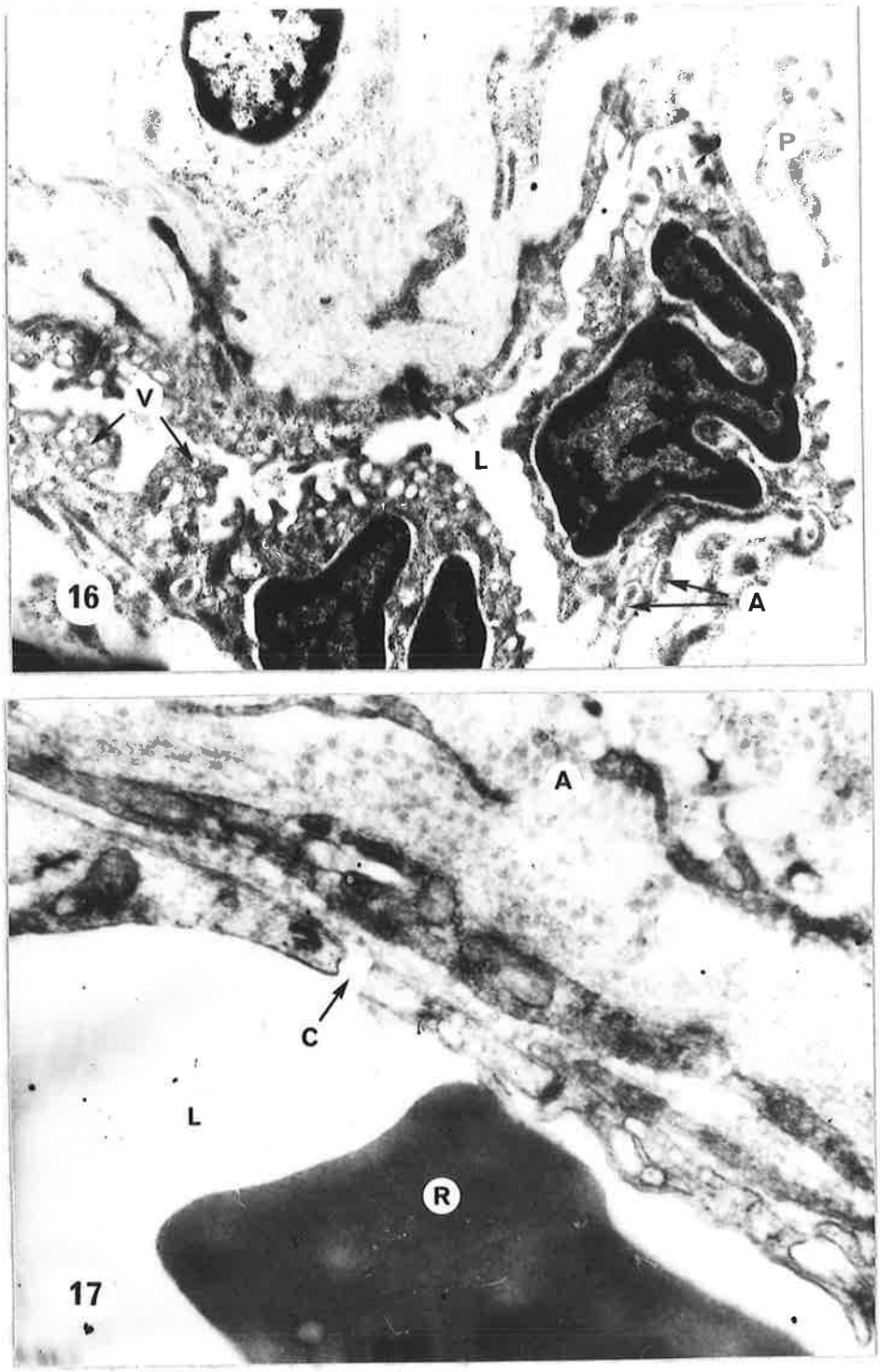
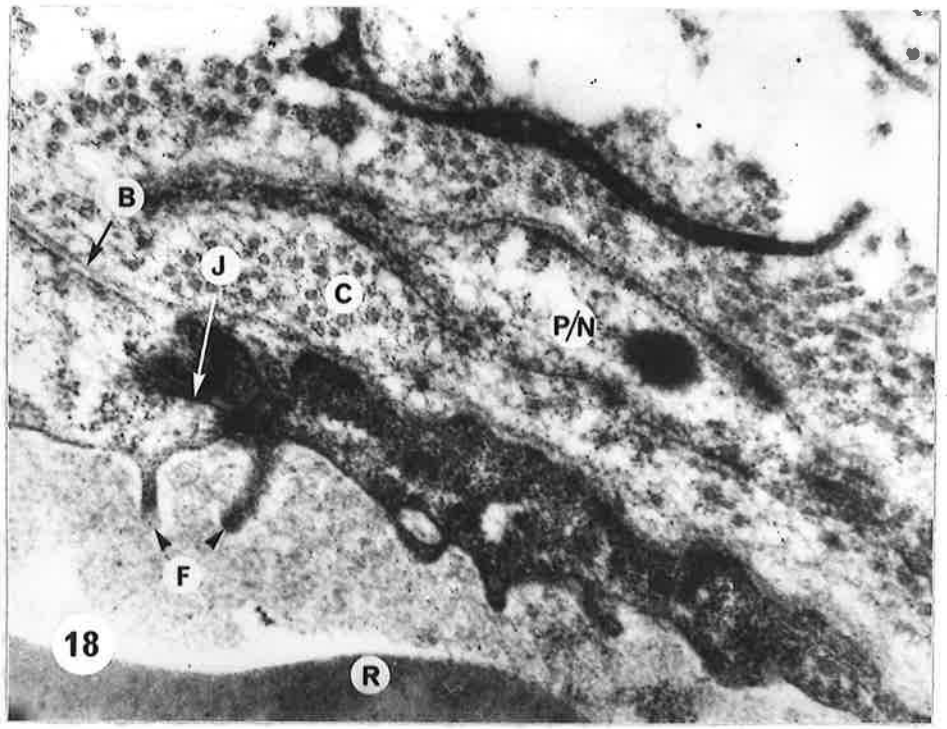


Fig. 16. Type I vessel: Showing luminal aspect vesicularisation. Vesicles (V), lumen (L), pericyte process (P), endothelial-pericyte close approximation (A). (6.10^3).

Fig. 17. Type I vessel: Showing a transendothelial channel (C), lumen (L), erythrocyte (R), adventitia (A). (18.10^3).



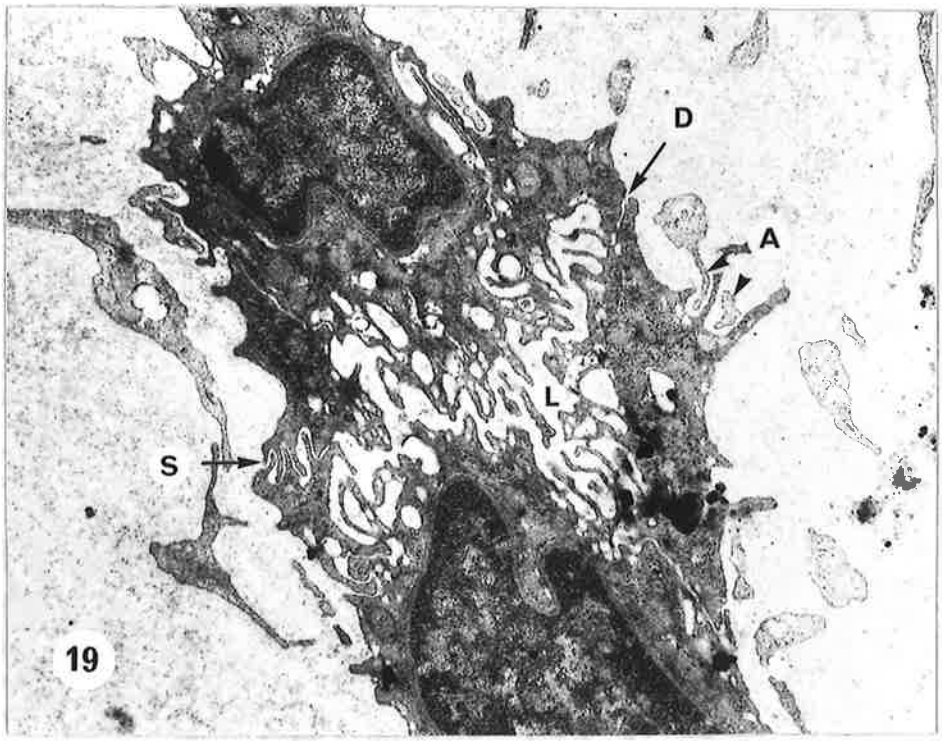
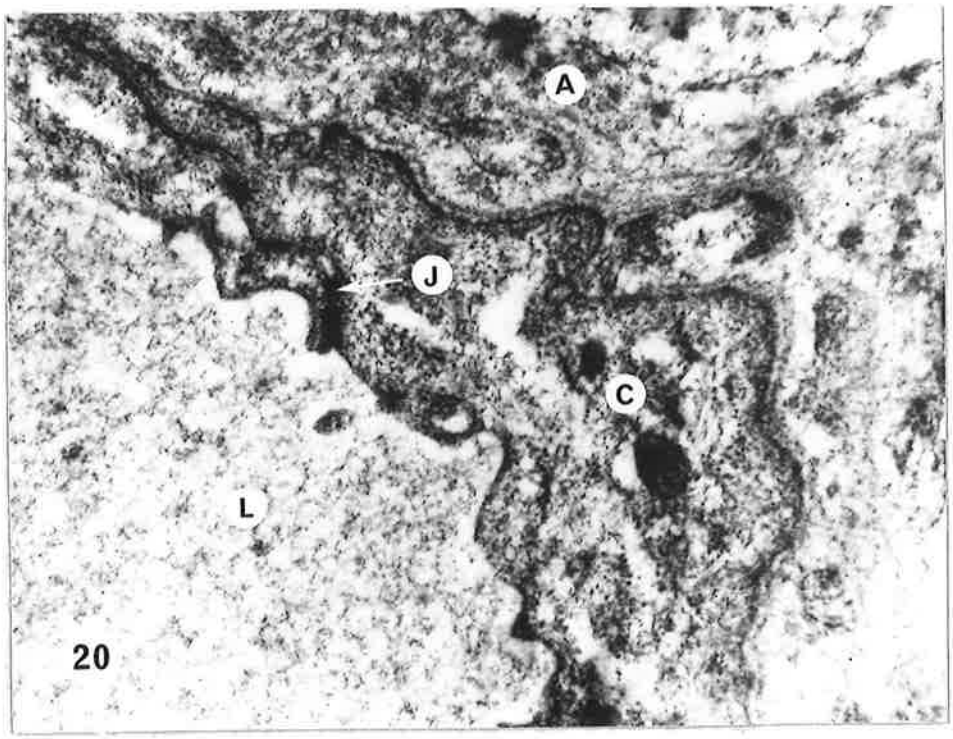


Fig. 18. Type I vessel: Showing luminal flaps. Erythrocyte (R), pericyte/nerve process (P/N), luminal flaps (F), interendothelial junction (J), basement membrane (B), collagen (C). (18.10^3).

Fig. 19. Type I vessel: Interendothelial junctions. Sinuous (S), direct (D), lumen (L), endothelial-pericyte approximations (A); note irregular gaps at (S). (8.10^3).



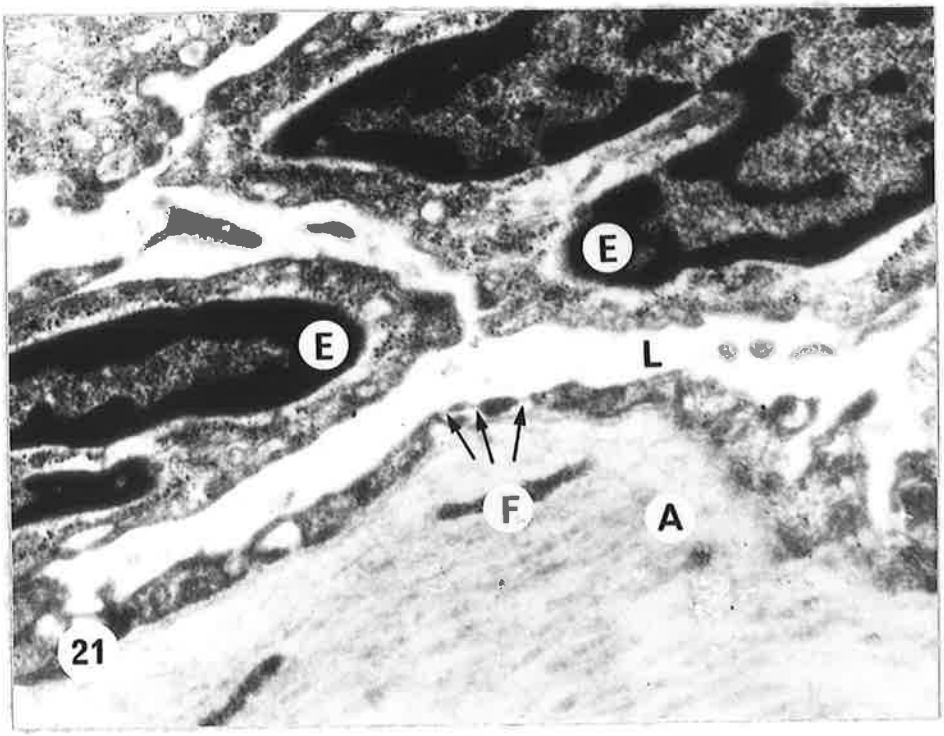


Fig. 20. Interendothelial junction: Showing zonula adherens/occludens type junctions (J), endothelial cytoplasm (C), lumen (L), adventitia (A). (17.10^3).

Fig. 21. Type I vessel: Showing intraendothelial fenestrae (F), lumen (L), endothelium (E), adventitia (A). (8.10^3).

would be reasonably apparent. The impression was gained that less than 5% of type I vessels were fenestrated. The fenestrae seemed quite typical, i.e. they occurred only at the thinnest sections of endothelium and they appeared to be closed by a diaphragm.

The abluminal surface of endothelial cells was characterised by a complete and typical basement membrane. Depending on the degree of collapse the abluminal surface was thrown into folds, and often the picture of the abluminal surface was quite stellate (Fig.10).

BASEMENT MEMBRANE INCLUSIONS

Within the endothelial basement membrane there were often seen inclusions of several types. In some the inclusion was, fairly obviously, a sectioning effect of a process of a periendothelial cell (pericyte). In this type the abluminal endothelial cytoplasm appeared vacuoleated but the contents of the vacuoles were very consistent with the ultrastructure of adjacent pericytes. (Fig.16) Examining the several forms of close approximation that endothelial and periendothelial cells entered into, it seemed most likely that the presentation was a sectioning feature. The types of approximations will be presented later.

The second type of inclusion within the endothelial basement membrane was harder to account for. It appeared as a piece of pale cytoplasm with a more electron dense, fairly discreet, centre. The entire structure lay inside the endothelial basement membrane. (Fig.22). The area of contact between the cell process and the

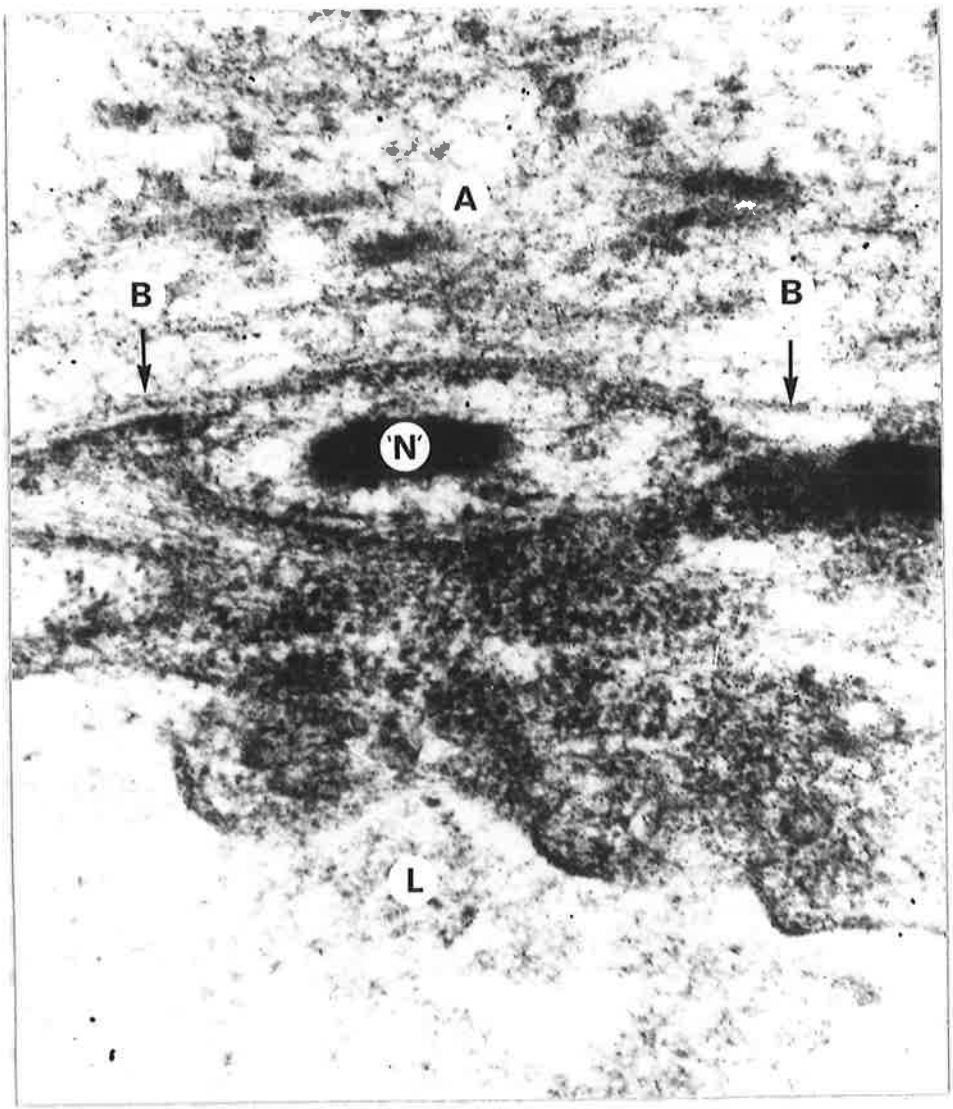


Fig. 22.

