THE FINE STRUCTURE AND DISTRIBUTION OF VESSELS IN A SMALL SEGMENT OF HUMAN PERIODONTAL LIGAMENT AND ALVEOLAR BONE.

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A report submitted in partial fulfilment
for the degree of Master of Dental Surgery.

Department of Dental Health,
The University of Adelaide,
1980.

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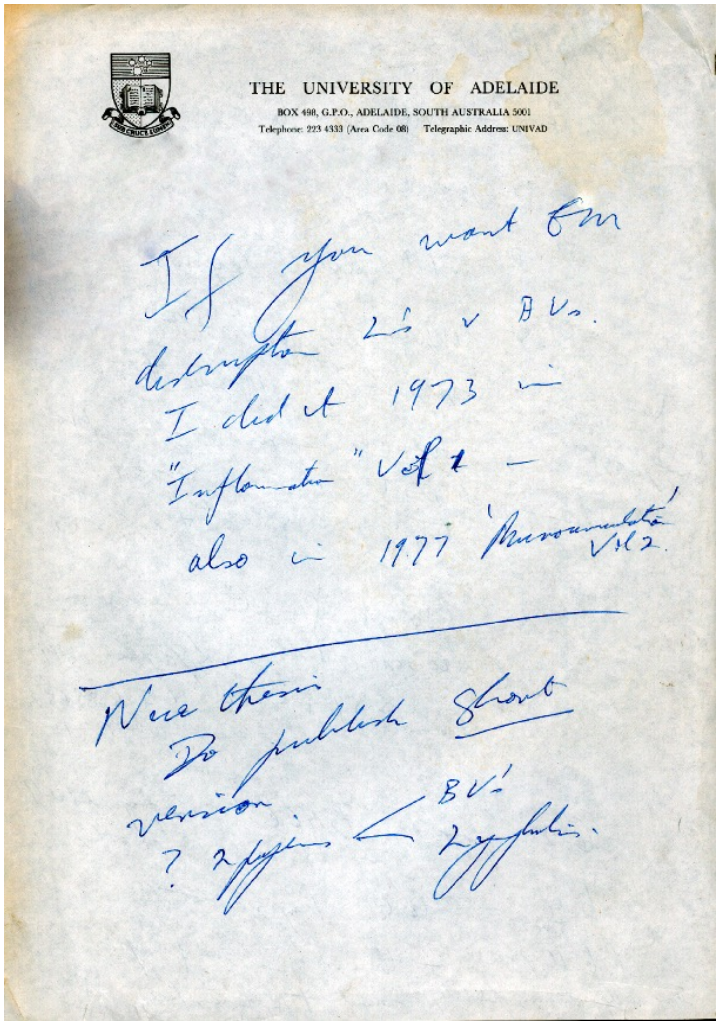
Addendum

Thesis Supervisor Dr John R Casley-Smith's handwritten comments c1980 on this thesis are reproduced and transcribed in this Addendum. His questions concerning 5.5 are answered by the author ('JB Ed') whose answers are transcribed in small print.

Part of this thesis was published in *Lymphology* vol 15 No.1 March1982 (1-13) under the title "Lymphatic Vessels in Human Alveolar Bone."



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If you want Em description Li v BVs. I did it 1973 in "Inflammation" Vol 1 — also in 1977 'Microcirculation' Vol 2.

Nice thesis
Do publish short version
? 2 papers - BV
- Lymphatics

"If you want electron microscopic description of Lymphatic Vessels and Blood Vessels, I did it in 1973 in
"Inflammation" Vol 1
also in 1977
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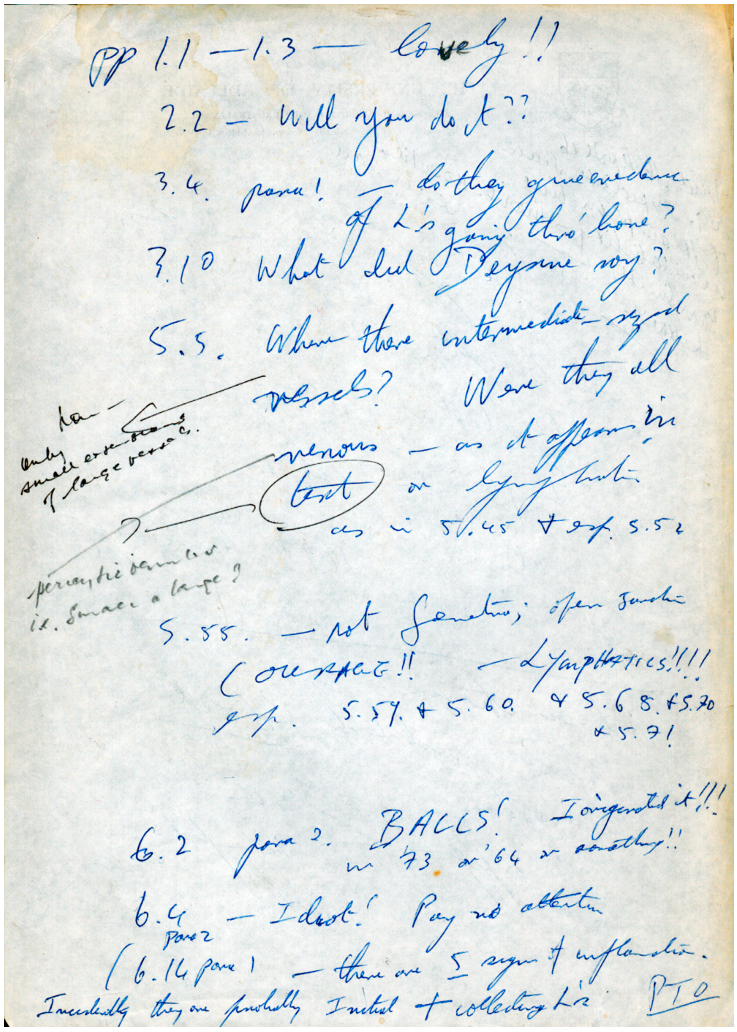


"pp 1.1 – 1.3 lovely!!

2.2 will you do it??

3.4 para 1 do they give evidence of lymphatic vessels going thro bone?

3.10 what did Deysine say?

5.5 'where' (were?) these intermediate sized vessels? 'no – only small extensions of large vessels.' (JB Ed)

were they all venous – as it appears in the text (here, JB Ed circles 'text' and questions his claim countering that they are pericytic venules i.e. small and large(?)

5.55 not fenestra; open junctions.
(OUTRAGE!! – LYMPHATICS!!!!
esp. 5.57 & 5.60, & 5.68 & 5.70 & 5.91.

6.2 para 2 BALLS! I originated it!!! in 1973 or 1964 or something!!

6.4 Idiot! Pay no attention.

(6.14 para1 there are 5 signs of inflammation)

Incidentally they are probably Initial + Collecting Lymphatics. PTO

LIST OF ILLUSTRATIONS.

SUMMARY.

From each of six teenage orthodontic patients one healthy, functional maxillary first premolar was surgically extracted, leaving biopsied buccal bone and periodontal ligament attached to each root.

The intact specimens were processed for viewing in the transmission electron microscope (TEM). Horizontal serial sections 1µm thick, for viewing in the light microscope (LM), were taken from the apical through to the cervical end of each specimen. Where vessels of any type were seen in either periodontal ligament or alveolar bone, silver sections of the same area and orientation were mounted on grids and photographed in the TEM.

Medullary tissue of alveolar trabecular spaces was found to communicate with the overlying gum tissues, and with the periodontal ligament, via foramina. Medullary interstitial tissue was identified as vestigial haematopoietic tissue, adipose and sinusoidal.

The most prominent feature of each specimen was the presence of large vessels which almost filled each trabecular space, and which ramified through the buccal cortex and the alveolar socket wall. The ligamentous

extensions of these vessels were the most obvious feature of the periodontal membrane, raising question that they have not been previously investigated. Long extensions of the large vessels linked ligamentous pericytic venules, which ran longitudinally in loose connective tissue columns within a stroma of principal fibres. The impression given was that ligamentous fluids were 'funnelled', via the foramina, into the large vessels of the trabecular spaces.

Some of the ligamentous pericytic venules passed through the foramina into the trabecular tissue, where they appeared to act as 'vasa vasorum' to the large medullary vessels.

The large vessels were tortuous and convoluted, being of the order of millimetres long, and tens of microns in diameter. They were greater than 2.10^3X the lumenal size of the pericytic venules.

No periodontal vessel was seen characteristic of the arterial side of the circulation.

Published ultrastructural criteria tentatively identified the large vessels as initial lymphatics. However, the presence of lymphatics in bone being without a

published precedent, suggestions are made how definitively to class these vessels.