Type I vessel: Subendothelial basement membranous nerve-like inclusion. Lumen (L), adventitia (A), endothelial basement membrane (B). Nerve like process with pale cytoplasm and darker central core (N). (30.10^3).

endothelium was usually quite distinct but often very narrow. When a gap was discernible there did not appear to be any contents of the gap, at least no contents analogous to basement membranous structures. It was suggested that on an ultrastructural basis these processes were most analogous to non-myelinated nerves. (Casley-Smith 1977b). Such nerves could often be definitively identified in the adventitia quite close to vessels.

Tunica media:

Of the detailed ultrastructure of pericytes very little could be seen at the type I level. It was infrequently seen that the nucleus of the pericyte was identifiable with a type I vessel. The cell processes seen were very fine and little intracellular detail could be ascertained. It was felt that the pericyte picture was consistent with the idea that it constituted a meshwork of fine processes over the abluminal endothelial surface.

ENDOTHELIAL-PERICYTE CLOSE APPROXIMATIONS

The junctions or close approximations entered into by endothelial cells and pericytes formed three fairly distinct groups, named for convenience:

1. finger-like,
2. horizontal depression,
3. perpendicular invagination.

Finger-like close approximations were the most simple, consisting of projections of cytoplasm from the abluminal surface of the endothelium and the pericyte

which came close together. Usually the main part of the endothelial cell and the pericyte process stayed fairly widely separated, often with general adventitial structures, e.g. collagen, between. The basement membrane appeared to be excluded from the areas of closest approximation, (Fig.23).

Horizontal depression types occurred as an abluminal surface indentation of the endothelial cell into which a pericyte process intruded and then spread out to a varying degree. They probably extended into the endothelium in a tunnel-like arranagement in the depression and under the endothelial basement membrane, (Fig.24).

Perpendicular invagination types consisted of a pair of close abluminal endothelial processes between which a process of pericyte projected. The degree that the pericyte protruded into the body of the endothelial cell varied, as did the length of the abluminal processes of the endothelial cell (Fig.25).

The nature of pericyte and endothelial approximations for type I vessels is deserving of some summarisation. To what extent these differences described are real is hard to say in the absence of topographical controls. However, it does seem reasonable to say that ultrastructural interrelationships between endothelial cells and pericytes are extensive, somewhat varied, and probably possess a degree of functional significance.

Tunica adventitia:

Apart from the areas where pericyte processes were interposed the adventitia abutted directly to the

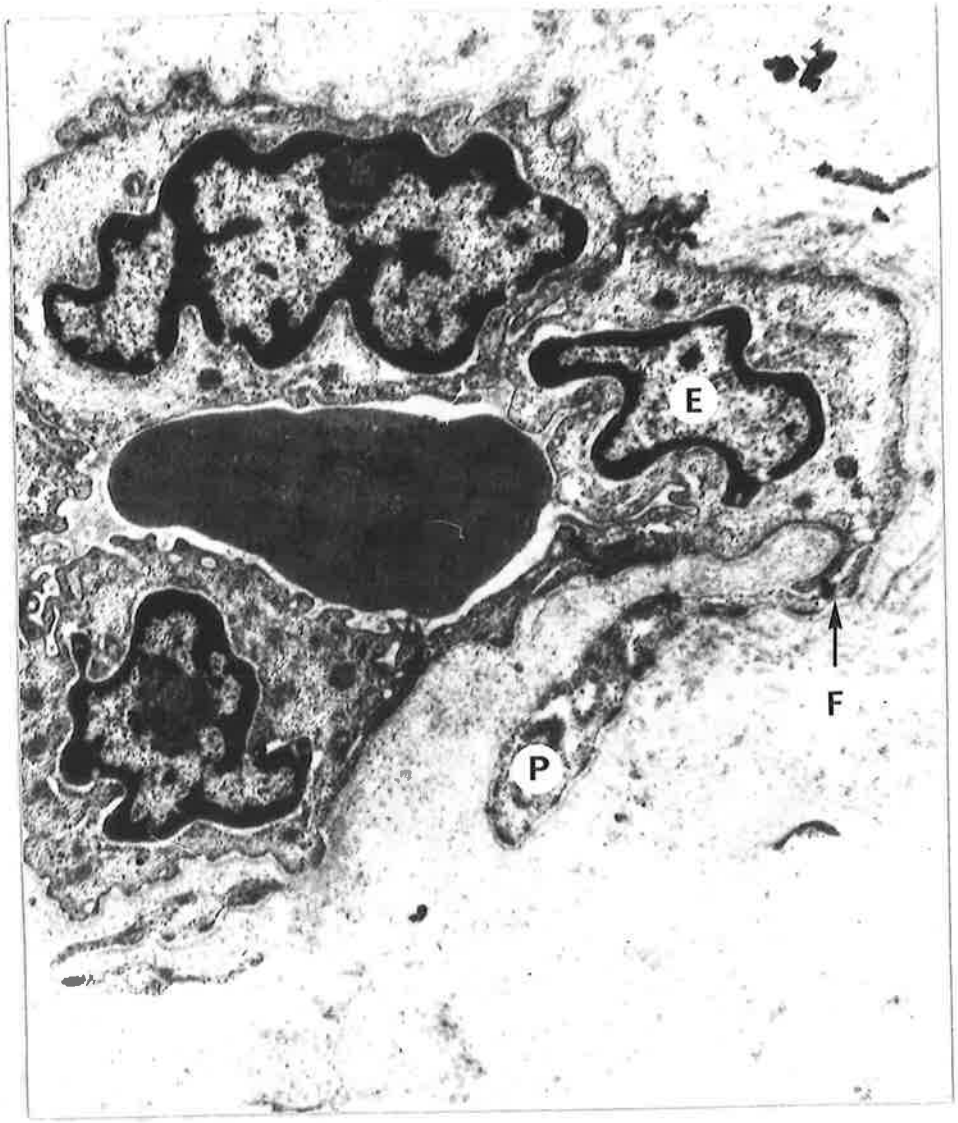
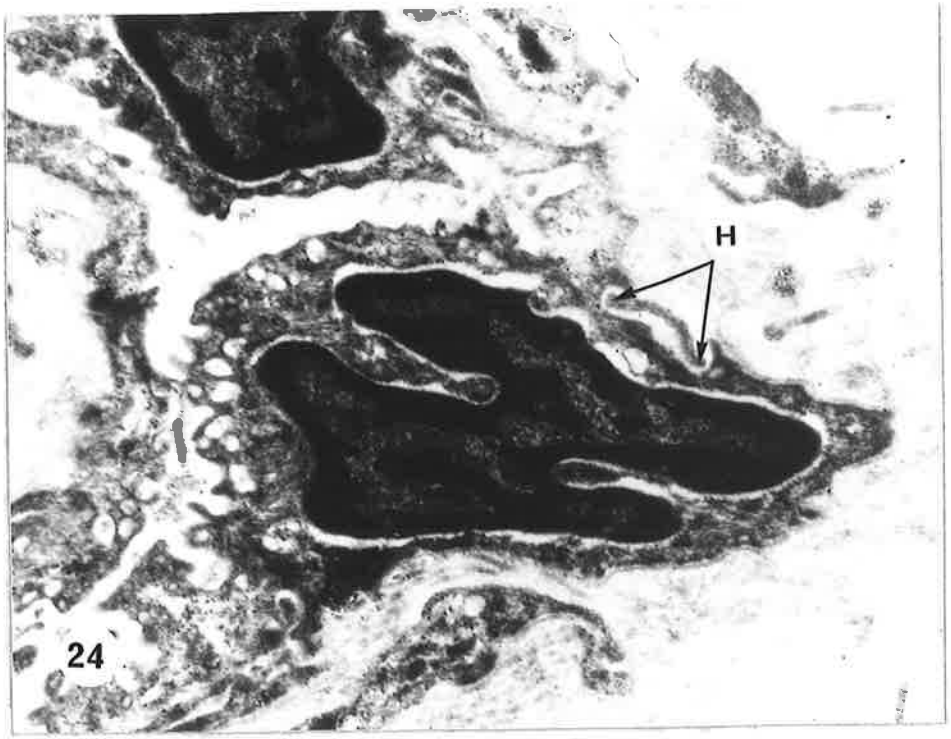


Fig. 23.

Type I vessel: A finger-like endothelial-pericyte close approximation (F), endothelial cell (E), pericyte process (P). (2.10^3).



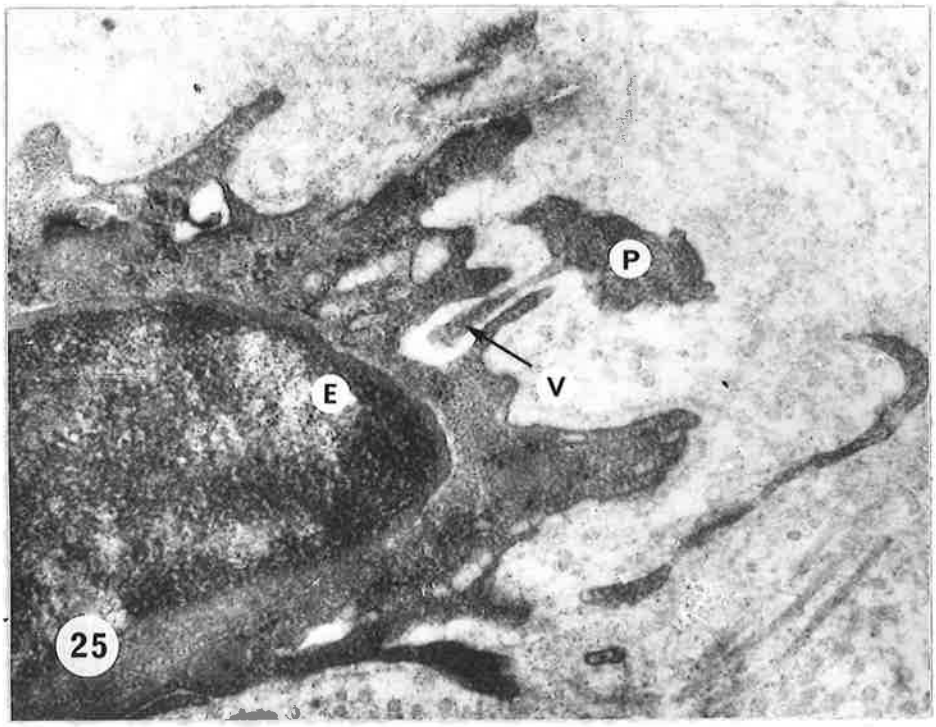
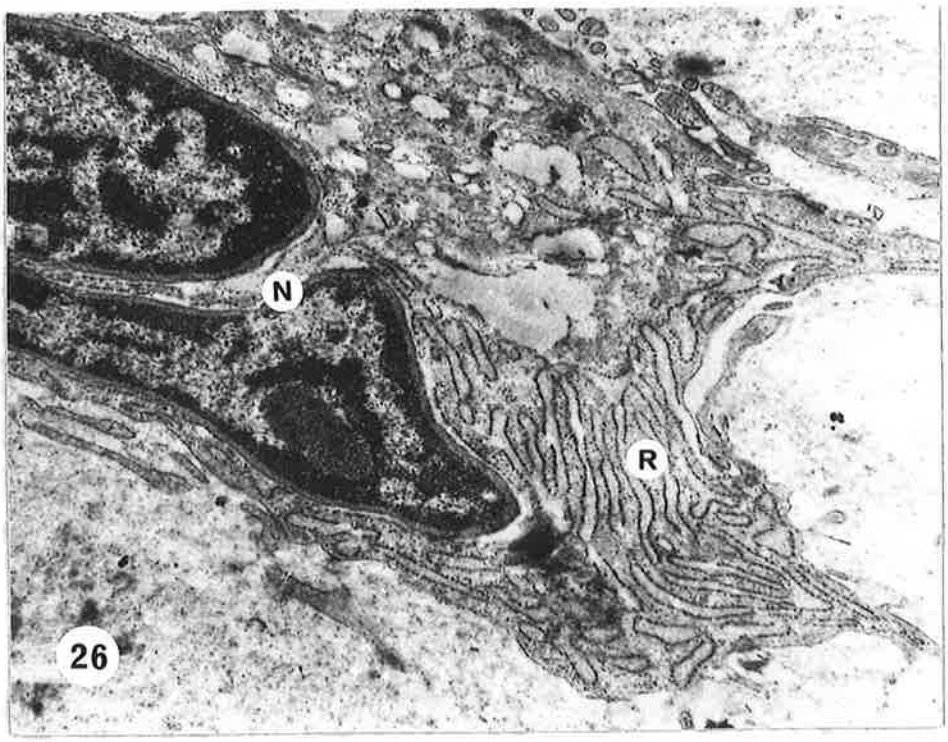


Fig. 24. Type I vessel: Horizontal depression-type endothelial-pericyte close approximation (H). (4.10^3).

Fig. 25. Type I vessel: Perpendicular invagination-type endothelial-pericyte close approximation. Pericyte process (P), endothelial cell (E), invagination (V). (20.10^3).

endothelial basement membrane. Adventitial structures varied according to whether the vessel was in an area of dense collagen or in an area of looser connective tissue. In the former locality mature collagen was seen directly adjacent to the basement membrane. Looser connective tissue between the principal fibre bundles contained more varied tissue elements and so there were more varied structures that came adjacent to the vessels. Where vessels were collapsed there was usually a space between the vessel and the general adventitia. It appeared as if this space represented the area occupied by the vessel when it was filled to a more functional diameter. When this feature was seen it was also apparent that some adventitial structures had collapsed with the vessel, so that the space seen had loose connective tissue on both sides. There would seem to be a prima facie case that those structures that had collapsed with the vessel were best considered components of the vessel. The components seen were loose collagen, cells, cell processes (fibroblasts or veil cells) and nerves. The appearance of the cells and processes was variable. Sometimes plump, active cells, with much rough endoplasmic reticulum (Fig.26) were seen. In other instances the cells appeared inactive with thin, elongated processes and a general paucity of organelles (Fig.14). These cells were devoid of a continuous basement membrane and the slender processes appeared to be densely packed with fibrils (Fig.27) to such an extent that it was often hard to discern the unit membrane at the periphery. The principal indicator of the cellular nature of these fibrillar structures was the appearance of periodic mitochondrion-like structures.



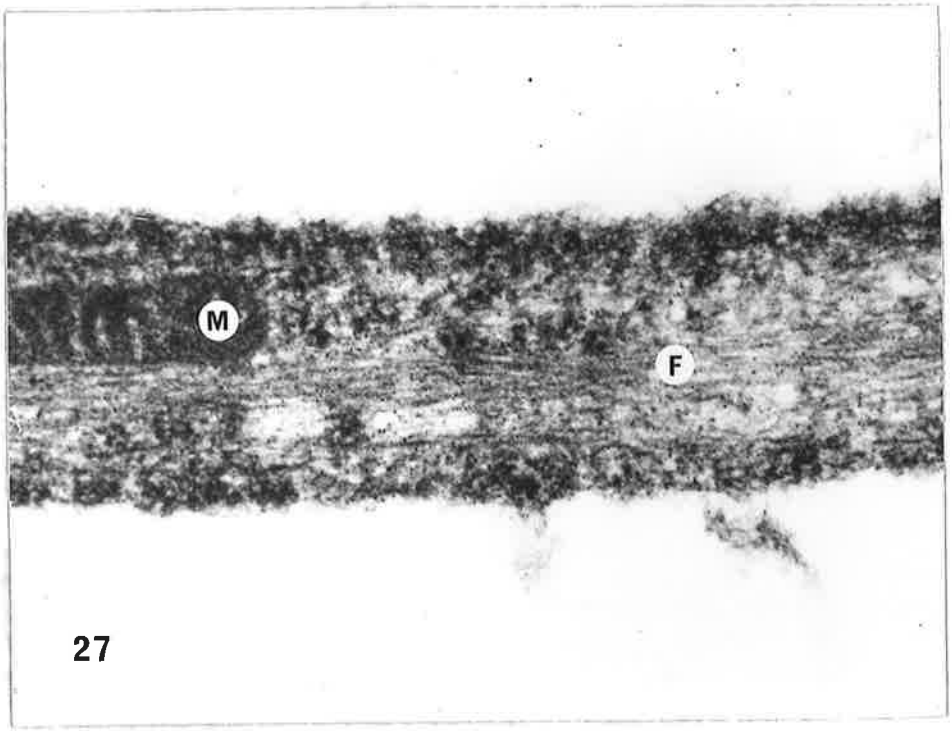


Fig. 26. Fibroblast: Rough endoplasmic reticulum (R) deeply cleft nucleus (N). (7.10^3).

Fig. 27. Adventitia: Fibrillar cellular process with mitochondrion-like inclusion (M), central fibrils (F). Note absence of definitive peripheral plasma membrane. (30.10^3).

The nerves that ran quite close to blood vessels of this type were usually non-myelinated and quite characteristic (Fig.28). Connections between these neural elements and neural-like elements in the tunica intima as described have not been definitively sighted.

Cells corresponding to macrophages were often seen, particularly on the adventitial aspect of type I blood vessels. They had a very irregular adventitial plasma membrane with deep invaginations and cytoplasmic processes which lay flap-like on their surfaces. They were characterised by electron-dense granules. These granules were relatively large and, within a range, fairly consistent in size. Internally these granules gave the impression of having a folded granulo-membranous nature, (Fig.29 and 30). Within the broad limits of macrophage ultrastructural variability these features were consistent with non-specific 'free' macrophages as described by Vernon-Roberts (1972) and Carr (1973). The dark granules corresponded to the lysosomes or H - granules described by Carr (1973).

That the abluminal plasma membrane should be thrown into folds as the vessel adopted the characteristic collapsed state was quite reasonable, but all folds did not seem to have been produced by the same mechanism. Some seemed random and quite passive. Others however, appeared to be highly specific (Fig.31). They consisted of marked projections into the adventitia. Around the ends of the projections there appeared to be condensations of extracellular material. These condensations appeared to be fibrillar in nature. Whether there was some specific

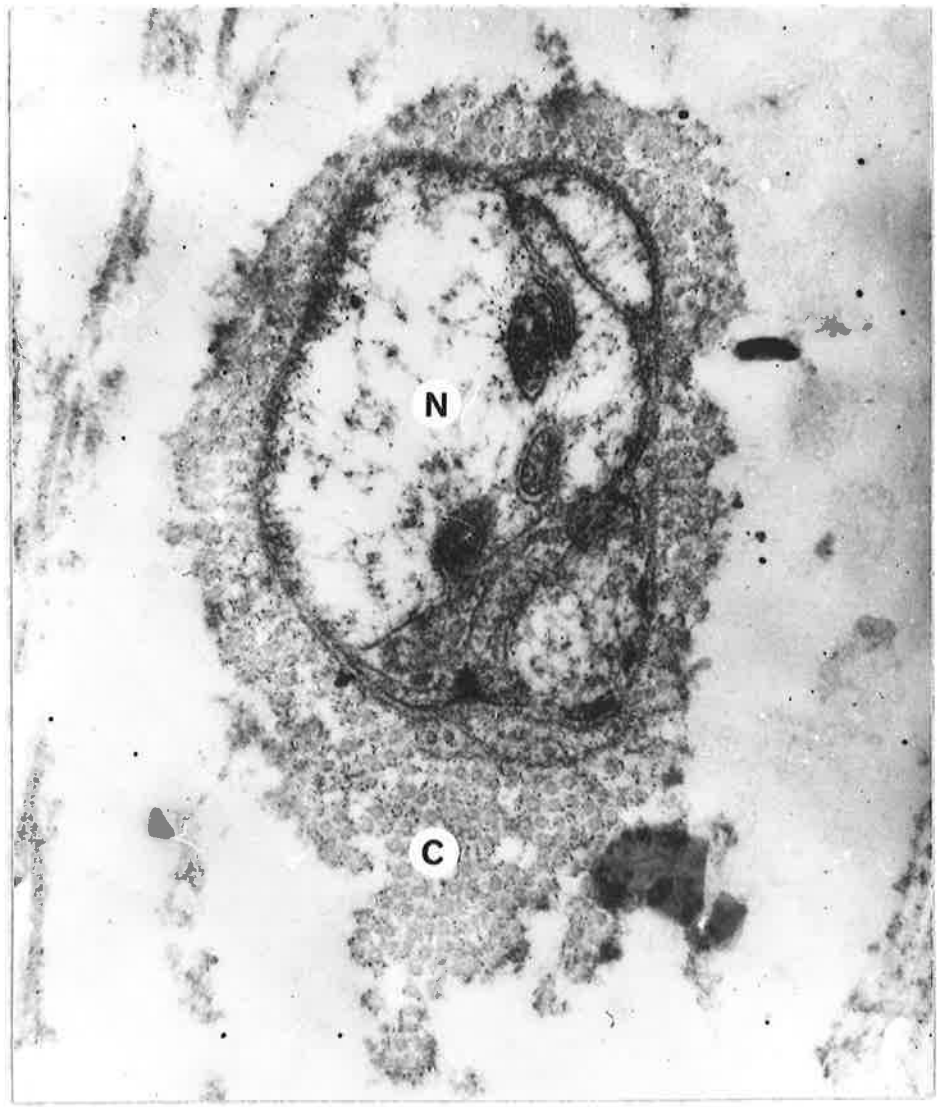
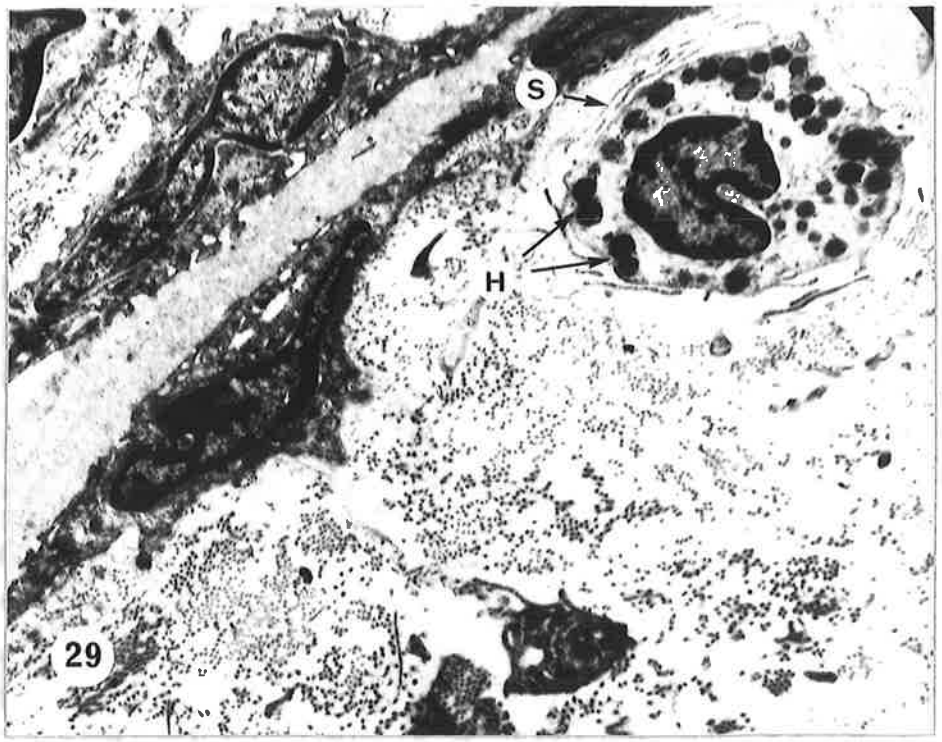


Fig. 28.

Adventitia: Non-myelinated nerve from perivascular connective tissue. Nerve (N), peripheral collagen (C). (20.10^3).



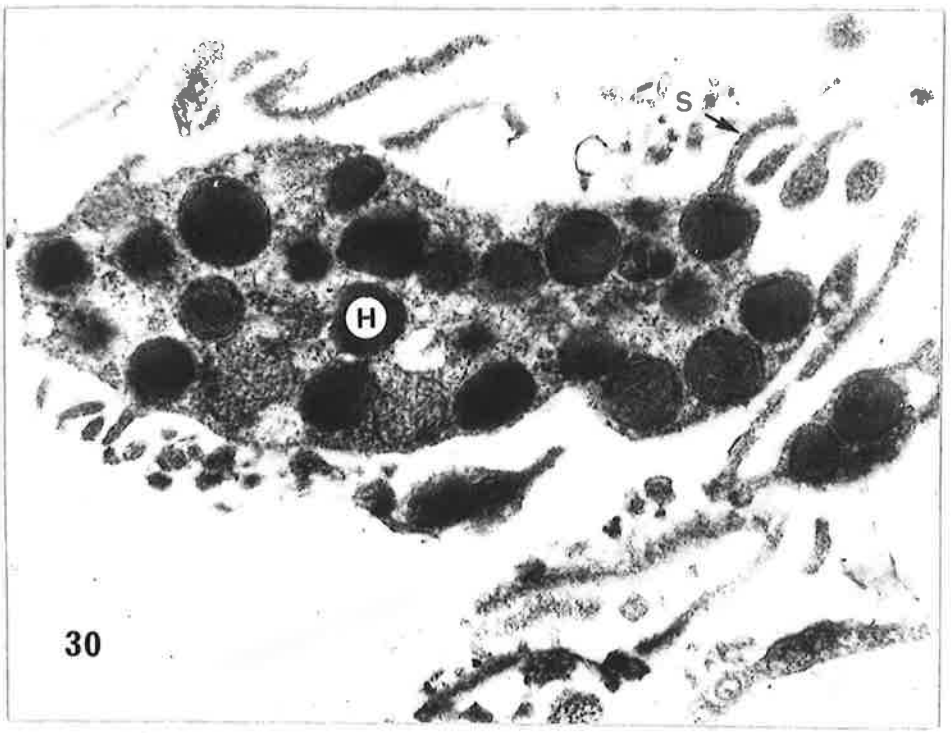
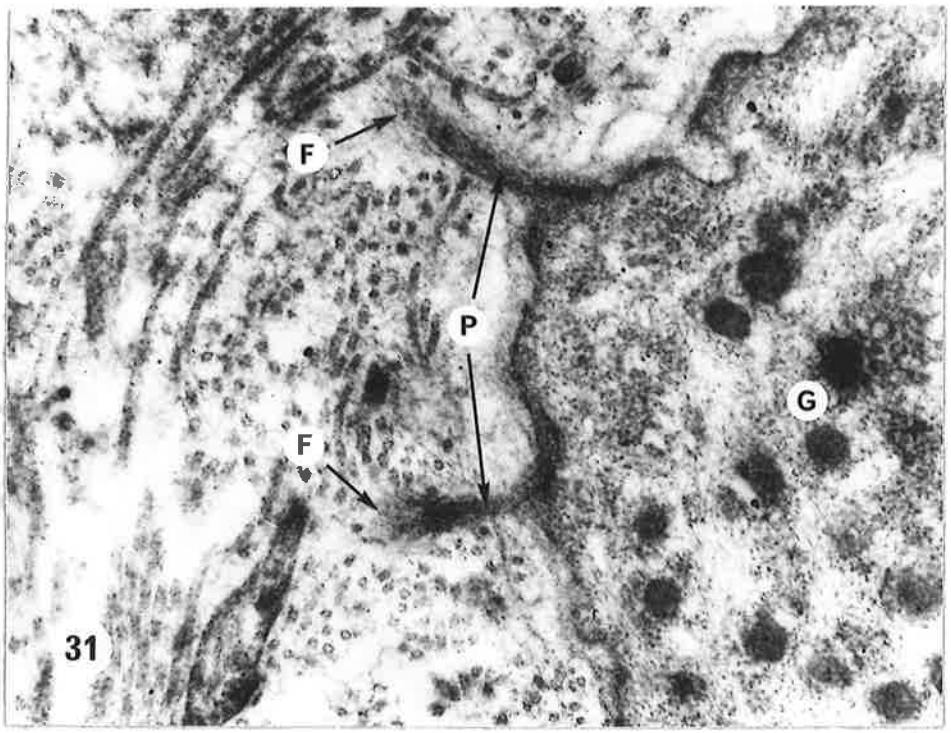


Fig. 29. Free macrophage: Adjacent to vessel. H-granules (H), surface flaps (S). (4.10^3).

Fig. 30. Free macrophage: Granulo-membranous texture of H-granules (H), surface flaps (S). (14.10^3).

connection between these processes and discreet extracellular structures or other cellular structures was not discernible. However, the fibrils were very similar to oxytalan fibrils. The fibrils might merely disseminate into, and interlock with, the general ground substance. From the prominence that these processes adopted when the vessel collapsed it would appear that the process-extracellular fibril feature described had some structural significance. In some specimens an unusual endothelial-adventitial interface was seen (Fig.32). It consisted mainly of an endothelial cell with a general smooth curve to its abluminal aspect and a dense granular material on the immediate abluminal aspect of the abluminal plasma membrane. Structurally the granular material appeared similar to basement membrane. The actual plasma membrane however, was extremely convoluted around this general smooth curve. Whether the convolutions were relatively stable structures or a transitory phenomenon involving extensive vesicularisation it was not possible to say. The appearance was quite marked and certainly atypical even compared with other adventitial areas of the same vessel. In type I vessels it was usual to find limited amounts of mature collagen between the processes of the pericytes and the abluminal aspect of the endothelial cell (Fig.23). Generally speaking, exclusive of their close junctions, the cell processes of the media appeared to be more closely adapted to the intima in type I vessels than other types to be described. The larger the pericyte fragment, the further away from the intima it stood and the more organised,



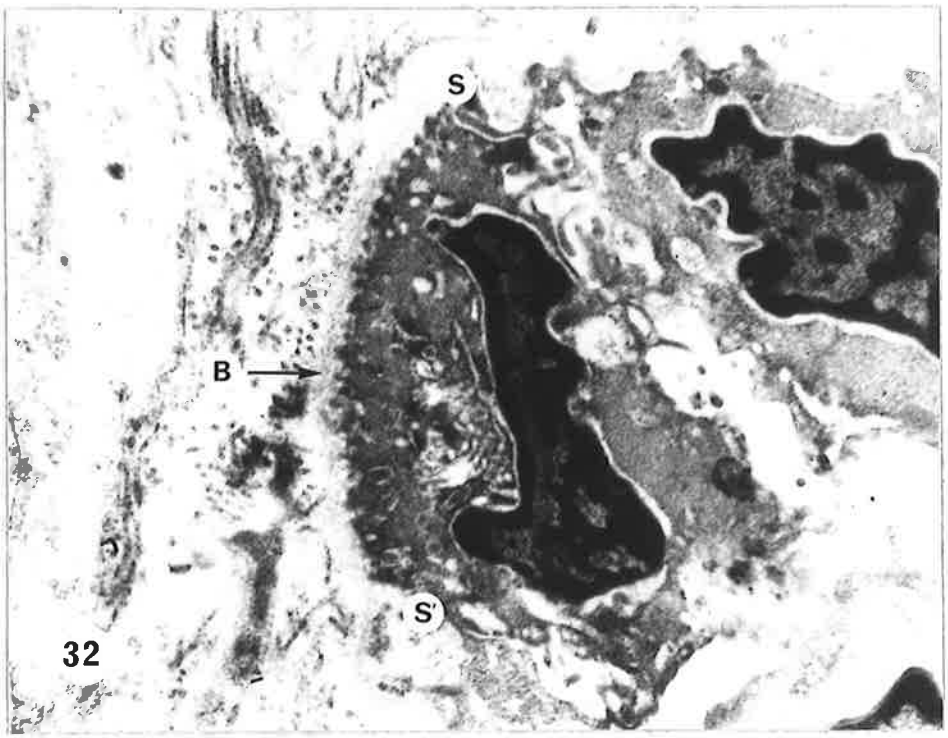


Fig. 31. Type I vessel: Non-passive abluminal projections (P), fibrillar condensation (F), endothelial cytoplasm with dark granules (G). (10.10^3).

Fig. 32. Type I vessel: Occasional abluminal presentation. Smooth curve (S-S'), vesicularisation and basement membrane thickening (B). (8.10^3).

mature collagen was to be seen between the two.

The propensity for endothelium to swell and encroach upon the lumen of microvessels was apparent in this study. Where only one, or at least a minority, of the endothelial cells had so reacted the process in a physical sense was easily discernible. However, when all endothelial cells had reacted the picture became confusing, to the point that it was problematical if the structure being contemplated was vascular at all. The obliteration of the lumen and its contents was, on occasions, so profound that an identification of a structure as 'vascular' was made on relatively empirical grounds. In difficult circumstances the following criteria were adopted:

1. A completely enclosed 'potential lumen space' could be identified.
2. Cellular components enclosing the 'potential lumen space' were enclosed by a complete basement membrane.

In cases of provisional identification of a vascular structure, demonstrating swelling of all endothelial cells, the structure was often circular with less evidence of gross collapse. The numbers of 'endothelial' nuclei varied from none up to three or four per vessel. Identification was often provisional between the limits of the type just alluded to and obvious vascular structures.

BLOOD VESSEL TYPE II

Vessels classified as type II in this report differed from type I in the following respects:

1. The periendothelial cellular investment was more obvious, with cell nuclei and perinuclear cytoplasm usually being seen.
2. Between pericyte nuclei, perinuclear cytoplasm of same and the abluminal aspect of endothelial cells, a specific arrangement of extracellular structures occurred.
3. Because of the differences 1 and 2 cited being seen in vessels:
    a) where the periendothelial layer of cell bodies and processes was incomplete.
    b) the periendothelial cellular investment was complete.

Type II vessels were divided accordingly into IIa (Fig.33) and IIb (Fig.34).

Tunica intima:

There appeared to be no structures in type II endothelium that did not exist in type I. There were however, some structures that did not appear in type II which were described under type I. Intraendothelial fenestrae and transendothelial holes and channels were not seen in type II. Analogous structures included interendothelial junctions, luminal flaps and dark granules.

The nucleus was similar with a promient nuclear membrane, nuclear fenestrations and peripherally condensed chromatin. An irregular nuclear outline with invagination of clefts was prominent and the nuclei were flattened longitudinally with the vessel. In the appropriate plane of

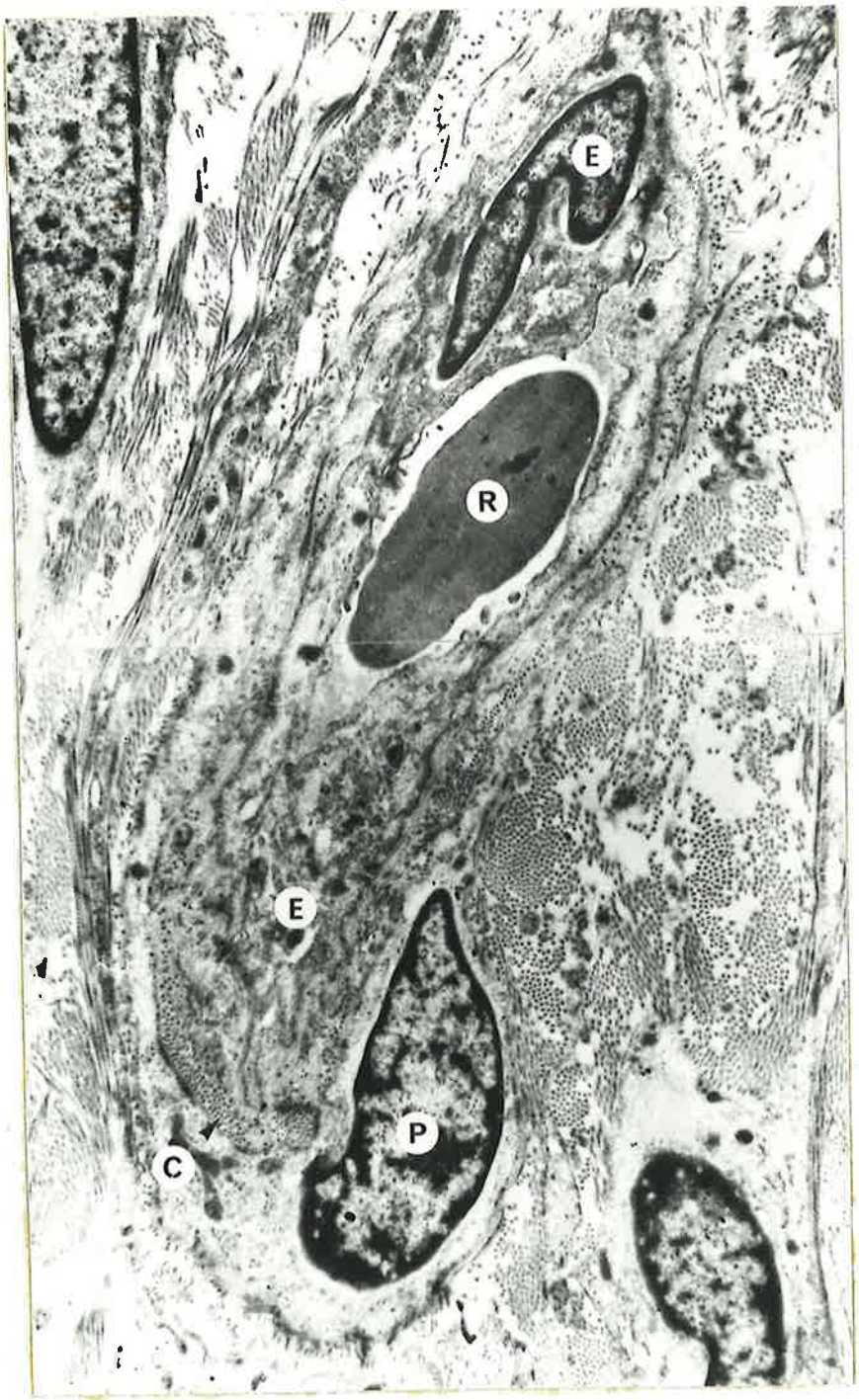


Fig. 33.