It is strongly suggested that ligament and bone are one unit, and that a proper periodontal functional analysis must await stereologic morphometric data for the combined tissues. However, based upon the reported findings is conjectured a simple microcirculation for the periodontium and, proposed, are the components of a system which probably controls alveolar responses both to changes in masticatory patterns and to orthodontic treatment.

Suggestions have been detailed to further investigate the reported findings.

ACKNOWLEDGEMENTS.

Dr. J.R. Casley-Smith and his assistant, Mr. K. Crocker, freely gave help without which this work would have been fruitless. They have my thanks not only for their assistance but also for permitting me to use the transmission electron microscope in the Henry Thomas Laboratory, The University of Adelaide.

My supervisor, Dr. M.R. Sims, gave needed guidance in the preparation of this report. Further cheerful help was given by Mesdames J. Aloia and L. McMahon, and Miss A. Smith.

Throughout the course, the biography of N.H. Molesworth (Willans and Searle, 1958) was a philosophical source of constant inspiration and guidance for my fellow students and me.

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.

J.H. BARKER.

SECTION 1.

INTRODUCTION.

There is no atlas of the histology or ultrastructure of human periodontal ligament. Very little seems to be known about the structure and function of the ligament, not only of humans but also that of animals. Modern textbooks reproduce little-modified descriptions of the ligament published twenty or thirty years ago. These descriptions are generally of gross anatomy, little space being given to the ligament's histology or ultrastructure.

Hence, there is much histological and ultramicroscopical work yet to be done on the ligament. This is exemplified by the existence of only two publications which try to identify, by LM and TEM, types of ligamentous vessels (Avery, Corpron and Lee 1975, Corpron, Avery, Morawa and Lee 1976).

Similarly, LM and TEM studies of medullary tissues in alveolar bone are rare. No attempts appear to have been made to identify and determine the orientation of vessels within this part of the periodontium.

Perhaps, in the past, it has been considered to be too difficult to carry out this type of study.

However, a dearth of direct information has not prevented, indeed, has probably stimulated, much speculation. An example of such surmise is as follows: periodontal ligament is living connective tissue; thus, like other connective tissues it must have within its structure arteries, veins, capillaries, lymphatics, nerves, fibrous tissues and ground substance. Some of these structures having been positively identified, quasi support has been given to the remaining contentions. Another example of this type of reasoning is, thus: there are three possible routes by which vessels and nerves may pass to and from the ligament ... via interseptal bone, via apical foramina, and via the gum tissue, the last being an extra-bony or cervical route. These, too, have seemed to be reasonable propositions and, without further supportive evidence, have been accepted as fact (Pindborg and Mjor 1973).

Based upon such surmised structural arrangements are 'theories' of periodontal function and performance, conjecture which is, consequently, as questionable as the precepts upon which it is based. The function of an organ or tissue depends entirely on its form; without proper knowledge of the structures within periodontium, one cannot properly analyse its function.

Consequently, no sound functional analysis of periodontal tissue can yet be made. A systematic morphometric and stereological analysis is required.

Eventually, the goal of all dental research work is to improve and to better understand the clinical treatment of human beings. To obviate problems of applying to humans data from animal derived analyses, it is sensible that a comprehensive qualitative and quantitative analysis should be made directly upon human periodontal tissues.

SECTION 2.

PROJECT AIMS.

The author was ambitious. Having decided that there was little basic qualitative or quantitative information about structures within human periodontal ligament, he set out to satisfy this need. A stereological and morphometric analysis of human periodontal ligament was commenced, an account of which is given in a preliminary report (Barker 1979). The tissue preparation techniques used enabled resin embedded sections to be taken through composite human specimens of tooth, bone and periodontal ligament, to permit accurate orientation of structures within the ligament. The technique was ideally suited to both LM and TEM identification and quantification of these components. Serial sections were taken of the first few specimens, attention being concentrated on the ligament. However, by this approach, previously unreported ligamentous structures were revealed, which were found to be connected to similar organisations of tissue within the adjacent alveolar bone. As a result of this, the original study was extended to become an analysis of the periodontium, incorporating both the periodontal ligament and attached alveolar bone.

Eventually, it became obvious to the author that completion of the stereological analysis, as he envisaged, was impossible in the time allotted for the MDS (Orthodontics) course. Hence, quantitation was set aside for completion at a later date.

Accordingly, sectioning of the periodontium was continued with the aim of presenting, in this dissertation, a description of some of the structures which have not been previously identified.

SECTION 3.

REVIEW OF THE LITERATURE.

(i) STRUCTURE OF HUMAN PERIODONTIUM.

In this dissertation, 'periodontium' includes alveolar bone and periodontal ligament. The ligament, the connective tissue which lies between alveolar bone and tooth, is an organisation of cells, an extracellular phase consisting of fibres and ground substance, and a blood and nerve supply. 'Principal' collagen fibres of the periodontal ligament insert into the socket wall of alveolar bone, which was defined by Scott (1968) to be "the parts of the jaws containing the sockets of functional teeth and the crypts of developing teeth". There are large cancellous spaces in human alveolar bone (Edwards 1977).

(ii) DISTRIBUTION OF MAMMALIAN PERIODONTAL VESSELS.

Mammalian ligamentous vessels have been described by Hayashi (1932), Sicher (1962), Kindlova and Matena (1962), Boyer and Neptune (1962), Castelli (1963), Castelli and Dempster (1965), Kindlova (1965, 1966, 1967), Birn (1966),

Carranza, Itolz, Carrini and Dotto (1966), Simpson (1966, 1967) and Folke and Stallard (1967). These authors have claimed that, for all mammals, there is a common arrangement of vessels, and that veins and nerves follow the same course within the ligament. They held that the ligament is mainly supplied with vessels which originate from arterial and venous trunks embedded in the jaw bones. These trunks subdivide to form smaller vessels which, on passing into the alveolar processes, branch to perforate the socket walls. Apical perforating vessels supply the pulp as well as the periodontal ligament of a given tooth. It was claimed that, at all levels within the tooth socket, vessels penetrate the alveolar wall to vascularise the periodontal ligament. Examining the distribution of perforations (foramina) in socket walls, Birn (1966) concluded that "the blood supply of the periodontal membrane is greatest in the gingival and least in the middle third. There are no significant differences in the blood supply to the four surfaces of a socket". It was further claimed that the perforating vessels give rise to a network which runs within the ligament parallel to the long axis of a tooth.

It has been commonly observed that ligamentous vessels lie in the loose connective tissue which fills the spaces between the bundles of fibres. Gilchrist (1979) commented, "principal fibre bundles irrespective of location are almost avascular".

The mammalian gingival blood supply is considered to originate separately from the alveolar blood supply. Accordingly, the mandibular and maxillary arteries are supposed to give rise to sublingual and palatal branches, respectively, which in turn divide into gingival branches. These branches anastomose freely with those of the periodontal ligament.

Castelli (1963) claimed that veins and arteries follow different paths within the ligament. Kindlova and Matena (1962) described an intraligamentous plexus of veins, and Castelli and Dempster (1965) agreed with them that the veins within the ligament either pass through the alveolar wall to join a central septal venous plexus or anastomose apically with the pulpal veins.

Levy and Bernick (1968) claimed that, in marmosets, ligamentous veins and lymphatics follow

the same course. They opined that "lymph capillaries" originate as blind endings in the stroma of the periodontal ligament and empty into collecting lymph vessels. Collecting vessels were thought to follow one of three pathways from the ligament: "passing over the alveolar crest to drain into the submucosal regions of the palate and gingivae, perforating alveolar bone to traverse spongiosa, or passing directly apical in the periodontal ligament".

Except for the work of Gilchrist (1979), studies identifying vessels as arterial, lymphatic or venous appear to have been inadequate, the classifications being subjective. Tracers were not used, and ultramicrography was avoided. Consequently, vascular identification was made either by the "apoxestic technique" (Simpson 1966, 1967) or by LM examination of formalin fixed, paraffin embedded histological sections, each of which was probably of the order of 8µm thick. Moreover, references were not given to experiments determining the stated origins of periodontal vessels, and no evidence was given for the cited existence of a "central septal venous plexus" (Castelli and Dempster 1965) or for the passage of vessels of

given types within alveolar bone.