Type IIa vessel: Endothelial nucleus and cytoplasm (E), pericyte nucleus (P), erythrocyte (R), collagen (C). Composite electron micrograph. (4.10^3).

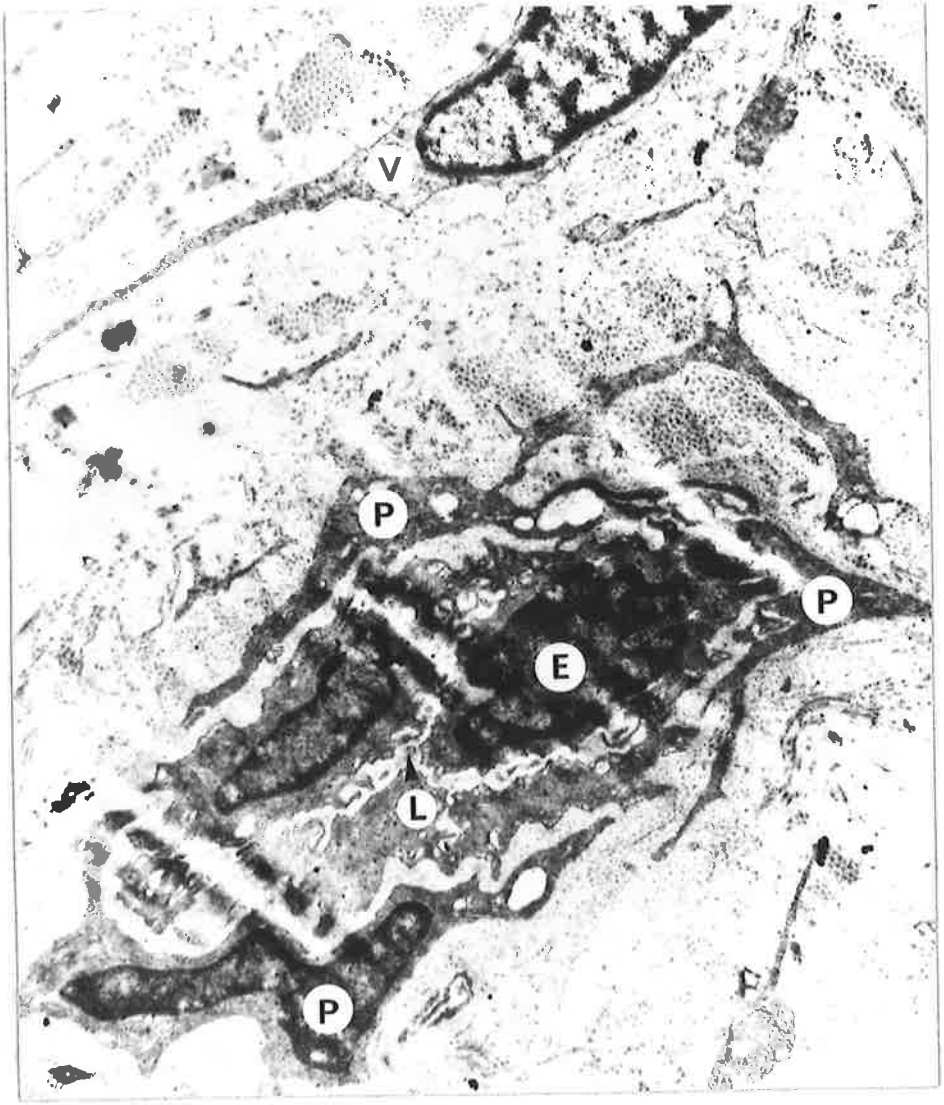


Fig. 34.

Type IIb vessel: Pericytes (P), endothelial cells (E), lumen (L), veil cell (V). (2.10^3).

section single nucleoli were seen.

The cytoplasm appeared similarly constituted with dark granules, abundant fibrils, peripheral vacuoles and luminal vesicles. In type II the degree of collapse was most extreme and the luminal plasma membrane could be thrown into an extreme number of small folds (Fig.35). On occasions vesicularisation of the luminal plasma membrane was profound (Fig.36). Basement membrane appeared to be directly analogous between types I and II.

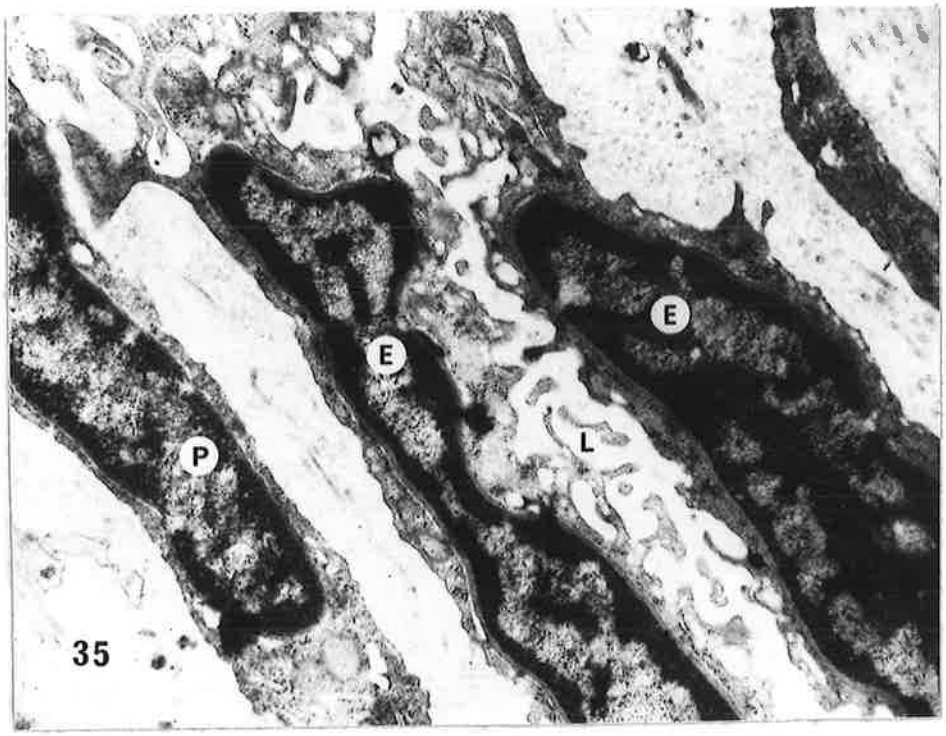
Tunica media:

Where cell processes of pericytes were seen close to the abluminal aspect of the intima the types of junction entered into were analogous to type I i.e. three types:

1. finger-like,
2. horizontal depression,
3. perpendicular invagination.

There was a fourth type, much more common with type II, involving an abluminal process of the intima opening out into a mushroom or 'T' shape against the pericyte (Fig.37). This type was not seen exclusively with type II but it was only at this level that it occurred frequently enough to justify a separate description.

Differential staining between cells was common to varying degrees in all vessel types but in type II it was at its most obvious (Fig.38). Most commonly (but not invariably) the pericyte processes and cell bodies were stained more darkly than the cells of the intima, or veil cells in the adventitia.



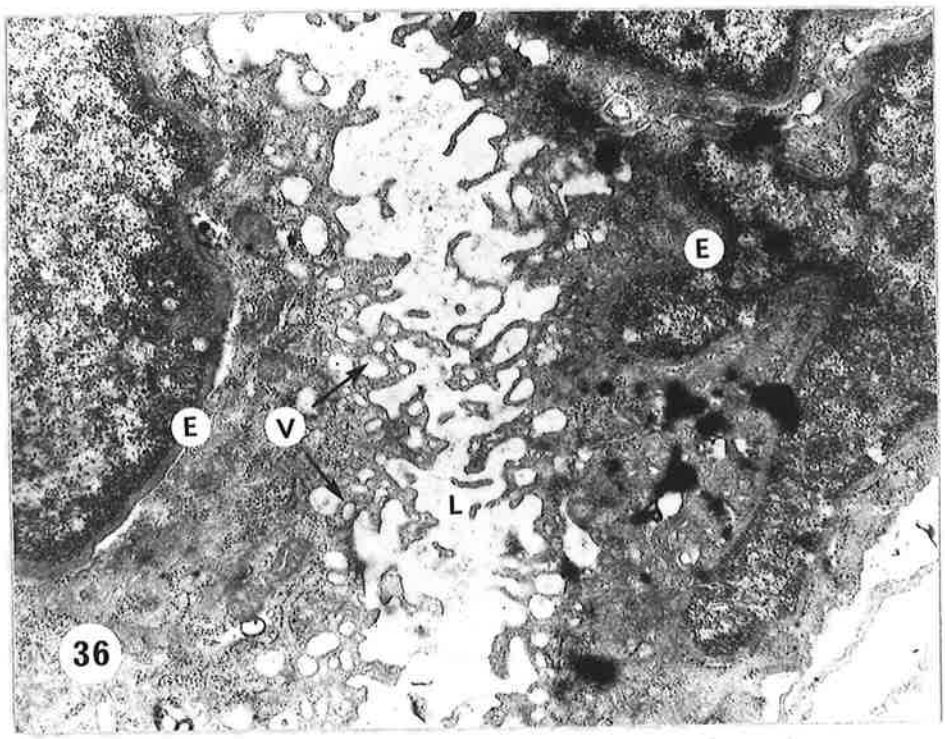


Fig. 35. Type IIa vessel: Irregular luminal endothelial border. Endothelial cells (E), lumen (L), pericyte (P). (2.10^3).

Fig. 36. Type IIa vessel: Intensive luminal vesicularisation. Vesicles (V), endothelial cells (E), lumen (L). (16.10^3).

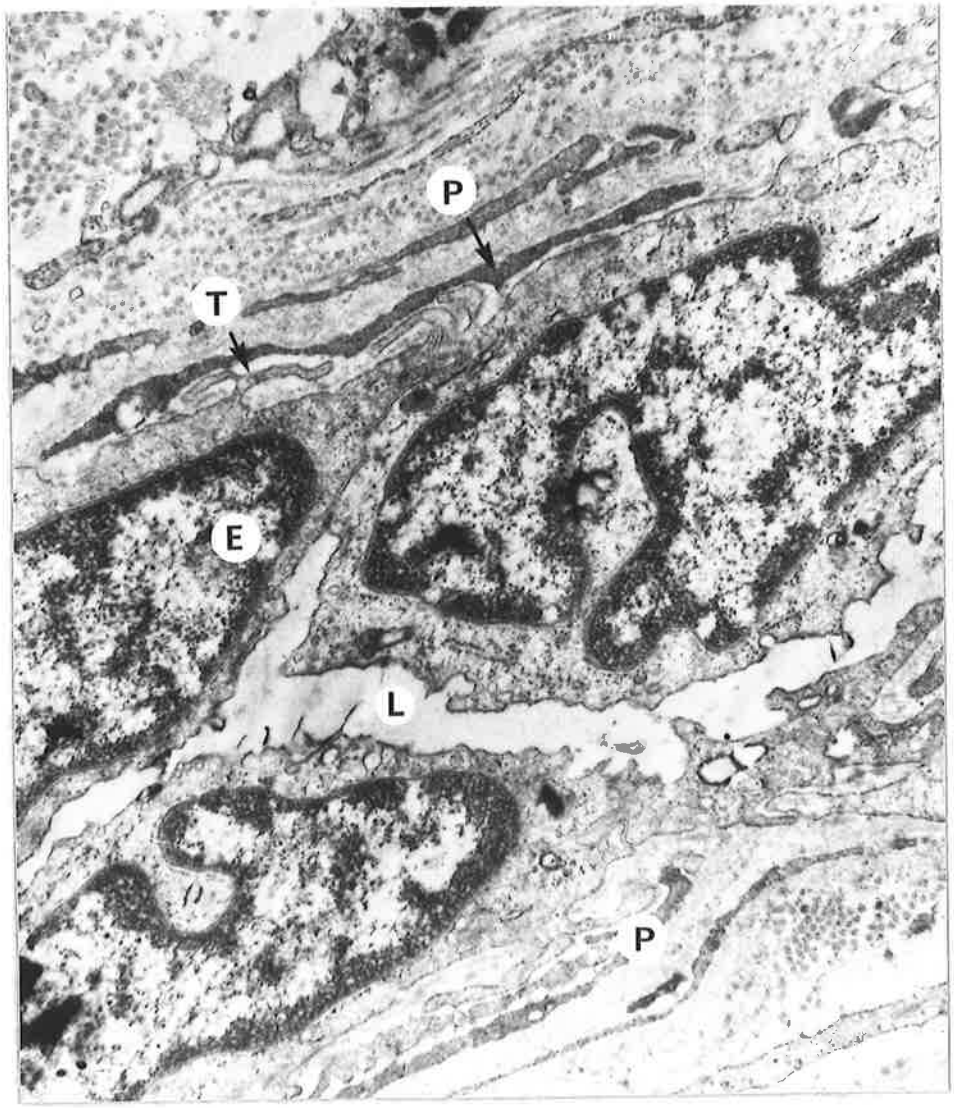


Fig. 37.

Type IIb vessel: 'T' type endothelial-pericyte approximation. Endothelial nucleus (E), pericyte processes (P), lumen (L), abluminal 'T' process of endothelium (T). (8.10^3).

Pericytes

The definitive cell of the media was the pericyte and at this level observations were not limited to processes only, thus a full description is appropriate.

The nucleus was flattened in accordance with the orientation of the pericyte to the vessel lumen (Fig.38). Peripheral condensation of chromatin was apparent as was a nuclear membrane with fenestrations. The membrane, however, was less obvious in pericytes and fenestrations seemed fewer than in endothelial nuclei. The nuclei though irregular were less irregular than in cells of the intima.

The cytoplasm seemed more granular and less fibrillar than endothelium (Fig.38). Dark granules were present, usually in those portions of cytoplasm closest to the nucleus. Rough endoplasmic reticulum was seen near the nucleus (Fig.38) but, apart from dark granules, cell processes did not seem overly endowed with organelles. The cell was enclosed by a continuous basement membrane.

FIBRO-COLLAGENOUS LAMINA

It was between the approximal surfaces of pericytes and endothelial cells that the most striking structures were seen. The abluminal surface of the endothelial cells was much more convoluted than the intimal aspect of the pericyte (Fig.39). The folds of endothelium that were thus thrown up were quite regular and between these folds were seen considerable amounts of mature collagen cut in cross section. At the abluminal tips of the endothelial folds the cytoplasm appeared to be

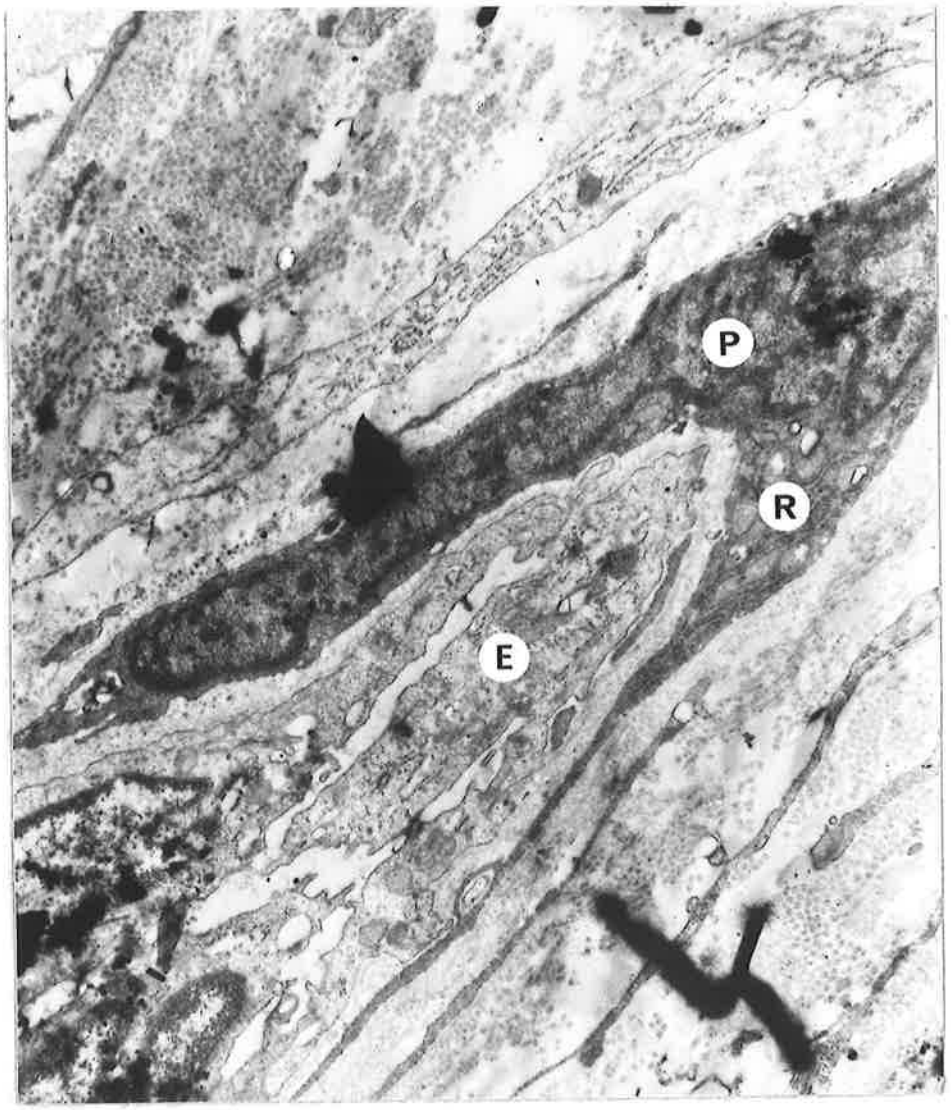


Fig. 38.