Consequently, it appears that much evidence is inconclusive upon which identification and distribution of periodontal vessels has been made. Thus, Saunders and Rockert (1967) stated:

> "There is still a marked lack of physiological information regarding the behaviour and role of the circulation in and about the tooth, which ... stems from the protection of pulpal and periodontal vessels by hard dense tissues and the difficulty of directly observing them.
> ..."With some few exceptions, we have as yet only morphological information regarding the vascular system of the dental tissues.
> ..."A review of the literature reveals that comparatively little work has been done on the major blood vessels supplying the human jaws and teeth, vascular studies having been largely restricted to the small vessels in and about the teeth. Current textbook descriptions provide a broad statement of the general blood supply of the jaws and teeth, but seldom give information regarding the materials used or the sources consulted. Indeed, the literature reveals a need for the systematic re-examination of the distribution, symmetry, and variations of these vessels, and a study of their vascular patterms over various age periods.
> ..."This situation has doubtless arisen from the difficulty of obtaining fresh dentulous

> human material for study, as well as the difficulty of visualising the vessels throughout their bony course.
> ..."Thus an account of the gross anatomy and distribution of the dental or alveolar arteries can at present be little more than a revision of textbook statements..." .

Concerning lymphatics within periodontal tissues, the attitude of Saunders and Rockert, and of this author, was well summarised by Mjor and Pindborg (1973) who stated: "The lymph drainage, although inadequately known, presumably follows the path of the blood vessels".

In summary, all that one can conclude concerning the distribution of periodontal vessels is:

(a) Blood vessels, as yet unidentified, are present in alveolar bone and periodontal ligament.

(b) Textbook descriptions of the afferent and efferent distributions of periodontal vessels are inadequately researched.

(c) Periodontal lymph vessels probably exist, but their distribution and their afferent and efferent connections have yet to be determined.

(iii) SMALL VESSELS OF THE LIGAMENT.

In this dissertation, 'small' vessels are defined as being in lumenal size roughly equal to the diameter of an erythrocyte. Thus, by Luft's classification (1973), small vessels are capillaries.

1. Distribution:

According to Gilchrist (1979) and Mjor and Pindborg (1973), ligamentous capillaries are situated in loose connective tissue which lies between the bundles of principal fibres.

2. Structure:

Ultramicroscopic studies of ligamentous capillaries are few. Gilchrist (1979) classified all human ligamentous capillaries present in his specimens as pericytic venules.

"Terminal vessels" in mouse periodontal ligaments were described in a TEM study by Avery et al (1975) and by Corpron et al (1976). They described "capillaries" which have finger-like projections into the lumen of the vessels, and endothelial cytoplasm which contains numerous scattered vesicles, ribosomes,

and small oval mitochondria. Occasional endothelial tight junctions were reported, but only in those vessels lined completely by a basement membrane and which are without a complete circle of pericytes. Fenestrae were specifically reported to be restricted to vessels which lie on the bony side of the ligament, and all these appear to have diaphragms; the fenestrae were reported to be 0.05µm in "length". Small "arterioles" were noted ... "near alveolar bone or near cementum" which display ... "an incomplete muscular coating and occasional myoepithelial junctions". However, Gilchrist (1979), examining human tissue, could find no evidence of smooth muscle within the walls of any vessels, and he saw few fenestrae. He reported some vessels to be surrounded by pericytes but that, most commonly, the pericyte layer is incomplete.

(iv) SMALL VESSELS OF THE ALVEOLAR BONE.

1. Distribution:

The present author could find no published material dealing with the distribution of capillaries in the medullary spaces of alveolar bone.

2. Structure:

No information appears to exist on the LM or TEM appearance of alveolar capillaries.

(v) LARGE VESSELS OF THE LIGAMENT.

In this dissertation, 'large' vessels are defined as being greater in lumenal diameter than small vessels by a power of greater than 2.10^3. Thus, by contrast with small vessels, at any cross sectional level a large vessel is capable of containing, within its lumen, many thousands of erythrocytes.

1. Distribution:

No information appears to exist on the subject of large ligamentous vessels. General references to "lymph capillaries", "arteries" and "veins" indicate that large vessels have, most probably, been seen in the past.

2. Structure:

Histological, TEM or other investigatory studies of ligamentous large vessels do not appear to exist in the literature.

(vi) LARGE VESSELS OF THE ALVEOLAR BONE.

(vi) 1. Distribution:

Unsupported references were made by Levy and Bernick (1968) to lymphatic collecting vessels in alveolar bone. However, no lymphatic vessels have ever been histologically, ultrastructurally or experimentally demonstrated in any type of bone in any animal (Abramson 1962, Vaughan 1975, Deysine 1980 personal communication). Consequently it appears that Levy and Bernick's opinion was based on surmise.

Kindlova and Matena (1962) and Castelli and Dempster (1965) referred to a "central septal venous plexus" fed by ligamentous veins; however, no justifying evidence was given to support these descriptions.

2. Structure:

There are no published descriptions of the structure of the large alveolar vessels.

(vii) MEDULLARY CANALICULAR RELATIONSHIPS IN LONG BONES.

Excluding the walls of osteocytic lacunae and canaliculi, Vaughan (1975) considered that the surfaces of all long bones consist of:

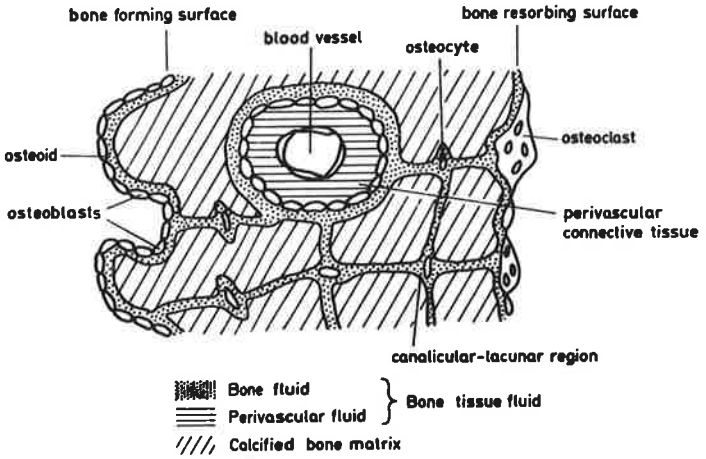
(a) osteogenic tissue:
(b) bone tissue fluid:
(c) osteoid tissue:
(d) mineralised matrix.

Osteogenic tissue was thought to consist of an inner layer of cells which forms a "functional membrane" covering the entire mineral matrix surface (Ramp and Neuman 1973). Cells of the inner osteogenic layer were thought to be in contact by close, open and tight junctions (Matthews, Martin, Collins, Kennedy and Powell 1973). In young, actively growing bone the osteogenic layer is several cells thick; however, in older bone, it is often only one cell thick (Sissons 1970).

Between the internal osteogenic layer and the mineral surface matrix is bone tissue fluid (Neuman 1969). This differs from plasma and extracellular fluid in both its mineral and protein content (Owen and Melick 1973, Triffitt and Owen 1973). "Bone tissue fluid between the osteogenic cells and the mineral matrix surface is continuous with the bone tissue fluid in the canaliculi, in the osteogenic lacunae of both trabecular and cortical bone, and possibly with the fluid in the vascular connective tissue spaces between the endothelial walls of the blood capillaries and the cells on the bone surfaces of the Haversian canals" (Vaughan 1975). This is shown diagrammatically in Figure 1.

Macromolecules, such as intravenously injected horseradish peroxidase and thorium dioxide, have been found to penetrate the canalicular-lacunar region, presumably by means of a transfer mechanism, to the bone tissue fluid (Doty and Schofield 1972). Moreover, Owen (1973) has shown that albumen, immunologically identical to serum albumen, is present throughout the bone tissue fluid region. Vaughan implied that albumen, the protein mainly responsible for binding calcium in plasma (Moore 1970), might penetrate to lacunae and canaliculi by a mechanism similar to that which transports horseradish peroxidase and thorium dioxide.

This evidence applies to long bones. There appears to have been no similar investigation of alveolar bone.



Part of the cross-section of the mid-shaft of the femur of a three-week-old rabbit, very diagrammatic and not to scale. Cell processes connecting osteocytes and cells on periosteal, endosteal and surfaces of Haversian canals are not shown.

Fig. 1. VASCULAR CANALICULAR RELATIONSHIPS.

(From Owen, Triffitt and Melick 1973).

SECTION 4.

MATERIALS AND METHODS.

(i) SAMPLE.

A maxillary first premolar, together with attached buccal bone and periodontal ligament, was biopsied from each of six (6) children. The subjects, males and females, ranged in age between 11 and 15 years. Removal of this tissue was necessary for orthodontic treatment purposes.

Prior to extraction, the teeth were in functional occlusion. They were healthy, and had not been subjected to any previous orthodontic procedure.

(ii) TISSUE PREPARATION.

(a) Biopsy Technique:

The cervical two-thirds of the root of the premolar was surgically removed, together with a sliver of intact buccally attached periodontal ligament cum buccal bone. This surgical technique has been detailed by Gilchrist (1979). To avoid unnecessary

treatment discomfort to the children, it was considered best not to include the apical one-third of the tooth in surgical removal. Advantages of the surgical biopsy technique were that a full thickness of periodontal ligament was available for sampling, and dental and bony attachments of these specimens remained undisturbed. Orientation of the tissue was easy. However, to ensure complete tissue fixation, it is unfortunate that the sample was restricted to a 1mm wide segment of the cervical two-thirds of the periodontium.