Type IIb vessel: Pericyte detail; differential staining between pericytes and endothelial cells is apparent. Elongated, adapted pericyte nucleus (P), endothelial cell (E), rough endoplasmic reticulum (R). (6.10^3).

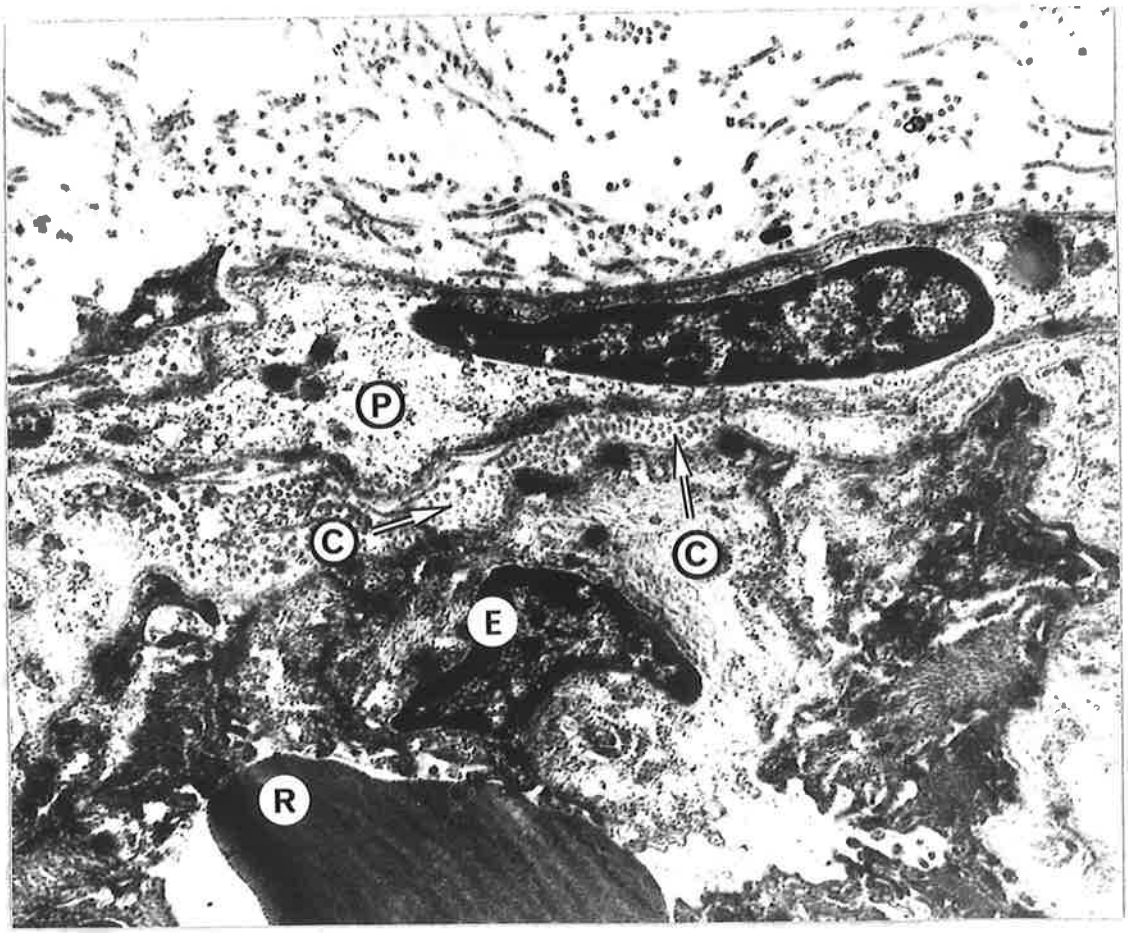


Fig. 39.

Type IIa vessel: Demonstrating organised mature collagen between pericytes and the abluminal aspect of endothelium. Pericyte (P), endothelial cell (E), erythrocyte (R), collagen (C). (6.10^3).

condensed. The continuity of the basement membrane was not disturbed however. The discreet bundles of collagen fibrils seen between the projections gave the impression of a tunnel-like arrangement, either longitudinally or as a large-pitch helix. These characteristic patterns of cells and collagen seemed to be confined to areas where a cell body of a pericyte was adjacent to the intima, rather than simply a pericyte process (Fig.40). The impression was gained that there was a strong functional relationship between the collagen in this location and the cellular components of the vessel. Varying amounts of collagen were usually seen between pericyte processes and the intima but nowhere else was the relationship so orderly.

Tunica adventitia:

The adventitia essentially comprised fibroblast cell bodies and processes (veil cells), collagen and nerves. The veil cells were typical in that they possessed no basement membrane. The perinuclear cytoplasm contained rough endoplasmic reticulum and a normal admixture of organelles. The veil cell processes however were of a dense fibrillar nature as described under type I. The degree of activity of veil cells varied considerably as exemplified by the cell shape, disposition and numbers of organelles seen. In type IIa vessels, nerve-like elements were seen under the intimal basement membrane, in very close approximation to the endothelium.

The distribution of type II vessels, particularly type IIb, was less ubiquitous than type I. Type II was almost exclusively confined to the middle annular third of

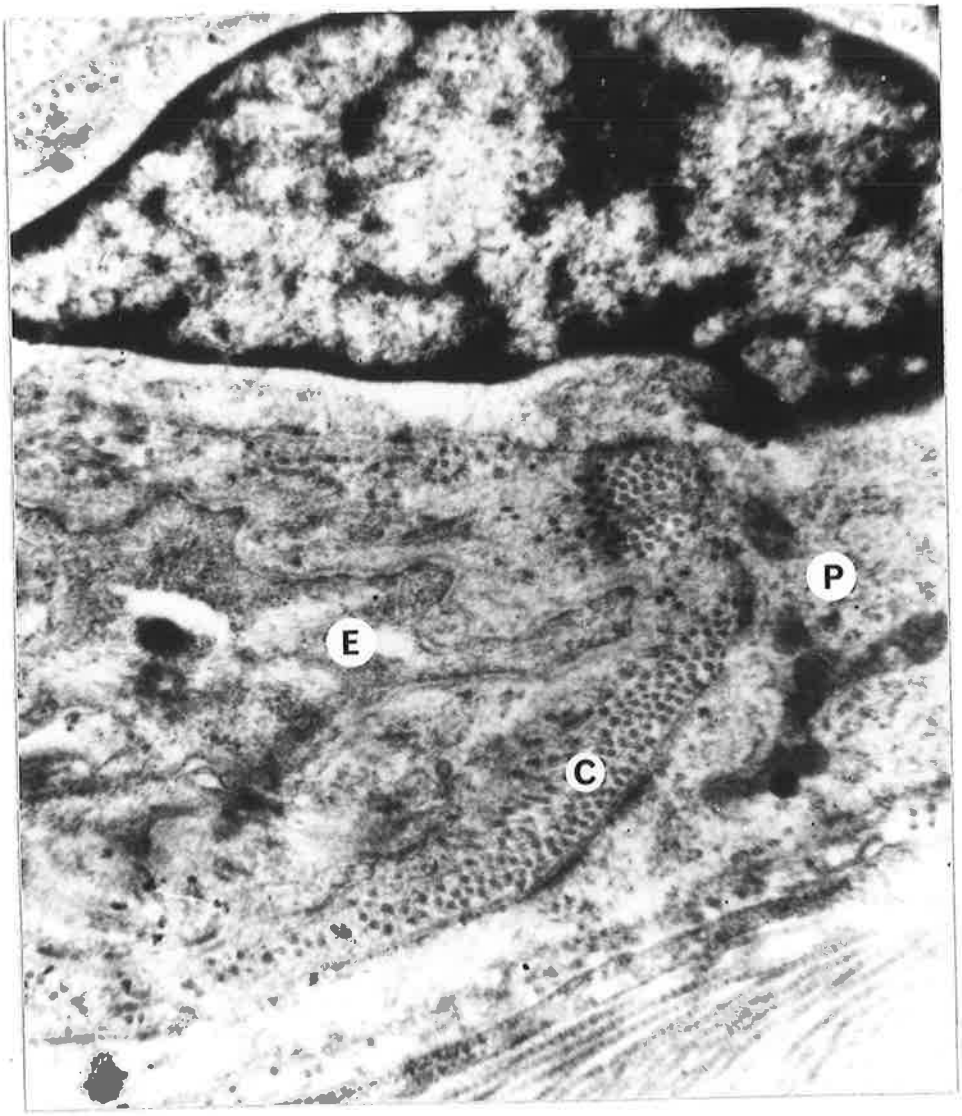


Fig. 40.

Type IIa vessel: Internal fibro-collagenous lamina. Endothelial cell (E), pericyte (P), collagen (C). (10.10^3).

the ligament space. A greater tendency to be located in areas of looser connective tissue between the principal fibre bundles was noted. Nerves in the regions close to type II vessels tended to be a mixed collection of myelinated and non-myelinated nerves (Fig.41). The latter were analogous to nerves which were identified running close to type I vessels.

Vessels, particularly type II, tended to be collected in clumps. While scanning, much relatively avascular tissue could be seen but when a type II vessel was located there tended to be several of them within the same grid square (200 mesh). About these collections of vessels it seemed that nerves and fibroblasts tended to be aggregated in larger than average numbers.

BLOOD VESSEL TYPE III

These vessels were the largest to have been identified in this study. They were characterised by a complete periendothelial cellular investment having a multilayered appearance. Type III were confined to the middle annular third exclusively, and tended to be axially oriented. The principal differentiation between types IIb and III was the nature of the periendothelial cellular investment.

In type IIb only the degree of completion of the periendothelial cell layer was critical whereas in type III vessels the principal component of the layer was cell bodies rather than mainly cell processes as in type IIb (Fig.42).

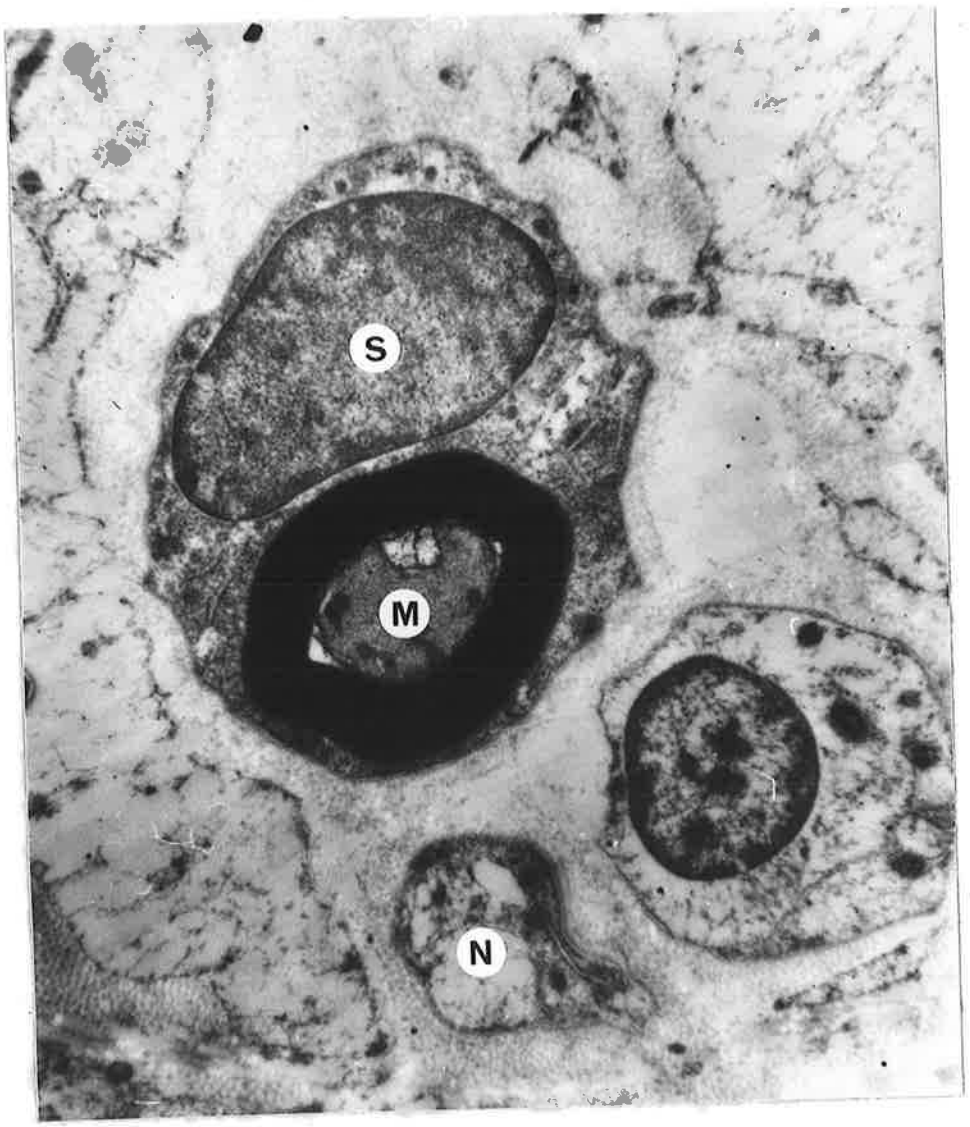


Fig. 41.

Adventitia: Nerves close to blood vessels. Non-myelinated (N), myelinated (M), Schwann cell nucleus (S). (4.10^3).

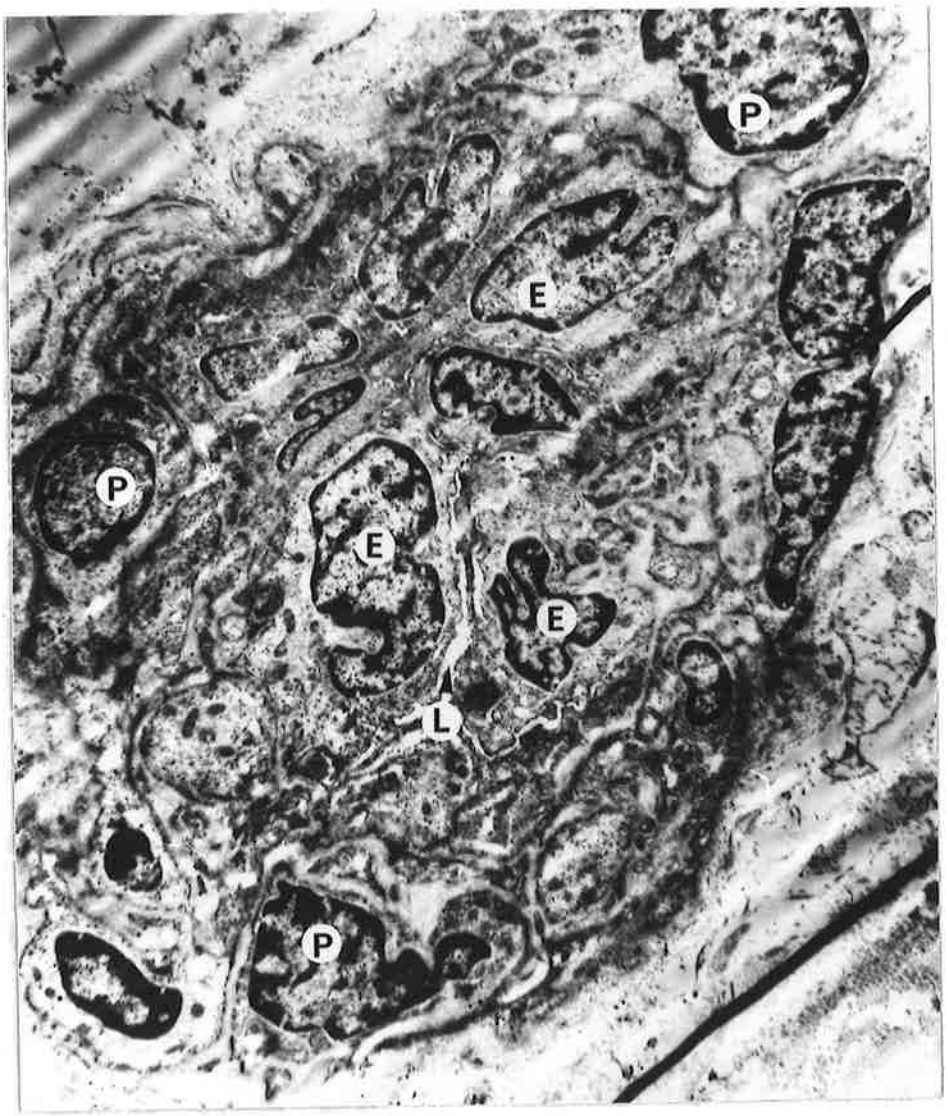


Fig. 42.

Type III vessel: Cellular tunica media.

Pericytes (P), endothelial cells (E), lumen (L).

(1.10^3).

Tunica intima:

At this level the degree of collapse was complete, usually only a thin, linear lumen was seen. The overall outline was approximately circular (Fig.42).

Nuclei tended to have lost most orientation to the vessel lumen in that they did not show any marked flattening when the vessel was cut in transverse section. Nuclear outline was irregular with a nuclear cleft. Peripheral chromatin condensation was observed. The nuclear membrane was easily discernible and fenestrations were present. There seemed however, to be fewer fenestrations per nucleus than in smaller vessel types.

Cytoplasmic contents seemed typical of periodontal blood vessels. Limited numbers of organelles and a routine complement of dark granules were seen. In contrast to other blood vessels, abluminal vacuoles and luminal vesicles seemed less prevalent, although still present in substantial numbers. Junctions between endothelial cells were sinuous and luminal flaps near the luminal aspect of the junctions were seen. It must be noted however that in type III vessels the collapse was so profound that at the extremities of the collapsed lumen it could be difficult to distinguish the lumen from the interendothelial junctions. Furthermore, the ability of endothelial cells to form junctions with cells with which they came into contact, as elucidated by Wolff (1977), can only serve to make more guarded any definitive conclusions drawn from observations presented here.

The abluminal endothelial plasma membrane was

irregular but the impression was gained that this was more a passive process due to collapse rather than a demonstration of underlying ultrastructure. Around the endothelial cell's abluminal aspect there was a complete, continuous basement membrane.

Tunica media:

Cells of the media tended to show more orientation to the vessel form than did intimal cells but they were still plump cells with only a minor orientation.

On a nuclear or cytoplasmic basis there seemed little, apart from shape, to differentiate these cells from any other pericytes. One feature presented however that had not been observed before. Pericytes in type III vessels showed a propensity to enter into moderately complicated interpericytic close approximations (Fig. 43). They seemed to be analogous to the 'finger-like' junctions observed between cells of the media and intima in other vessel types. Close approximations between pericytes and endothelial cells were less numerous but were of routine types described in other vessel types.

Tunica adventitia:

Type III vessels had an adventitia equivalent in all respects to type II vessels.

These vessels were found in the same locality i.e. middle annular third in both the coronal area and the middle third (apicocoronal). The frequency of sighting such vessels was quite low. It is estimated that the functional diameter of this vessel (Fig.42) would be of the order of 35 to 50 μm.

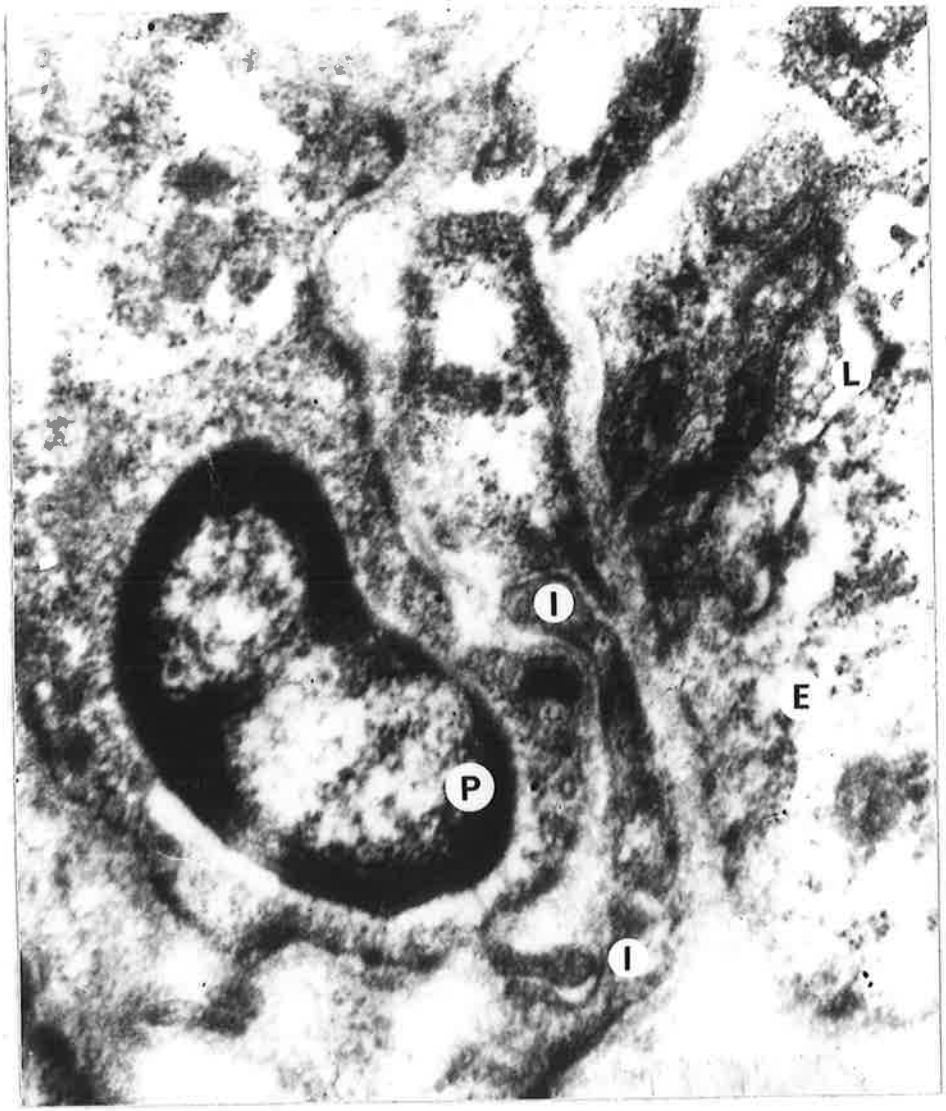


Fig. 43.

Type III vessel: Interpericytic close approximations. Pericyte (P), interpericytic close approximations (I), lumen (L), endothelium (E). (12.10^3).

LYMPHATIC TYPE VESSELS

Ascribing lymphatic status to a vessel in the light of problems of differentiation as explained by Casley-Smith (1973 and 1977b) is fraught with uncertainty. The general criteria used in this study were as follows:

1. A large potential diameter to wall thickness ratio.
2. Relatively sparse periendothelial cellular investment.
3. Presenting a markedly stellate picture when collapsed but with few (3-5) points.
4. Minimal blood cellular contents identifiable.
5. The presence of a larger number of relatively open interendothelial junctions.
6. Relatively sparse basement membrane.

Vessels complying with those criteria were present in some numbers. They seemed to be rather more variable in their orientation to the tooth surface than did blood vessel types. There seemed no profound differences between the coronal and mid-root specimens (Fig.44).

Tunica intima:

Endothelial cells of lymphatic-type vessels were very thin and flattened. While substantially enclosed in a basement membrane it was not possible to say the enclosure was complete. The membrane was thinner and much less distinct.

Nuclear characteristics were typical of other endothelial cells i.e. peripheral chromatic condensation, prominent nuclear membrane, nuclear fenestrations, nuclear

clefts and generally oriented, elongated form.

Cytoplasmic appearances were non-specific, dark granules were not abundant. Occasional mitochondria were seen. Vacuoles in the abluminal cytoplasm were not prominent but luminal vesicles were moderately common.

Inter-endothelial junctions tended to appear more direct, less sinuous and less complicated. Open junctions as described by Casley-Smith (1977a) were seen but were not a feature. It is possible that the lymphatics seen were not of the 'initial' type where a high proportion of open junctions has been described.

Tunica media:

Considering the potential distended size of some lymphatic type vessels the periendothelial cellular investment was scant. Close approximations between the intima and media were fewer and mostly of the simple finger-like type. The scarcity of lymphatic pericytes means that observations on their ultrastructure are based on fewer examples. They appeared however to be largely analogous to blood type I and II pericytes.

Tunica adventitia:

The adventitia comprised of a collection of fibroblasts, nerves, cell processes, collagen and occasionally blood vessels.

ASSOCIATED ADVENTITIAL STRUCTURES

The principal structures deserving comment and exemplification include:

1. Cells and cell processes.
2. Extracellular fibres.

CELLS: Certain cellular structures are mentioned for a variety of reasons.

It may be necessary to differentiate them from vascular tissue with which they share some features. The two such structures most frequently seen were fat cells and some neural structures:

a). Fat cells were seen as large, roughly circular representations of a probably spheroidal structure (Fig.45). They seemed to be a cell-bound or intracellular globule of uniform, moderately electron-dense material. There seemed to be only one associated cell. When the section was not diametric the appearance and size was not inconsistent with the electron density and general appearance of red blood cells.

b). Neural components took a multiplicity of forms in the periodontal ligament. On occasions being circular in cross section within the size range of periodontal vessels (Fig.46). At other times they might resemble a distended fenestrated capillary (Fig.47). Critical observation of the contents usually presented definitive reasons for a specific vascular or non-vascular interpretation.

Cells seemingly functionally related to blood vessels were veil cells. There did not seem to be any specific distinction, other than proximity, between veil cells and general periodontal fibroblasts. However, the adventitial collagen and cells that persisted in close proximity to the vessel when it collapsed would seem to

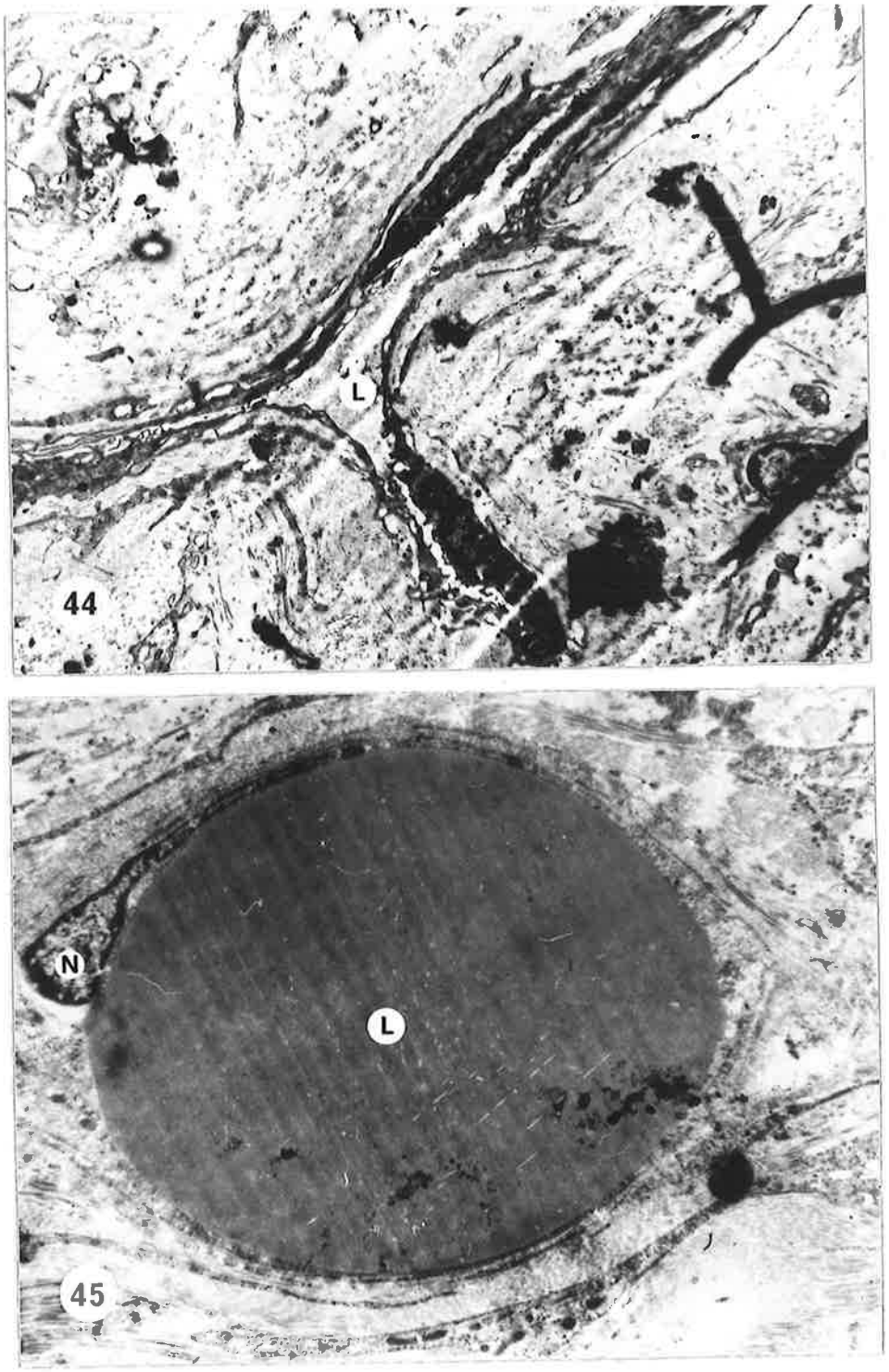
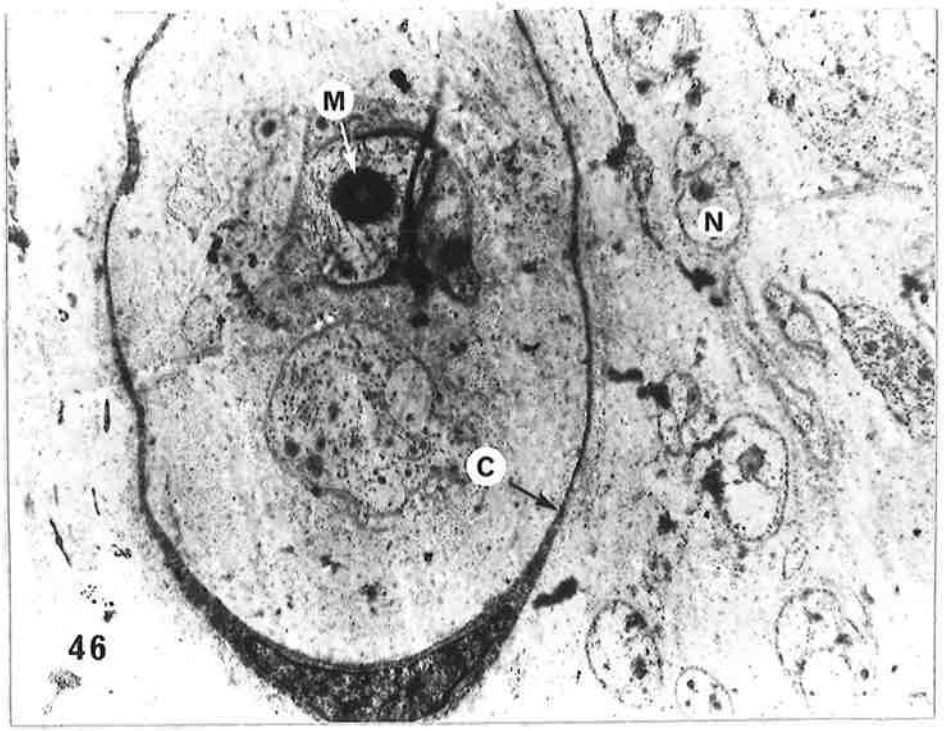


Fig. 44. Lymphatic-type vessel: Lumen (L). (5.10^2).

Fig. 45. Adventitia: Periodontal fat cell. Lipid (L), fat cell nucleus (N). (1.10^3).



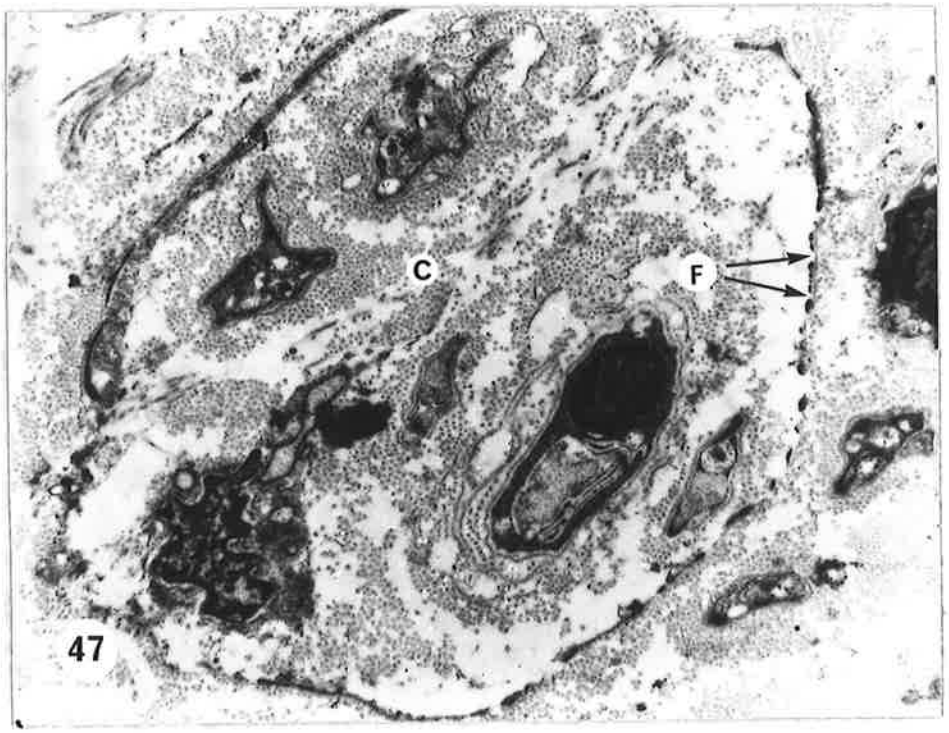


Fig. 46. Adventitia: Neural-type structure. Cellular envelope (C), myelin-like structure (M), non-myelinated nerves (N). (1.10^3).

Fig. 47. Adventitia: Neural-type structure with 'fenestrated' envelope (F), collagen within the envelope (C). (3.10^3).

indicate that those structures were part of the vessel wall.

CELL PROCESSES: Nerve processes were closely associated with vessels of all types. Both myelinated and non-myelinated nerves were seen. They occurred singly and in collections. Usually collections of nerves were an admixture of both types.

Processes that were probably fibroblastic in origin were regularly seen. They appeared as nearly parallel sided and occasionally branched. At their periphery they were relatively electron-dense and amorphous. Centrally they appeared to consist of fine fibrils with a wavy consistency but the net direction being parallel to the axis of the process. The interior structure was so similar to the reported T.E.M. appearance of oxytalan as shown by Sheetz et al. (1973) that the identification of the structures as fibroblast processes must be construed as provisional. Sometimes inclusions that resembled nerves in cross-section or, alternatively, mitochondria were seen.