(b) Anaesthetics:

All tissue was biopsied under local anaesthesia. For comparison in the electron microscope, a local anaesthetic containing a vaso-constricting agent (1:80,000 adrenaline, 2% lignocaine HCl; Xylocaine, Astra Chemicals Ltd., North Ryde, N.S.W.) was used to excise five specimens. One specimen was biopsied using a local anaesthetic containing no added vasoconstrictor (Citanest 15; Prilocaine 4%, NaCl 6mg, methyl-hydroxybenzoate 1mg in water; Astra Chemicals Ltd., North Ryde, N.S.W.)

Eventually, it was found that the vaso-constrictor had no apparent effect on the histology or fine structure of the biopsies.

Preparation of Specimen:

The steps are described in chronological order, as follows:

(c) Fixation:

Teeth and attached periodontal ligaments were washed briefly in physiological saline at room temperature. Within 20sec of excision they were immersed in the primary fixative.

The primary fixative used was 4% buffered glutaraldehyde (Taab Laboratories, Reading, England) pH7.4 at $4^{o}C$.

(d) Buffer:

Two different buffering solutions were used. They were 0.06M cacodylate (Sodium cacodylate; BDH Chemicals Ltd., Poole, England) and Clark's Buffer (see Appendix I). Where a tissue fixative incorporated a particular buffer, this same buffer was used for all subsequent buffering and washing procedures.

(e) Agitation:

The composite specimens of tooth, buccal bone and attached periodontal ligament were wrapped lightly in gauze, immobilized, and submerged in solutions which were gently agitated, during all procedures, with a magnetic stirrer. This minimised distortion and tissue damage, and prevented separation of tooth, bone and periodontal ligament. The specimens spent no longer than 12hr in the primary fixative.

(f) Washing:

Tissues were removed from the primary fixative and washed for 10min in buffer at $4^{o}C$.

(g) Decalcification:

The composite specimens of tooth, bone and intact periodontal ligament were decalcified in 0.1M buffered EDTA (Ethylenediaminetetraacetic acid di-sodium salt; Ajax Chemicals, Sydney, N.S.W.) in 2.5% glutaraldehyde pH6.0 (Anax Digital pH Meter) at $4^{o}C$. The decalcifying solutions were changed each day for the first two weeks, and then on each alternate day. At all times during the decalcification procedure the tissues were agitated with a magnetic stirrer.

(h) Trimming the Composite Specimens:

When the tooth enamel became soft, the composite-specimens were trimmed with a sharp scalpel or diamond disc to isolate the tooth-bone-periodontal ligament sample.

(i) Decalcification End-Point:

The decalcification end-point was determined radiographically. The standardised technique was described by Fejorskov (1971) using a dental occlusal radiograph, a long cone, a line voltage of 50KV, a current of 15maSec and an exposure time of 0.5sec. When the specimen was uniformly radiolucent, decalcification was judged to be complete.

(j) Washing:

The specimens were washed for 5min in each of two rinsing solutions of the appropriate buffer pH7.4 at $4^{o}C$.

(k) Post Fixation:

Cacodylate-buffered specimens were postfixed with 2% buffered osmium tetroxide (Johnson Chemicals Ltd., Royston, Hertfordshire, England) at $4^{o}C$ for 1hr.

(l) Block Stain:

Cacodylate-buffered specimens were stained for 1hr at room temperature with uranyl nitrate (Ajax Chemicals, Sydney, N.S.W., Australia) 1% in 70% ethanol. This stain is considered by Casley-Smith (personal communication, 1979) to improve the electron density of endothelial junctions and to decrease stain detritus.

(m) Embedding in Epon:

Cacodylate-buffered specimens were dehydrated as follows:

15min	70% acetone	rotated/agitated	room temperature
15min	70% acetone	rotated/agitated	room temperature
15min	80% acetone	rotated/agitated	room temperature
15min	80% acetone	rotated/agitated	room temperature
15min	90% acetone	rotated/agitated	room temperature
15min	90% acetone	rotated/agitated	room temperature
15min	100% acetone	rotated/agitated	room temperature
15min	100% acetone	rotated/agitated	room temperature

The last two solutions were stored over anhydrous copper sulphate for 24hr prior to use.

Epon 812 (Ladd Research Industries Inc., Vermont, U.S.A.) was used to embed these specimens in the following manner:

- infiltration for 12hr at room temperature in Epon resin: 100% dehydrated acetone = 1:1 with continuous agitation;
- infiltration continued for a further 12hr at room temperature by rotating or agitating tissues in 100% Epon;
- tissues embedded in silicon moulds with final Epon embedding resin;
- embedded tissue incubated at 37^{o}C for 48hr;
- tissue finally incubated at 60^{o}C for 48hr.

(n) Embedding in Spurr's Resin:

Specimens which were buffered with Clark's Buffer were dehydrated in a sequence of acetone solutions as follows:

10min	70% acetone	rotated/agitated	room temperature
10min	70% acetone	rotated/agitated	room temperature
10min	70% acetone	rotated/agitated	room temperature
10min	90% acetone	rotated/agitated	room temperature
10min	100% acetone	rotated/agitated	room temperature
10min	100% anhydrous acetone	rotated/agitated	room temperature
10min	100% anhydrous acetone	rotated/agitated	room temperature

The last two solutions were stored over anhydrous copper sulphate for 24hr prior to use.

Spurr's resin (Ladd Research Industries Inc., Vermont, U.S.A.) was used to embed these specimens in the following manner:

- infiltration for 4hr at room temperature in Spurr's resin: acetone = 1:1 with continuous agitation;
- infiltration continued for a further 14hr at room temperature in 100% Spurr's resin;
- tissues embedded in silicon moulds using 100% Spurr's resin;
- embedded tissue incubated at 70^{o}C for 6 days.

(o) Orientation:

The composite specimens of tooth, periodontal ligament and bone were incubated with coded paper, which marked the orientation of the tissue in the mould.

(p) Storage:

The coded blocks were stored at room temperature in similarly coded glass vials.

(q) Microtomy:

Each of the six blocks was serially sectioned, horizontally through tooth, bone and periodontal ligament. Specimens about 1µm thick for LM examination were cut at an angle of 3^{o} on a Reichert OMU3 ultramicrotome. Sections of high quality were obtained for this purpose by using glass knives prepared on an LKB Knifemaker, Type 7801B.

For the purpose of orientation, the block face was trimmed to the form of a trapezium, the divergent sides being of unequal length to prevent confusion due to the inevitable lateral inversion of the sections. These specimens were flattened with chloroform vapour, picked up on the sharp point of a

de-waxed scalpel blade (Gillette; E/11), and mounted in a pool of double-distilled water on a glass microscope slide. Sections for light microscopy were stained as follows:

- dried on a hot plate at 90°C;
- stained with (1% toluidine blue+1% borax) and heated, without drying, on a hot plate at 90°C for 1min;
- rinsed with double-distilled water;
- differentiated in 50% ethanol;
- dried on a hot plate at 90°C;
- cleared in xylene;
- protected with glass cover slips mounted using pix, xylol and dibutyl phthalate medium (XAM).

Each of the serial sections was viewed by LM to determine whether vessels were present in either the alveolar bone, the periodontal ligament, or in both.

(r) Ultramicrotomy:

If vessels were present in the block face, the sample was ultramicrotomed. Final

identification and classification of vessels were made in the TEM. To enable ready equation of TEM viewed material with light micrographs, the same mesa used for glass knife sectioning was used unmodified for ultramicrotomy.

Sections for viewing in the TEM were cut with a diamond knife (Diatome Ltd., Bienne, Switzerland) at a clearance angle of 8^{o} and a cutting speed of less than 1mm/sec. Sometimes successful sections for electron microscope viewing were taken with a glass knife at a clearance angle of 3^{o} and a cutting rate of less than 1mm/sec., but these occasions were few and quality suffered.

The thickness of the ultramicrotomed sections was about 70nm. They were floated in a bath of double-distilled water and diffracted the fluorescent light source, used at the time, as a silver colour (measurement of section thickness by Crocker and Casley-Smith, personal communication; 1979).

To flatten the silver sections, a camel-hair brush impregnated with chloroform was gently waved close to them as they floated in the bath.

(s) Grids:

Using a stainless-steel loop, the sections were transferred to the dull surfaces of uncoated, 200-mesh copper grids, diameter 2.3mm (01 210; Balzers Union).

By experiment, it was determined that this type of grid was best. The absence of a slightly opaque supportive coating increased the clarity of micrographs, and resulted in a reduction in the amount of detritus left by staining materials. Furthermore, it was found that the coarseness of the mesh reduced the probability of grid bars interfering with the field being examined.

(t) Final Stain:

Grid-mounted tissues were floated, face down, in filtered (No. 40 Millipore filter paper) 5% uranyl acetate (Ajax Chemicals, Sydney, Australia) for 30sec in a moist environment at 37^{o}C.

The grids were then washed by passing them slowly, tissue side foremost, through double-distilled water 37^{o}C for 30sec, after which they were gently dried by blotting, face-down,

on filter paper.

The tissues were then lead-stained by immersing them, face-down, in modified Reynolds' Solution (see Appendix II) at room temperature for 30sec. To reduce precipitation of lead carbonate on the grids, it was found necessary to carry out this staining procedure in a covered petri-dish, to which CO_2-absorptive sodium hydroxide pellets had been added not less than 10min before.

The grids were again washed in double-distilled water at room temperature for 30sec, and dried gently by blotting.

The specimens were then ready for viewing in the electron microscope.

(u) Specimen 'Conditioning':

Grids were mounted in the electron-microscope, with their dull sides toward the viewing platform. The instrument used was a Siemens Elmiscope 1, using 60KV and a point emitting filament.

However, tissues mounted on 200-mesh uncoated grids are poorly supported. Under the

pressure of a high intensity electron-beam the soft Epon-embedded tissue sags between the sparsely distributed grid bars.

A conditioning procedure was used to harden the Epon and thereby to stabilize the tissue. Thus, the total surface area of each specimen was exposed to a low intensity electron-beam at low magnification for 5min. When grids treated in this way were removed and examined in the light microscope, it was found that the conditioning procedure had caused the Epon to become a pale, translucent brown colour; furthermore, the tissue could no longer be restained.

Following the conditioning treatment, the material could be viewed at the highest electron intensities and magnifications without movement or tearing of the specimen. This was made manifest by the excellent clarity of well-focused micrographs, each of which was exposed for 4sec.

(iii) MICROPHOTOGRAPHY.

Light micrographs were taken with an Axiomat

(Zeiss, West Germany). The 1µm thick resin sections were only lightly stained with toluidine blue, with the result that coloured photographs were of poor contrast. Consequently, all light micrographs were made on high contrast black and white film (Recordak, Kodak). Ultramicrographs were taken only of good quality specimens. The degree of TEM magnification of each ultramicrograph was assessed by photographing at the same microscope settings a grating replica of 2160 lines/mm (Ernest F. Fulham, Schenectady, NY).

SECTION 5.

RESULTS.

In this section, explanation of the micrographs is contained within the text.

(i) DISTRIBUTION OF HUMAN PERIODONTAL VESSELS.

The ligament consisted of dense collagenous 'principal' fibres through which ramified 'columns' of connective tissue, relatively low in fibre content and high in ground substance. In a transverse serial histological section, a column appeared as an 'island' of poorly stained material surrounded by a densely stained mass of the principal fibres (Figs. 2, 3A, 3B, 4 and 8). Large and small vessels were contained within the columns (Figs. 2 and 4). From serial sections of the limited area of periodontium examined, it appeared that the connective tissue columns merged to pass through foramina in the alveolar bone, eventually to combine with the flimsy looking connective tissue of the trabecular spaces (Figs. 2, 4, 5A, and 19A to 19I). Some of the ligamentous vessels were seen to communicate, via the columns, with similar vessels in the alveolar spaces.

(ii) SMALL VESSELS OF THE LIGAMENT.

1. Distribution:

At a distance from the foramen, ligamentous small vessels occupied the cemental one-half of the periodontal space. Individual small vessels were contained in small islands lying close to the cement. In the centre of the ligamentous space lay the largest islands, each of which contained numerous small vessels (Figs. 2, 3A, 3B and 4). The entrance to a foramen consisted of one large connective tissue island, in which were situated ligamentous small vessels of the greatest external diameter (Figs. 4 and 5A).

2. Structure:

All ligamentous small vessels were characteristic of pericytic venules. No other type of small vessel was seen. As is demonstrated by comparing Figures 5C, 6, 7, 8, 9 and 10, the venules varied in appearance. However, each consisted of one or several endothelial cells surrounding a lumen, which was wide enough to contain an erythrocyte. The endothelium of these vessels was plump, and bulged into the lumens. In one example

(Figs. 5C and 5D), the endothelium was distinctly columnar. Basement membranes were well defined (Fig. 5D) and the vessels were either partly or completely surrounded by pericytes. Serial histological sections demonstrated that there was little change in the size or character of a small vessel throughout the ligament. Collagen fibres parallelled the vascular endothelium, but did not insert into it (Figs. 16 and 17). Interendothelial junctions were either close or tight. No open junctions were observed.

(iii) SMALL VESSELS OF THE ALVEOLAR BONE.

1. Distribution:

Some of the ligamentous pericytic venules passed through foramina in the alveolar socket wall to enter trabecular spaces (Figs. 19A to 19I). Within the alveolar spaces, the columnar arrangement of the ligamentous interstitial tissues gave way to a loose, flimsy connective tissue (Figs. 5A, 24, 25, 26C, 26D, 27B, 27C and 27D). Large vessels and fat spaces occupied the major part of the volume of the trabecular spaces (Figs. 4, 22A, 22B, 23A, 23B, 23D, 26A, 26B and 27A). Alveolar small vessels,

likewise identified as pericytic venules, were situated to within a few microns of the endothelium of the large vessels (Figs. 12, 14, 15 and 27E). Thus, one is tempted to term the alveolar venules 'vasa vasorum', for no reason other than that this association existed.

2. Structure:

Although the structure of alveolar pericytic venules varied (Figs. 11, 12, 13, 14 and 15) in basic appearance, they differed little from their ligamentous counterparts. Endothelial cells bulged into the lumen, basement membranes were well defined, and tight and close junctions only were observed. Accompanying collagen fibres were rare; however, as with ligamentous small vessels, fibres, where present, ran parallel to the walls of the venules.

(iv) LARGE VESSELS OF THE LIGAMENT.

1. Distribution:

Large vessels were, by far, the most obvious histological structures seen in the periodontal ligament. They were of greatest size opposite their point of entry, via alveolar

foramina, into the trabecular spaces (Figs. 2, 4, 5A, and 19E to 19H). At a distance from foramina, large vessels did not exist within the periodontal ligament (Fig. 22A). Thus, in any histological cross section of the periodontal ligament, ligamentous large vessels were, or were not, seen depending on the proximity of the section to a foramen. As serial sectioning proceeded closer to a foramen, the most distant branches of large vessels were seen to surround pericytic venules in the loose connective tissue islands (Figs. 2, 4 and 5B). Further sectioning revealed that the outlying branches anastomosed to form ligamentous large vessels, many hundreds of microns in diameter (Figs. 19A to 19I), near the opening of the foramen.

On one occasion, a foramen was seen which transmitted no vessel of any description (Fig. 3B). Figure 2 illustrates an isolated example of a 'cemental pearl' found to be present opposite a foramen.

2. Structure:

The walls of ligamentous large vessels consisted of a single layer of endothelial cells

supported by a poorly defined basement membrane (Figs. 18A and 18B). There was no adventitia. The endothelial cytoplasm was wide, containing numerous organelles and fine fibrils. Open junctions (Fig. 18B), as well as the usual close and tight junctions, were seen, and the vessels were invested with an incomplete layer of pericytes. The tissue supporting ligamentous large vessels was more fibrous than that which contained alveolar large vessels. In general, collagen fibres parallelled the vessel walls. However, at intervals, collagen fibrils were seen to insert into the endothelial lining, fine cytoplasmic fibrils being orientated in the same direction as the extravascular insertions (Fig. 18B). Ligamentous large vessels contained particulate matter in which occasional erythrocytes were seen (Figs. 18A, 18B, and 19A to 19I).

(v) LARGE VESSELS OF THE ALVEOLAR BONE.

1. Distribution:

Large vessels, together with the closely adjacent fat spaces, occupied the main volume of the trabecular spaces (Figs. 4, 20A, 22A, 22B, 23A, 23B, 23C, 26A and 27A). Every

trabecular space contained a large vessel which, on evidence derived from serial sections (Figs. 19A to 19I), eventually ramified into the periodontal ligament through a foramen in the alveolar socket wall. Hence, with the one reported exception, associated with each foramen a large vessel was seen to extend into the trabecular spaces, on the one hand, and into the periodontal ligament, on the other. In one specimen, where the full thickness of alveolar bone was retained, a trabecular extension of a ligamentous large vessel was seen to penetrate the buccal plate of bone through a foramen (Figs. 23A to 23C).