EXTRACELLULAR FIBRES

Collagen, of course, was by far the most common such fibre. In transverse section it appeared to consist of a number of nearly circular fibrils. In longitudinal section the characteristic cross banding was seen (Fig. 48). As mentioned, some collagen is apparently an ulta-structural feature of the tunica adventitia. The peculiar relationship that mature collagen had between tunicas media and intima in type II vessels is a further connection

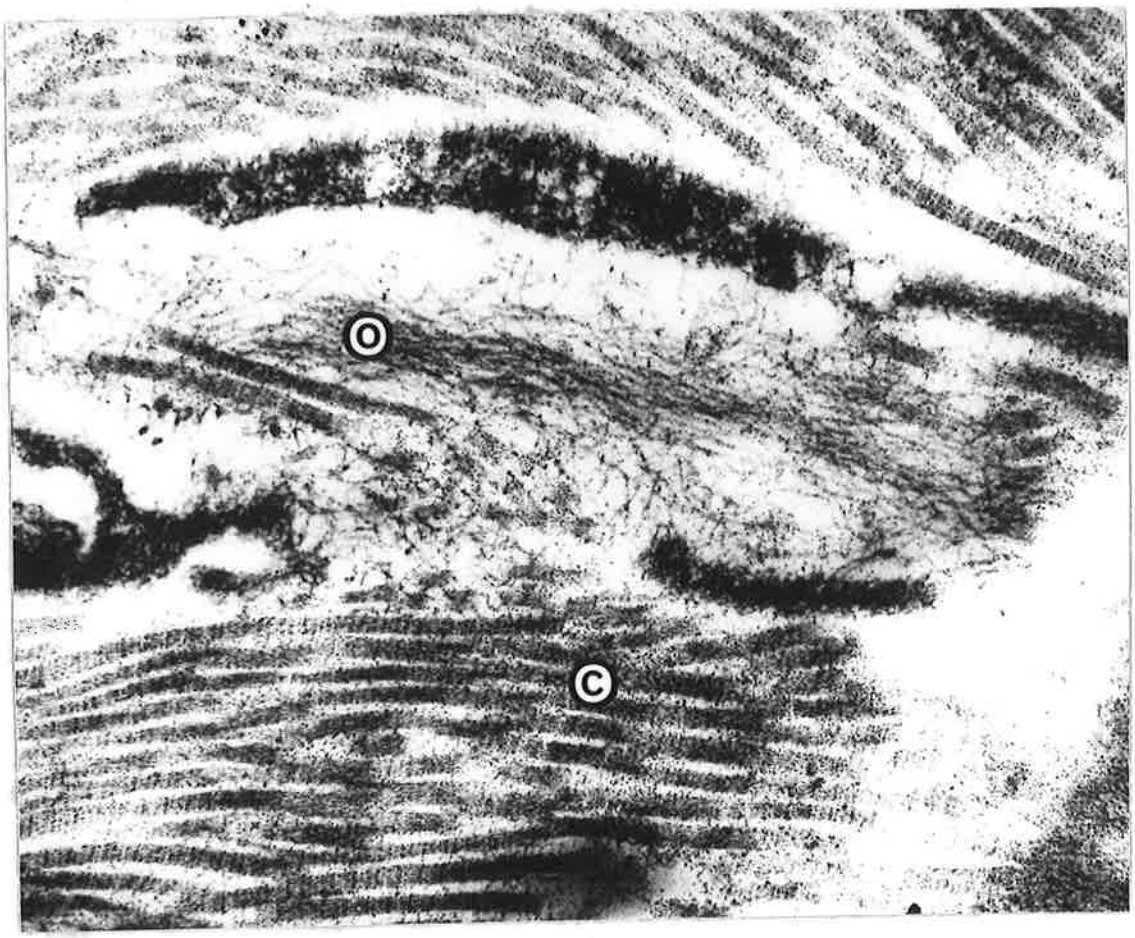


Fig. 48.

Extravascular fibres: Collagen with cross banding (C), reticular oxytalan fibrils (O). (17.10^3).

between vessel ultrastructure and connective tissue.

Extracellular fibrils very similar to the Sheetz et al. (1973) picture of oxytalan were seen. Such fibres have been identified at the cemento-periodontal interface (Fig. 48) and in the adventitia close to vessels.

Figure 48 shows collagen with cross banding near the dentoperiodontal interface together with oxytalan seen as a much more reticular pattern. This is analogous to the material shown by Soames and Davies (1978).

SECTION 6

DISCUSSION

The amount of material considered in this study numbered approximately 800 sections. Sections examined came from all areas within the blocks from the apical to the coronal aspect. Many sections yielded no definitive vascular structures at all, particularly those toward the apical aspect of the specimens. It must be remembered that 'apical' in this sense is an aspect not a locality. The farthest apically that specimens were taken was about midroot. There is fairly uniform agreement that the midroot area is the least vascular of any segment of the periodontal ligament (Saunders and Rockert 1967).

That the project has been successful in its stated aims is demonstrable. Firstly, by many examples of human periodontal vessels and secondly, by the detection of characteristics possibly peculiar to the periodontal ligament or like tissues. The questions it has raised rather than the answers it proposes form a most interesting part of the project. The questions are related to two facets of the project. How best to prepare material for examination in T.E.M.? In other words, technical and methodological problems. The second aspect is essentially centred around interpretation of findings. The principal obstruction to objective analysis is topographical control. Topographical control is the ability to accurately locate in a cartesian coordinate system features, or sequences of features, irrespective of technique of examination. It

can immediately be seen that perfect topographical control is an ideal to aim at rather than a serious short term goal. Thus observers are always operating within an elective but limited segment of topographical control. It is critically important to keep in mind that these elective limitations impose observational limitations which, while not invalidating conclusions, do tend to limit that validity. It can be seen that anatomical validity in this study falls between Rhodin's elegant technique and previous studies utilising periodontal remnants scraped from acutely avulsed teeth. As for laboratory technology, the permutations and combinations of the processing variables are extremely numerous.

NOMENCLATURE

The structure that lies between the dental cementum and the alveolar bone goes under two common names:

1. periodontal ligament,
2. periodontal membrane.

Noble (1969) considered that in the light of the microscopic orientation of the connective tissue that the term 'membrane' was inappropriate and that the term 'periodontal ligament' was more correct. The term periodontal ligament has been adhered to throughout this report. Noble also suggested that there were two prime facets of periodontal ligament function:

1. to attach the tooth to its investing alveolus,
2. a more complex function of receiving and transmitting the forces generated during

dento-facial function.

Melcher and Walker (1976) list the components of the periodontal ligament as being typical of connective tissues. The structure comprises:

1. cells,
2. extracellular substances,
   a) fibres
   b) ground substance
3. blood vessels and lymphatics,
4. nerves.

It is the extracellular substances that have been of prime interest to Melcher and Walker. On a proportional basis the amount of collagen in periodontal ligament is much lower than other connective tissues, for example tendons. These authors felt that the role of ground substance may be very much underrated in periodontal function.

The principal concern of this project is a consideration of the ultrastructural aspect of the vascular components of the periodontal ligament. The second objective is to consider comparatively these structures with vascular structures reported in other tissues.

As anyone who has had, or performed, a dental extraction can testify, the resultant haemorrhage demonstrates that the vascular connections of the periodontal ligament seem extensive. The small volume of tissue involved and the highly intertwined nature of its components render it difficult to examine one aspect of the whole, such as the vessels, without at least making passing observations of associated structures.

Relatively few references were located whose prime objective was a study of periodontal ligament vasculature. Of these, fewer were found where the study was of a histological nature. Electron microscopic findings are rarer still. In the light of the statements of Saunders and Rockert (1967) it can be concluded that the understanding of periodontal ligament ultrastructure and, by extrapolation ligament function, is in its infancy.

Considering the highly specialised physical and physiological environment of the periodontal ligament it would be surprising if there were not some discernible evidence of adaptive variations of its components. The atypical proportion of connective tissue elements found by Melcher and Walker (1976) is possibly an example of specialised morphology and composition reflecting specialised function. To facilitate the comparative aspect of this project, the literature review was divided into two parts:

1. the structure and function of the microvasculature in general, and
2. the structure and function of the periodontal vasculature in particular.

The advent of the T.E.M. with its greatly superior resolution has opened new horizons for the microanatomist and histopathologist. The T.E.M. has allowed an unprecedented look at biological ultrastructural detail.

The vessels considered in the literature review ranged from the smallest arterioles with a continuous internal elastic lamina through the terminal vascular bed

to small collecting veins. This range probably is somewhat arbitrary. Overall, in the vascular system, the larger the vessel in physical dimensions the less important it is to the function of the vascular system as a whole, which is biochemical exchange across the microvascular membrane. Any system of vasculature can be subdivided into two parts:

1. those components of the vascular system whose role it is to conduct blood to and from functionally related groups of tissues,
2. those components of the vascular system that allow the biochemical transactions between the blood and the tissues.

In the final analysis, as Majno (1965) has said when considering the ultrastructure of the capillary endothelium, 'The circulatory system conceptually and anatomically culminates at the level of the capillary wall'. A problem arises because there seems to be no uniform agreement as to exactly what a capillary is and where it starts and ends. Important microvascular functions are found outside the 'capillary' bed. For example, in vascular permeability, venules are known to be far more important than arterioles and capillaries combined (Casley-Smith 1973, citing Siperstein, Unger, and Madison, 1968), so that obviously our concept of 'culmination' should be extended to venules as well. The fact of the matter is that as our observational devices become more sophisticated the conceptual 'capillary wall' expands outwards, particularly downstream, from the capillaries

themselves. It is important to keep an open mind, thus reference to the 'conceptual culmination' will be by the term 'microvascular bed'. It is felt to be appropriate not to put too fine a line as to where it commences upstream or concludes downstream.

Most classifications of vessels are based upon their transmural structure and overall dimensions. This method has considerable precedent to support it (Zimmerman 1923, Zwiefach 1937, Bennet et al. 1959 and Rhodin 1967). Nevertheless, it is not entirely adequate. The reasons for this are varied but revolve, in part, around the fact that the differentiations made by observers are not absolute. Vessel types used to exemplify the various archetypes of a classification do not have discreet interfaces with adjacent types. There is a progressive merging of structure and size so that delineations can only be reasonably sure at each side of a zone of transition. Within this zone, characteristics of both adjacent vessel types are seen and the differentiation into one definitive type or another is thus somewhat speculative.

There is another way of classifying vessels. It is more abstract and relatively few authors in the literature have been seen to allude directly to it. On the matter of capillaries Luft (1973) selected a system based upon pragmatic interpretation. If the structure of a vessel led him to the conclusion that this vessel had the capacity for capillary type function then he considered that it was a capillary.

This type of concept is implied by other workers

e.g. Majno (1965), Rhodin (1967, 1968) and espoused directly by Wiedeman (1963) and Luft (1973).

In a functional classification the vascular system can be divided into two broad areas:

1. conductive a) afferent
   b) efferent,
2. transactional.

Casley-Smith (1977a) considered that the division of capillaries into arterial and venous limbs was only a statistical concept. Zwiefach (1977) proposed that two key features separated microcirculation from larger blood vessels, namely:

1. the capacity to adjust flow to metabolic requirements, and
2. the capacity to adjust and stabilise flow and pressure.

He further proposed that the term microcirculation be reserved for vessels that possessed certain functional features rather than structural features. The microvasculature of a tissue or organ was best considered as a separate independent functional component of the vascular system as a whole.

The basis of this type of classification would be ultrastructural evidence of the ability to perform 'transactional' acts. This type of extrapolation, to a limited extent, would appear to be within the bounds of current knowledge linking structure and function.

It is quite apparent while reviewing the literature that terminology is fairly consistent but the question

can be posed, are all workers assigning the same terms to the same, or analogous entities? With blood vessels having been so extensively studied there are many classifications of vessels. Most classifications are based on the ultrastructure and relative proportion of the three layers of any vessel. That is, the tunicas intima, media and adventitia, and discernible variations such as the number of cell layers, cellular organelles together with the presence or absence of basement membrane. On the arterial side, classifications such as Movat and Fernando (1963), Fernando and Movat (1964a,b), Rhodin (1967) and Laguens and Gomez-Dumm (1969) are based on these parameters.

Wiedeman (1963) studied vessels from a more quantitative aspect. She looked at average length, average diameter and numbers of branches. This information is displayed in table form below.

Vessels Considered	Average Length mm	Diameter µm	Number of Branches
Artery	17	52.6	12.3
Small artery	3.5	19.0	9.3
Arteriole	0.95	7.0	4.6
Capillary	0.23	3.7	3.1
Postcapillary venule	0.21	7.3	0.0
Venule	1.0	21.0	5.0
Small vein	3.4	37.0	14.1
Vein	16.6	76.2	24.5

Table 2. From Wiedeman, (1963).

This concept is very interesting but a little impractical

when examining material in histological slices as the classification is essentially three dimensional. It does, however, give insight into some interesting volumetric considerations. For example, 80 per cent of the blood at any time is in the venous side of the circulation. The number of postcapillary venules is three times the number of capillaries. Thus the majority of the 'conceptual' end of the microvascular bed is on the venous side of the circulation.

Baez (1977), however, felt that there was "uniform agreement" that:

1. venous capillaries were formed by the confluence of two or three capillaries;
2. postcapillary venules were formed by the confluence of two to four venous capillaries.

This author however has found little evidence of this "uniform agreement" either in a numerical or terminological sense. It is thus apparent that, in spite of intensive research, the last word on fundamental microanatomy, including elementary details such as numbers of branches, and nomenclature in general has not been said.

It is known that the average diameter of the smallest vessels in the microvascular bed is 3.7 μm which is about half of the mean diameter of erythrocytes. This indicates that the biconcave discoid form of circulating erythrocytes is highly flexible. This is supported by a review (Spector, 1956) cited by Wiedeman (1963) which gave a minimum diameter of the microvascular bed of four different mammalian species as between 3 and 5 μm.

Most other classifications (Majno, 1965; Rhodin 1967, 1968) incorporate a diametric component. However, this investigation has found that from a structural point of view most vessels were collapsed and few were in a near normal, functionally filled state. In defense of the validity of results independent of a metric component Wiedeman (1963) has said, "It is meaningless to define an artery or arteriole on the basis of its diameter, or to define a capillary in this manner. The assignment of a name to a specific vessel should be determined by its position and function in the vascular system".

HYPOTHESES

When reviewing the theories related to periodontal function several theories of vascular contribution have been presented that require further discussion. It is possible that the interstitial fluid phase may have a role, in conjunction with the perforations in the cribriform plate of the dentoalveolar socket, in a hydraulic damper type system (Birn 1966).

The hypothesis of vascular constriction by collagen (Bien, 1966) raises the possibility that, if this mechanism functions, there might be a facility for the rapid movement of fluid across the vascular wall. Thus, some functional adaptation to provide for the facility would not be unreasonable to postulate. Otherwise, the 'damping' would have a take-up time of zero thus acting as instantaneous resistance to movement, i.e. no 'damping' at all. On the other hand, such a system may act as a sequence of restricted flow apertures acting in the direc-

tion of flow or, perhaps, even in a retrograde direction. The problem of extreme intravascular pressures, and thus transmural pressure gradients, would then be raised. Some functional adaption to prevent rupture and extravasation might be thought possible. However, on the other hand, the pressures generated in the interstitial fluid phase may approximate those generated intraluminally. Thus, no functional adaptation may be necessary as there may be no large transmural pressure gradient.

The literature as related to normal loads and normal root surface areas, as they apply to the anatomical location of this study, can be distilled thus:

1. Hillam (1973) calculated root surface areas for all teeth and compared his work to 3 other studies. Hillam's finding was an average of 204 mm^2 for human maxillary first premolar teeth. Hillam cited findings of studies by Tylman (1970), Jepson (1963) and Watt et al. (1958) were 149, 234 and 219.7 mm^2, respectively. These figures average out to approximately 200 mm^2.

2. Watt, MacGregor, Geddes, Cockburn and Boyd (1958) reviewed the literature with respect to biting forces, citing Dennis (1894), Haber (1926) and Dietz (1920) who produced figures for premolar segments. Using their averages a normal masticatory load of 33.8 kg per tooth can be calculated. Combining this load and the surface area, a calculation reveals that premolar teeth under normal masticatory function are capable of transmitting 170 gms/mm^2 to the connective tissue elements of the periodontal ligament. If such forces were to be

transmitted across blood vessels they would be sufficient to cause occlusion of all vessel types constituting the microvascular bed.

The material examined in this study can lay only limited claims to being "topographically controlled". Thus, any conclusions as to the definitive and relative locations of structures described must be guarded. While ultrastructural categories of vessels can be identified, it is impossible to be authoritative about vascular sequence. Although such categorizations are convenient it is fatuous to propose that these differentiations are in any way real. Differences between types are arrived at through indistinct intermediate stages and cell changes in the tunicas are continuous and progressive. Even dimensional aspects are invalidated unless topographically controlled examination techniques are used (Wolff, 1977). Cliff (1976) mentioned that regions of membrane fusion of the "zonula occludens" type can occur between luminal contents and the luminal aspect of the endothelium. This matter is raised to illustrate two factors:

1. Care must be taken in making extrapolations relating a piece of dead, fixed and highly processed tissue to ultrastructural features as they would be in vivo.
2. Changes do occur 'post fixation'. For example, vesicularisation has been shown to procede 20 minutes after the commencement of perfusion fixation (Wolff, 1977).

In the absence of adequate topographical control

conclusions about vessel classification and location within the vascular network must be guarded. Also, the small amount of tissue examined in each section, that is 0.5mm by 0.5mm and about 75nm thick, an observer is limited simply by the number of sections that he examines. Even in the most exhaustive study, the percentage of tissue examined with respect to both structure size and specimen size is very small and the results presented must be seen in this light. Also, an important variable is the fact that this study is highly localised to a 1mm strip of periodontium from the alveolar crest to the mid-root area. More apical structures, mesio-distal tissues, lingual and bifurcational areas have not been examined. These considerations must further be allied to the fact that grid bars eliminate approximately 20 per cent of all sections suitable for examination.

TRENDS

In this study several trends have appeared:

1. Vessels of any type were much more common in the crestal third than in the middle third.
2. Vessels were collected in concentrations. In between the vessels were areas of lower density, i.e. less collagen and more cells.
3. Neural elements, both myelinated and non-myelinated, were often seen in association with vessels. In the middle third fewer neural elements were seen in association with vessels.
4. Principal fibre bundles, irrespective of

location, were almost avascular.