The loose connective tissue surrounding the ligamentous extensions of large vessels was continuous with the flimsy connective tissue of the trabecular spaces. Within a foramen which transmitted vessels, collagen fibres were common, although they were sparse when compared with the dense principal fibre bundles in the bulk of the ligament. However, in the trabecular spaces far removed from a foramen, collagen fibres were few, and often only individual fibres were seen (Figs. 26D, 26E, 27B, 27C and 27D). The volume of periodontal

large vessels was not estimated; however, indication of their size is given in Figure 26A, where the length of part of a sectioned alveolar large vessel is 1.3mm and its average width is 150μm. Relative to the volume of the excised alveolar tissue, alveolar large vessels were very large indeed. They contained particulate matter, a few scattered erythrocytes, and occasional inclusions which were possibly fat globules (Figs. 24 and 25).

2. Structure:

Similar to their ligamentous extensions, alveolar large vessels consisted of a single layer of endothelial cells on a basement membrane which, in trabecular spaces far removed from the foramen, was apparently non-existent (Figs. 20F to 20L, 24, 25, 26D to 26K and 27D). The vessel walls were exceedingly thin, as a result of which few vesicles (Figs.26H and 26I) or organelles were evident. So slight was the investing endothelium that apparent fenestrae were often seen (Figs. 20L and 26H). Where they were recognisable, most junctions were close or open (Figs. 20K, 21D, 26F, 26G and 26J). Supporting collagen fibres were

few but, where present, were often seen to insert into the endothelial lining (Figs. 20F to 20L, 21D and 26E). An incomplete layer of pericytes was common.

Occasionally a vesicle, apparently containing fat, was seen in the walls of alveolar large vessels (Fig. 20F), although it is possible this might also represent an erythrocyte passing through the endothelial wall.

The extremely delicate structure of alveolar large vessels and their supporting tissues was impressive; it seemed to be incompatible with the vessels' great size.

(vi) MEDULLARY CANALICULAR RELATIONSHIPS IN ALVEOLAR BONE.

Generally, the walls of trabecular spaces were lined with cells (Figs. 20M, 22B, 27D, 28A and 28B). In some light micrographs it appears that this investing layer was torn, either during excision of the specimen or block processing. This type of artefact was well illustrated by Vaughan (1975). Consequently, it has been difficult to determine whether endosteal cells lining the bony walls coupled as the endothelial cells of alveolar large vessels (Figs. 19I, 20B, 20M, 22A, 22B, 26B, 28A and 28B). However, in other

examples (Figs. 27D and 27E), it is apparent that the endothelial walls of large vessels assumed a close anatomical relationship with trabecular endosteal cells. Thus, whether or not they were endothelial, the cytoplasm of the endosteal cells was closely related to the cytoplasm of osteocytic processes running within the bony canaliculi (Figs. 20M, 28A, 28B and 28C). No ultramicrographs were taken of the actual junctions between these cells, the necessary ultra serial sectioning not having been part of the experimental protocol. However, the approximation of the two cytoplasmic processes exemplified by Figures 28A to 28C, and the proximity of alveolar large vessels to the endosteal trabecular cells, makes it probable that communication, of some sort, existed between alveolar large vessels and osteocytes embedded within the lacunae of the alveolar bone.

(vii) FAT SPACES.

Common to all specimens was the presence of large 'fat spaces' (Figs. 4, 5A, 20A, 20B, 22A, 22B, 29A and 29B). Generally circular or polygonal in transverse section, these spaces were of the order of 100μm in diameter. Under the ultramicrotome dissecting microscope, such spaces within the block face were seen to be filled with globular

material, the impression given being that of a bunch of grapes (Fig. 20B). In future, it should be quite simple to dissect this material from unfixed, nondecalcified, unstained, frozen alveolar bone, sectioned with a sharp razor blade; the material could then be analysed, perhaps by X-ray diffraction, to determine its crystalline structure and, hence, its composition. However, in this dissertation, and without this type of evidence, the material contained within the spaces will be designated 'fat', the spaces being termed 'fat spaces'. Whether cholesterol, triglyceride, or some other fat derivative, the ultrastructural appearance of this material is shown in Figure 20E. In the opinion of Casley-Smith (1980, personal communication), the fine structure of these deposits is typical of a fat derivative.

All fat spaces were seen only within trabeculae, none having been seen in the periodontal ligament.

The fat spaces lay close to the walls of alveolar large vessels (Figs. 19I, 20A, 20B, 22A and 22B), and were extravascular. Where the fat bulged into the wall of an alveolar large vessel, the fatty material was lined with endothelium and a thin layer of connective tissue (Fig. 20D). Within foraminal interstitial tissue, the wall separating

two fat spaces was fibrous and well organised (Figs. 29A to 29D). However, in travecular spaces proper, the walls were of the flimsy, loose connective tissue (Fig. 20C). There appeared to be no cytoplasm to separate the fat from its containing walls (Figs. 20D and 29D).

What appeared to be 'fatty inclusions' existed within the lumens of alveolar large vessels (Figs. 24 and 25). Ultrastructurally, these inclusions were similar in appearance to the material contained in the fat spaces. Such inclusions were not seen in ligamentous large vessels. Occasionally, fat was seen in the vesicles of endothelial cells which lined both large and small vessels of the alveolar bone and periodontal ligament. Illustrated in Figures 10B, 10C and 20F, the exact nature of this material cannot, again, be stated. However, its ultrastructure was similar in appearance to the fat contained in the trabecular fat spaces.

(viii) SUMMARY.

LM and TEM observations of six specimens of human buccal periodontal tissue excised together with a functional maxillary first premolar revealed common findings:

(a) In the cemental one-half of the periodontal ligament, pericytic venules lay in loose connective tissue between the principal fibre bundles.

(b) Some of the ligamentous pericytic venules, together with their investing loose connective tissue, passed through foramina in the alveolar wall of the tooth socket to the trabecular spaces where, by their close association with the walls of alveolar large vessels, they became 'vasa vasorum'.

(c) 'Large' vessels, of dimensions measured in millimetres, ramified throughout the trabecular spaces, communicating via foramina both with overlying buccal tissues (seen in the one specimen where the buccal plate was retained) and with the periodontal ligament. Ligamentous extensions of alveolar large vessels surrounded pericytic venules. Large vessels were the most obvious feature of the periodontal ligament. Collagen fibrils inserted into the endothelial linings of periodontal large vessels.

(d) Together with alveolar large vessels, 'fat'

contained in 'fat spaces' occupied the greatest volume of the medullary tissue. Fatty inclusions also were seen in the lumens of alveolar large vessels, and within vesicles of the endothelium lining both alveolar large vessels and ligamentous pericytic venules.

(e) The cytoplasm of endosteal cells lining the bony walls of trabecular spaces, and the endothelium of alveolar large vessels, assumed a close anatomical relationship with canalicular osteocytic processes.

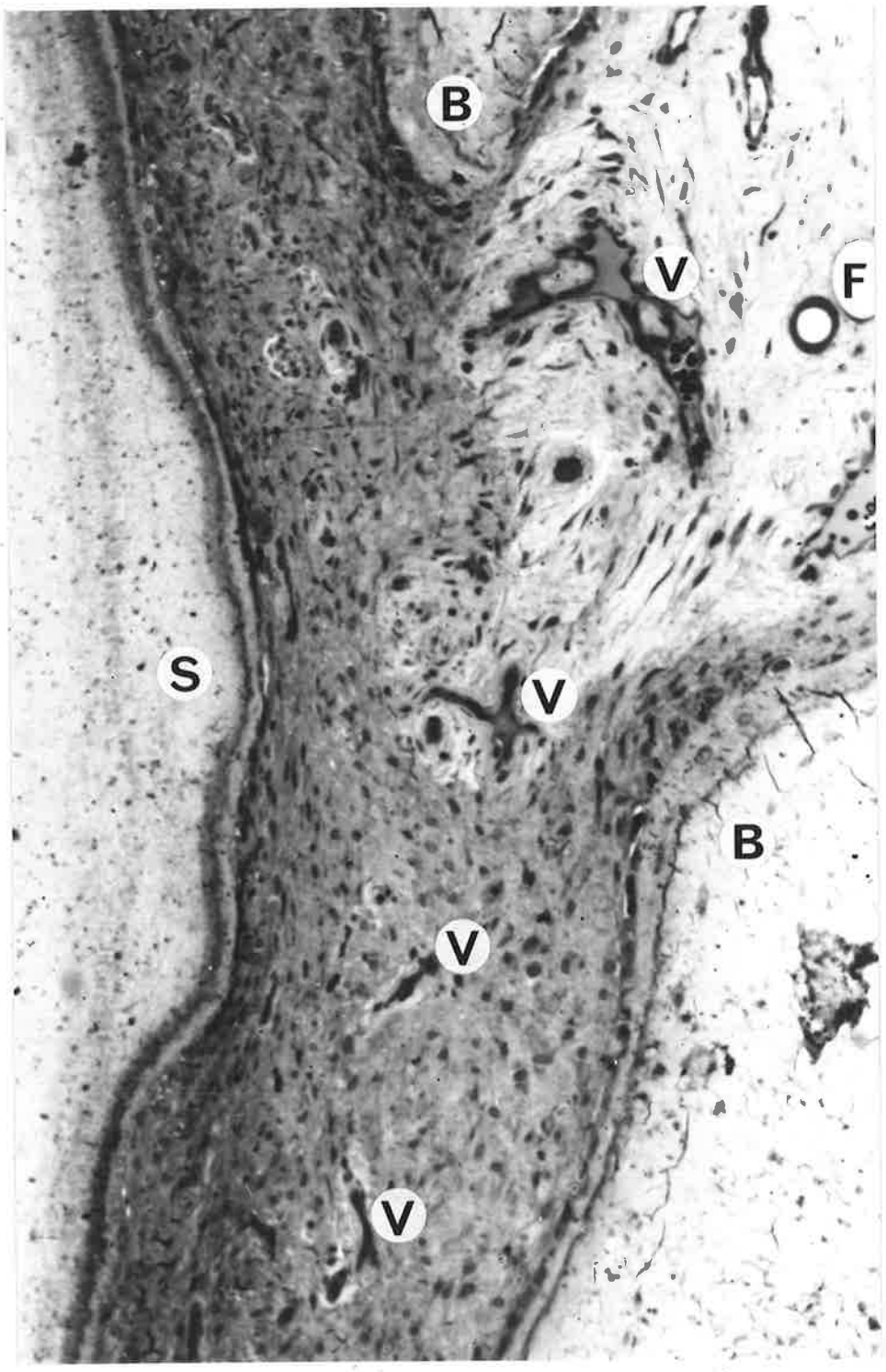


Fig. 2. Section of Periodontium:

Ligamentous large vessels (V) surrounding pericytic venules in loose connective tissue islands, fat spaces (F), foramen between walls of alveolar bone (B,B), cemental pearl (S).

Light micrograph. Toluidine Blue.

Decalcified. (400X).

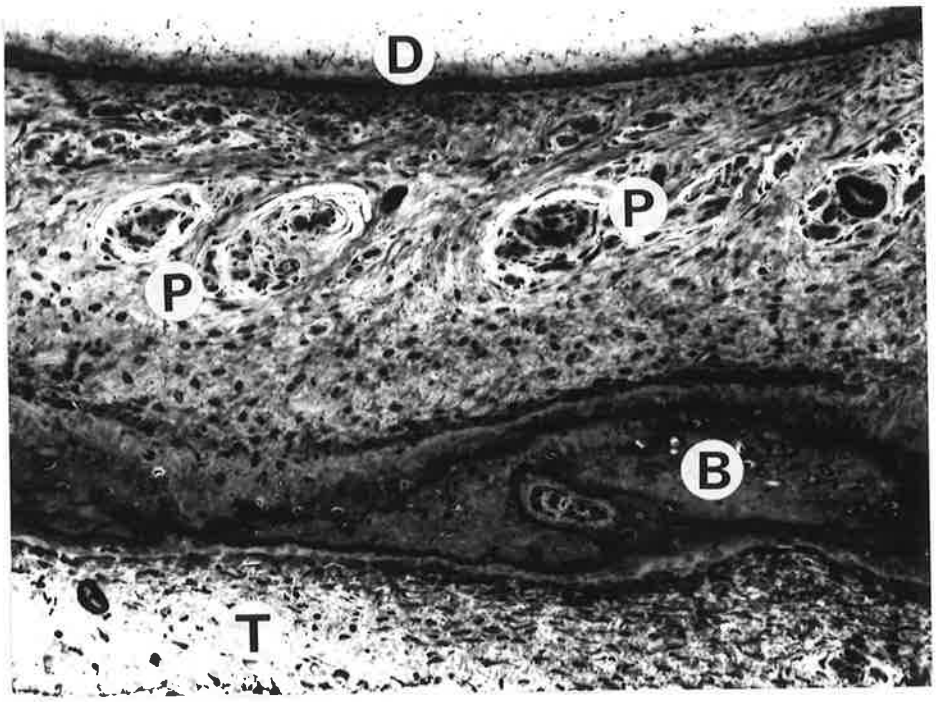


Fig. 3A. Section of Periodontium:

Alveolar bone (B), trabecular space (T), dentine (D), pericytic venules (P) in loose connective tissue islands.

Light micrograph. Toluidine Blue.

Decalcified. (200X).

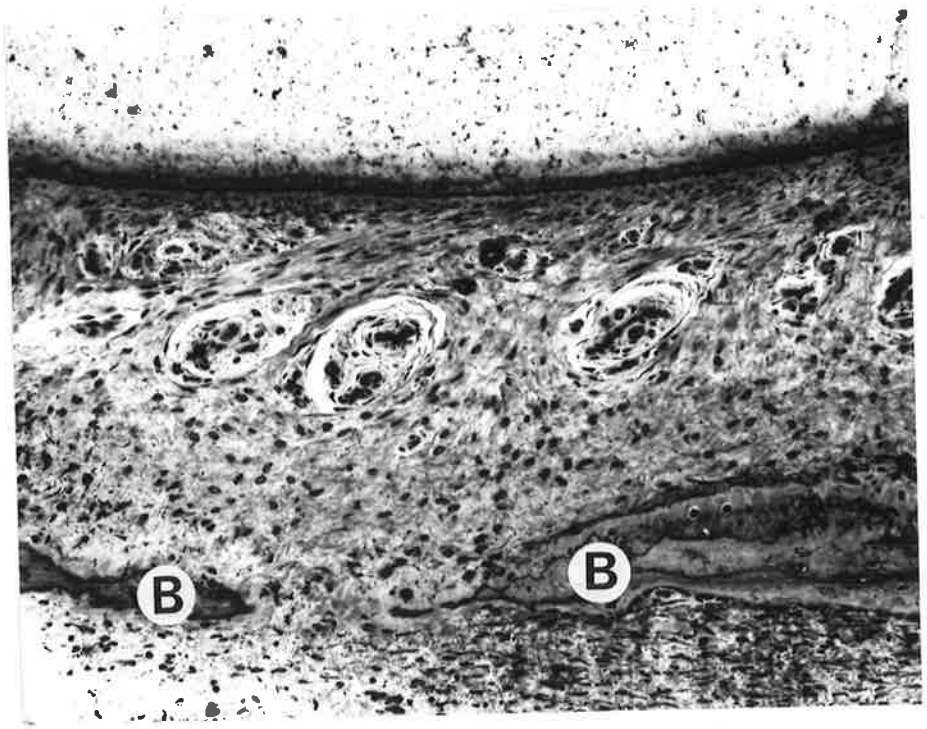


Fig. 3B. Semi Serial Section of Periodontium in Figure 3A:

Foramen (B,B). No vessels pass through foramen.

Light micrograph. Toluidine Blue.

Decalcified. (200X).