5. The differentiation, so obvious with light microscopy, between osteoid, cement and dentine in the periodontal ligament was not as well defined in transmission electron microscopy, particularly the ligament/bone interface. Dentine was well marked by a fine granulo-fibrillar matrix, dentinal tubules and the acellular nature of its substance. The attachment of periodontal fibres at the dento-periodontal interface was plainly seen and from this feature, the general orientation of the specimen was determined.
6. Oxytalan fibres were periodically seen. This infrequent observation was probably more due to their undistinguished appearance when stained with uranyl acetate and lead citrate and then sectioned transversely.
7. Erythrocytes were commonly seen in the medullary spaces of alveolar bone but few discreet vascular structures were identified in the bone.
8. No perforating vessels were identified but this was not considered to be surprising due to the low numbers of perforating vessels and the scale of tissue involved.
9. Few fenestrae were observed. It was felt that despite the collapsed vascular state, any fenestrae present would be readily apparent.

Thus, it was inferred that fenestrae were not a common vascular feature in this area.

10. Vessels with even a single layer of well-differentiated smooth muscle cells were not observed. Whereas, vessels with a complete pericyte layer were seen. The most common feature was an incomplete periendothelial cellular investment.

Rhodin (1967, 1968) showed that between the venous capillary and the incompletely described arterial capillary there would seem to be no worthwhile differentiation to be made. Venous capillaries were endothelial tubes with a few highly branched periendothelial cells. In postcapillary venules the periendothelial cellular investment was complete, while at the level of muscular venules it could be seen that the investment was characteristic smooth muscle. In the light of the extreme variability of arteriolar ultrastructure, as alluded to by Wolff (1977), it seemed that there was no differentiation on a pure ultrastructural basis to be made between the smallest muscular arterioles and venules.

PERIODONTAL MICROVASCULAR FEATURES

Given the limitations alluded to above, what can be said about the ultrastructure of periodontal blood vessels?

1. In general they conform to the picture presented by earlier workers using various techniques. That is, the vascular net of human maxillary premolars is

principally in the middle areas of the periodontal ligament.

2. Smooth muscle cells in the periendothelial areas (tunica media) are absent from the material examined in this study. This contrasts with the report of Avery et al. (1975) who reported small, muscle coated vessels and some with an incomplete muscular tunica media. Few details of location were incorporated in their publication.

3. The presence of details such as intra-endothelial fenestrae, vesicularization, fibrils, basement membranes and interendothelial junctions seem quite typical of connective tissue vessels in general.

4. A feature observed with some consistency, seemingly peculiar to periodontal ligament, is the apparent multilayering of nonspecific periendothelial cells in the larger vessel types. Apart from this feature, there is no obvious ultrastructural evidence to suggest that periodontal vessels are modified in any way to accommodate large transmural pressure gradients.

Utilising Rhodin's (1967, 1968) classification, vessels described in detail in this report could be classified in the following manner. In the absence of definitive smooth muscle cells in the media of any vessels described, the general limits within which vessels described in this report are fixed. The limits are, downstream of the most terminal afferent blood vessel possessing a smooth muscle tunica media and upstream of the smallest efferent vessel having a smooth muscle media. Thus periodontal ligament blood vessels must be classified between, but not including precapillary sphincters and muscular venules.

Classification of individual vessel types gave the following.

Type I: equivalent to Rhodin (1968) venous capillaries. (In the absence of any comprehensive description of an arterial capillary, it seemed best to consider it a transition rather than a separate type).

Type IIa: equivalent to Rhodin (1968) postcapillary venules.

Type IIb: equivalent to Rhodin (1968) collecting venules.

Type III: equivalent to Rhodin (1968) collecting venules.

The range of functional diameters represented by this sequence is 7 μm to 50 μm with a minimum of perhaps 4 μm. This range is not inconsistent with diametric impressions gained in this study even though most vessels were collapsed.

The principal problem of an attempt at classification is that all vessels identified are on the venous side of the circulation. The enigmatic question to be answered after classifying all vessels seen as venous types is, "How does the blood get in?" On a purely pragmatic basis the periodontal ligament must have an arterial supply. In the presence of limited topographical control this study found no ultrastructural evidence of it when applying the classification criteria of workers such as Rhodin. All arterial structures down to the level of precapillary sphincter had, according to Rhodin, well differentiated, continuous layer(s) of smooth muscle cells. However, it is pertinent that Wolff (1977) alluded to an ateriolar vessel devoid of a smooth muscle tunica media.

CLASSIFICATION OF PERIODONTAL VESSELS:

Applying the Bennet et al. (1959) capillary classification to vessels seen in this study provided the following interpretations:

Type I: A; 1; α

A = complete continuous basement membrane

1 = No fenestrae or perforations

α = Incomplete periendothelial cellular investment

Note: when considering vessels that did exhibit fenestrae the classification would be A; 2; α.

Type IIa: A; 1; α.

Type IIb: A; 1; β. (β = complete periendothelial cellular investment).

Type III: A; 1; β.

It may be questionable to apply a capillary classification standard to type III vessels. But there seemed to be no ultrastructural reason why all vessels seen could not undertake biochemical and fluid transactions between the luminal contents and adventitial structures.

Two vascular ultrastructural features that seemed characteristic of the periodontal microvascular bed were:

1. non-myelinated 'nerve-like' inclusions within the endothelial basement membrane,
2. tunnel-like and sheet-like arrangement of collagen between the tunicas intima and media.

NERVE-LIKE INCLUSIONS:

On the evidence presented in this report, a definitive conclusion that the 'nerve-like' inclusions are in fact nerves is not justified. However, the possibility that they are justifies discussion. Such a relationship allows the premise that, since no evidence of the independent contractility of endothelium is proposed by any worker, the relationship could be ultrastructural evidence of sensory efferent output from some vessels in the periodontium afferent to a higher neural centre. Such a receptor could augment proprioceptive sensory output of other, more specifically neural, receptors in the periodontium, like the mechanoreceptors described by Griffin and Harris (1974) and Harris and Griffin (1974).

Whether such a vascular proprioceptor might be sensitive to transmural pressure gradients or mechanical distortion of the vessels in function is purely speculative at the moment.

Should the 'nerve-like' presentations prove to be sectioning features of some of the complicated and intimate endothelial-pericyte close approximations (E.P.A.), the possibility of direct vascular mechanoreception becomes less likely.

The considerable complexity of E.P.A.'s is challenging. While it is hard to say what the appearance of such approximations might be in the normal state of distention via transmural pressure gradients; it is possible that intimate cellular associations such as E.P.A.'s might act as a means of conducting information

possibly afferent with respect to endothelial cells but more likely efferent. The contact between a cellular transducer and definitive nerves could then be more adventitially oriented and, therefore, remain undemonstrated, as far as this study is concerned.

FIBRO-COLLAGENOUS LAMINA:

The existence of an internal fibro-collagenous system in some vessel types invites speculation that there is a specific relationship between connective tissue and vascular elements. Such an association could serve several potential functions:

1. the fibro-collagenous lamina could be purely structural i.e. some sort of biological reinforcement against deformations and forces produced during function.

2. The link between the vessel and connective tissue elements may be a feature of a general connection between elements of the connective tissue and vessels. The connecting agents here might be cellular, fibrous or neural. The relationship of the oxytalan fibres to vessels, particularly in the middle annular third, as described by Sims (1975), must be a potential co-respondent in such a connective tissue - vascular association.

Rodbard (1970) proposed that various parameters of rheology and function imposed certain ultrastructural features on the transmural vascular wall and connective tissues immediately peripheral to the vessels in question. His thesis basically proposed that function imposed form on an ultrastructural basis. Cells and tissues arranged themselves quantitatively and qualitatively to best cope

with the rheological and functional peculiarities of the vessels and tissues concerned. By example he considered that:

1. absence of stress promoted fat cells and ground substance,
2. mild compressive forces promoted basement membrane,
3. tension produced collagen which was oriented along the stress lines and the amount of collagen was proportional to the amount of stress,
4. elastin was deposited where rates of change of tension were high.

Moreover, he considered that tensile forces in vessel walls were greatest in the inner layer and diminished abluminally. Rodbard was considering primarily tension circumfrentially, rather than longitudinally, but it would not seem unreasonable to propose that the collagen pattern seen in the periodontal ligament was laid down primarily in response to the functional forces that prevailed. Compressive forces tending to collapse vessels due to hydraulic forces would seem to be more a possibility than pressure gradients going the other way. An extrapolation of this theory is quite compatible with large thin walled vessels with moderate amounts of collagen oriented longitudinally close to the tunica intima.

As to the mechanism whereby such a functional orientation might be mediated, Rodbard had no suggestion to make but he did propose a negative feed-back control of

the mechanism whatever it was.

Forces that might be important in the periodontal ligament seem to be twofold:

1. compression: via hydraulic forces
2. tension: as the principal fibre bundles came under masticatory loads.

Wills, Picton and Davies (1972) have concluded that irrespective of the direction of force, the collagen response is basically tensional. Indeed, it would seem reasonable to suggest that the principal fibre bundles themselves are produced and maintained under the auspices of the basic mechanism alluded to by Rodbard (1970).

PERICYTES:

There are several characteristics of pericytes which bear consideration:

1. differential staining,
2. differential collapse,
3. mature collagen intervening between pericytes and endothelial cells.

Differential staining: While interesting, this phenomenon must remain of a highly speculative origin. The stains used in this study are relatively non-specific, although, differential staining was noted between endothelial cells in the same specimen. More sophisticated staining techniques will be required before some light can be shed on this aspect of pericyte ultrastructure.

Differential collapse: Using such a term implies passive differential collapse of endothelial tubes along with their periendothelial cellular investments. This need

not necessarily be the case although it is a potential reason for the appearance. If pericytes were contractile, the same presentation could result with endothelial cells crumpling passively inside the annular compression due to pericyte contraction. No specific evidence of contractility on an ultrastructural basis was found in this study. It is, however, widely held that fibrils seen intracellularly in endothelial cells and pericytes are composed, to an extent, of 'contractile' proteins (Cliff 1976, Casley-Smith 1977b). The question of a transducer for the proposed contractility must be raised. Fine non-myelinated nerve fibres are ultrastructurally unremarkable and their existence in some numbers close to periendothelial layers has been described in this report. Neural/pericyte junctions were not observed but their existence cannot be dismissed on that basis alone. The possibility of a non-neural mediated exchange cannot be discounted. There may be cells or cellular transducers free in the ligament space capable of releasing a chemical transmitter substance able to induce pericytic contraction. The other immediately apparent chemical route is direct haematogenous transmission across the microvascular membrane.

The existence of mature collagen between pericytes and the abluminal aspect of endothelial cells is an unusual finding. However, there is no specific problem in accounting for the existence of collagen in this location. Pericytes are thought to be able to synthesise their own basement membrane and recent work has shown that the basement membrane is largely collagen (Cliff 1976, Casley-Smith

1977b). It must be stated that by this criterion endothelial cells themselves may be capable of contributing to collagen synthesis in this locality.

The low numbers of endothelial fenestrae seen is thought by Casley-Smith (1977b) to approximate the occurrence of this structure in other relatively non-specific areas such as skin. This finding agrees with Rhodin (1967, 1968). Interendothelial junctions seemed unspecialised when mechanisms for rapid transport of solute or fluid are proposed.

TECHNICAL PROBLEMS

Problems will be considered in a sequence and the central cause, where it is discernible, will be exemplified.

Considering the general lack of technical information related to the preparation of decalcified sections for the T.E.M., this author feels that there are several avenues which, if employed, may produce higher quality sections more reliably.

1. Sections cut best when the hardness of the embedding medium and the specimen itself are close approximations of one another. At the same time resins of low viscosity are needed to fully perfuse the specimens. It is felt that the Spurr's resin employed adequately fulfils the perfusion requirement but falls short in being of variable hardness when polymerised. It is suggested that further work may be fruitful using harder resins while varying preparation techniques to ensure full perfusion.

2. The demineralisation end point determinations

described in this text are those accepted for light microscopy. It is by no means accepted that these standards are adequate for the ultramicrotomy required for the T.E.M.. It is suggested that for directly analogous specimens the effect of further prolonged decalcification times, for example 30 days, should be assessed. The inconvenience of Clark's buffer, due to its complexity and short shelf life, must be equated with a possible marginal inferiority to more common, simple and stable buffers such as Millonig's. The value of a buffer containing divalent cations (Ca^{++}, Mg^{++}) in the presence of a chelating demineralising solution must be considered.

There is evidence of early degenerative change in some cells. It is thought that this degeneration is of a highly nonspecific nature. The changes seen are of two main types:

1. swollen cells with loss of detail of intracellular organelles,
2. presence of a very irregular lumenal endothelial border with many vesicles.

It is probable that both of these types are stages along the cloudy swelling, vacuolar degeneration and hydropic change sequence. This sequence is a nonspecific cellular response to a variety of factors (Walter and Israel, 1970). Factors which may have arisen in this study included.

1. Anoxia: The duration of time between severing all vascular connections and final fixation allows the possibility of anoxic or

hypoxic cell damage. At what stage the tissue vitality is compromised by hypoxia is difficult to say. It may be as early as infusion with local anaesthetic; in this case containing a vaso-constrictor. Certainly the chronologically proximal limit is surgical amputation of all vascular connections. Some future work could no doubt assess an anaesthetic without a vaso-constrictor.

2. Surgical trauma: Using the bur to outline the specimen must inevitably cause micro-impacts to be transmitted into the specimen as a shock wave. Some preliminary trials were performed using various burs and other bone removal techniques e.g. chisels. The feeling of this author is that all bur techniques were superior to chisels in speed, minimising trauma, and patient acceptability. The technique outlined in Section 4 was felt to be minimally traumatic and as expeditious as possible.

3. Heat: Must be considered as a possible cause of degeneration. It must be remembered that heat sinks in the form of irrigation do not modify the absolute temperatures achieved at the point of contact i.e. in this case the contact point between bur and bone or tooth. The heat sink acts to steepen the temperature

gradients as they extend into the bur and tissues. In this project, copious normal saline irrigations at room temperature (20°C approx.) were used. Future workers may find an improvement by using lower temperature normal saline irrigations, e.g. 4°C.

The relative contributions of the factors mentioned and others unknown to this author are difficult to assess as the changes are non-specific and it is not unlikely that all factors play a part. This author's conclusion is that the preparation sequence is not without its hazards for the cells involved and that using, in future, local anaesthetics that do not contain vasoconstrictors and also using low temperature irrigations while cutting tooth and bone may offer some diminution of the incidence of degenerative changes seen in this study.

However, the expression of a pathological state, by luminal irregularities, vacuolarisation, and vesicularisation, is difficult to assess. Wolff (1977) and Gabbiani and Majno (1977) both referred to such findings in "normal" endothelium. The presentation, particularly of lumenal irregularities, may be compounded by collapse or the active contraction that produces the characteristic pattern of vessels as seen in this study. Thus, these features may not infer any pathogenic feature inherent in the methodology of this project.

In the material presented in this report the majority of vessels seen were in a partially collapsed

state. Some were seen with no discernable intraluminal contents. Others, however, were seen with erythrocytes, but in such a configuration that it was reasonable to postulate that the erythrocytes had been compressed to conform to a luminal configuration superimposed over the normal pattern by some other factor. In extreme cases it became problematical as to definitive identification of the vascular nature of a structure in the viewing field. Potential reasons for this appearance are as follows:

1. Surgical trauma to the tooth and/or buccal plate of bone literally pumping all, or most, of the blood out of all, or most, of the vessels in the specimen. Where intraluminal contents were seen, the vessels were usually among the smallest types identified i.e. they were seen relatively close to the cementum rather than in the middle areas of the periodontal ligament.
2. The copious, low viscosity irrigation used during surgery may tend to flush the blood out of the vessels. This may act via the severed ends of the microvascular bed which were progressively revealed during surgery.
3. The vaso-constrictor in the local anaesthetic may cause constriction of most of the vessels in the area, thus squeezing most blood out, before surgery at the same time as inhibiting, by arterial vaso-constriction, refilling of the vascular bed.

There are several other potential causes of collapse which are rather more abstract but it is proposed that they not be analysed further in this report.

Given that using human material makes fixation by perfusion impossible, the problem of vascular collapse would seem to be inevitable to a degree. Rapid, minimally traumatic surgery which was employed, and non vaso-constricting local anaesthetics seem to be the only factors which would minimise this problem. This is not felt to be of paramount importance as the collapsed state in no way precludes accurate typing of vessels on an ultrastructural basis. It does, however, preclude a diametric dimensional component to the study. In future studies, this author feels that perfusion with primary fixative with or without some electron-opaque vascular marker in non-human material would be a valuable adjunct to the findings presented herein. It must be remembered that perfusion fixation is not without problems e.g. of a hydrostatic and osmotic type.

SECTION 7

CONCLUSIONS

A highly specific relationship existing between vascular and connective tissue elements, particularly fibres, is concluded. There is a possibility that the association is either structural or proprioceptive.

The possibility that endothelium is innervated is raised. The microvascular tree may have a role in the generation of sensory efferents. A case can be made to propose that in function the periodontal ligament microvasculature is multi-purpose.

The relationship between pericytes and endothelial cells appears complex with many and varied presentations of intercellular close associations.

The existence of organised collagenous structures as an internal fibrocollagenous lamina is concluded to be a rheologocial and functional feature with the orientation being secondary to function.

Periodontal ligament microvessels would not appear to be involved in ligament function to the extent that their ability to withstand large transmural pressure gradients was critical.

If, in applying a vascular classification, all vessels are classified as venous or capillary types the classification can be concluded to be non-applicable to the tissue in question. General connective tissue standards like Rhodin (1967, 1968) are of little value as a guide to

vascular sequence. On a pragmatic basis the periodontal ligament must have an arterial supply but general vascular classifications were not helpful in the identification of it in the locations examined.

The periodontal ligament should be considered a specialised organ in its own right. Little understanding of its function will be forthcoming by attempting to describe its function under anything other than full anatomical and biochemical context. In such a context topographically controlled methods must be used to describe a vascular nomenclature suitable to the specialised function of the organ as a whole.

It is concluded that the best techniques for the obtaining and preparation of human material of this type for the T.E.M. in its most topographically valid form is far from completely determined.

To the understanding of general topography this study could make little contribution. The problems of topographic control are immense under the most ideal circumstances. In human studies with their attendant considerations these problems become almost insurmountable under routine circumstances. Models of general topography will need to be developed either in animal or human postmortem studies and extrapolations made to the human system in vivo.

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