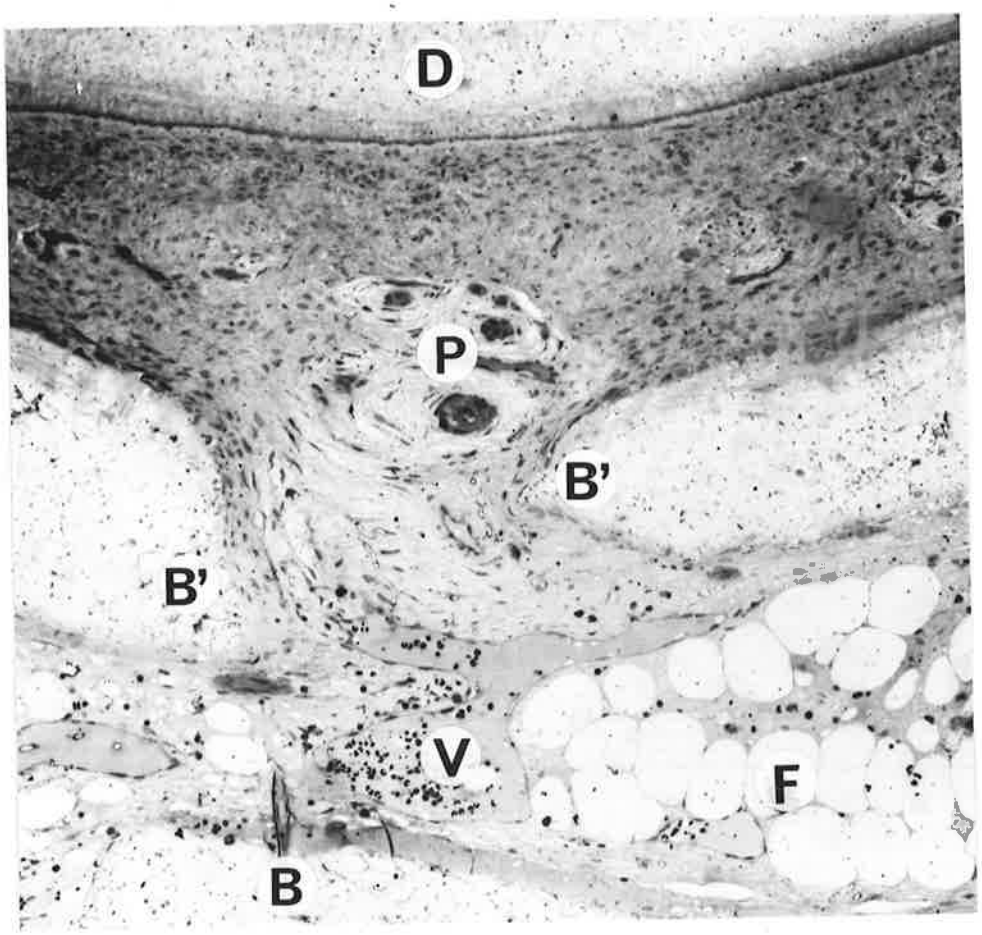


Fig. 4. Foramen in Alveolar Wall of Tooth Socket:

Dentine (D), foramen between (B',B'), in alveolar bone, large vessel (V), fat spaces (F), pericytic venules (P) in loose connective tissue islands.

Light micrograph. Toluidine Blue.
Decalcified. (125X).

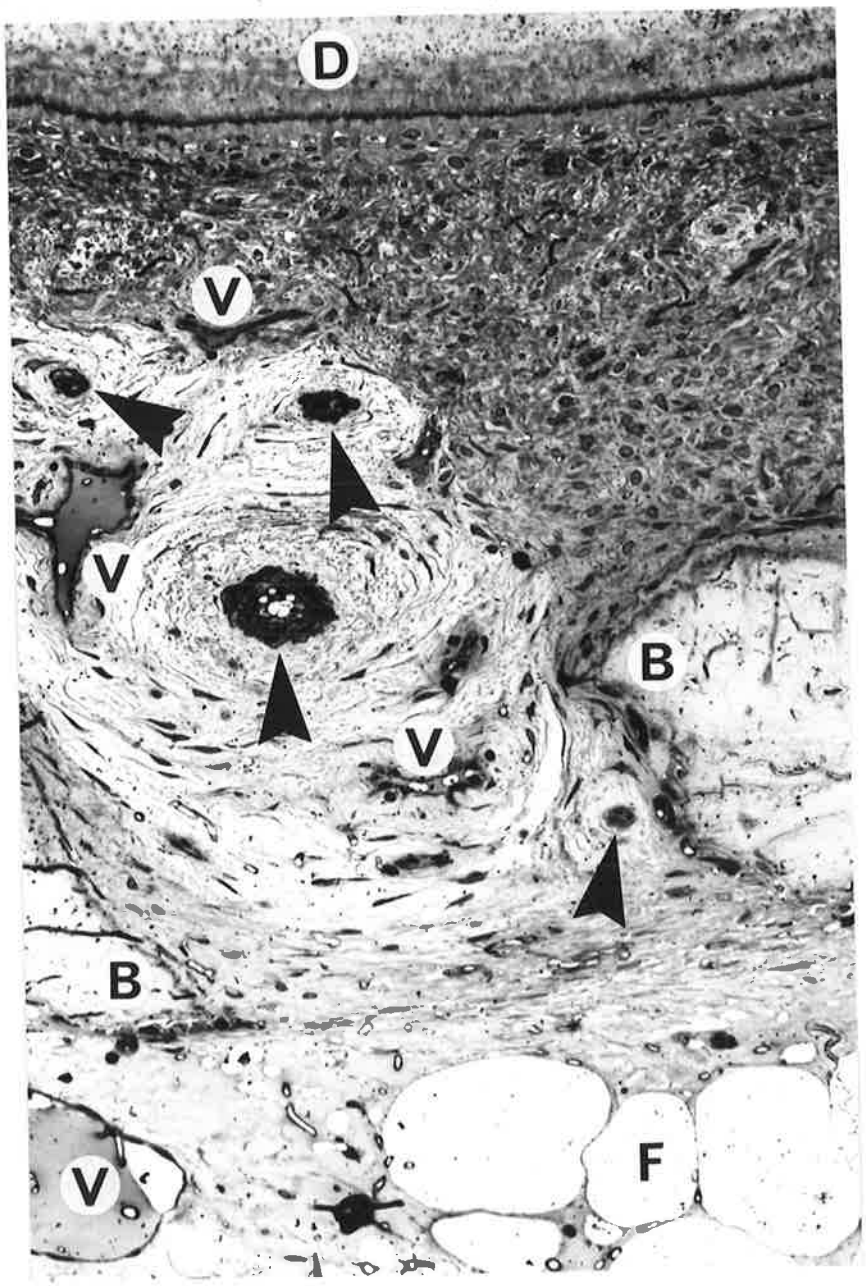


Fig. 5A. Ligamentous Pericytic Venules in Foraminal Loose Connective Tissue:

Foramen in alveolar bone (B,B), fat spaces (F), large vessel extensions (V), pericytic venules (arrowed).

Light micrograph. Toluidine Blue.

Decalcified. (250X).

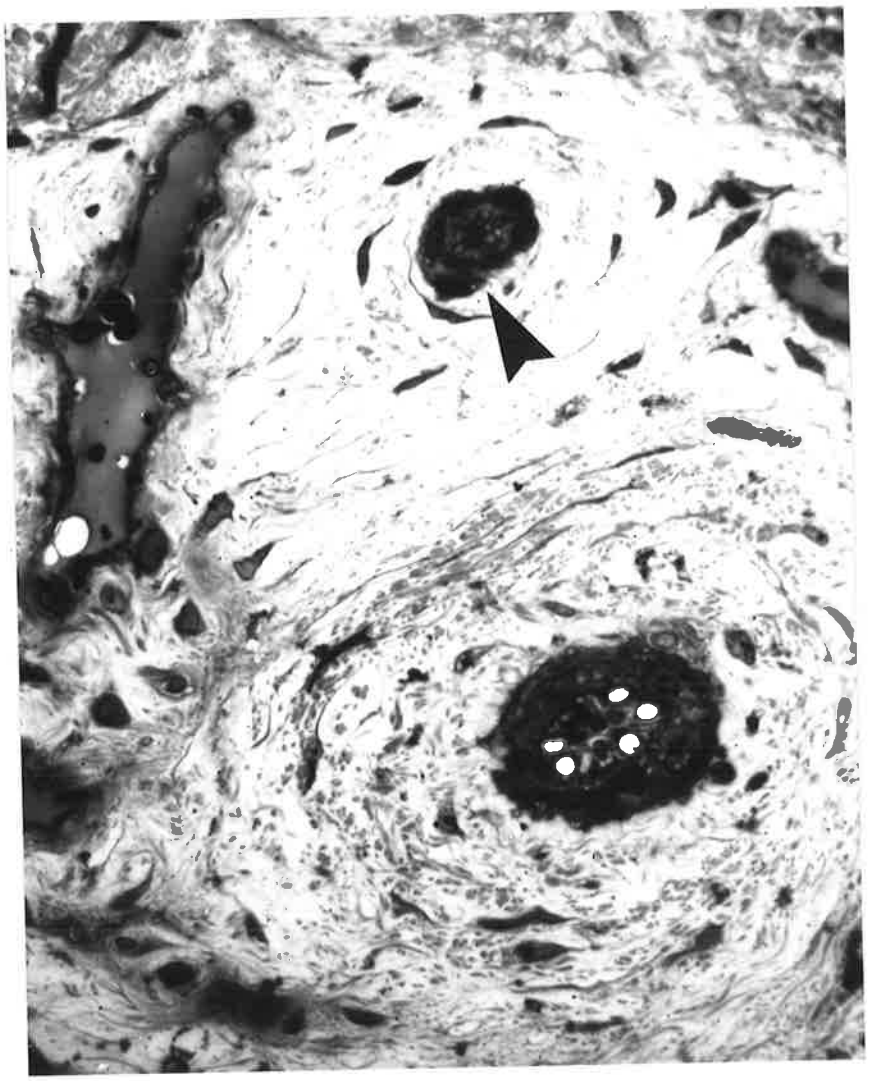


Fig. 5B. Detail of Pericytic Venules Arrowed in Figure 5A - Serial Section:

Light micrograph. Toluidine Blue.
Decalcified. (500X).

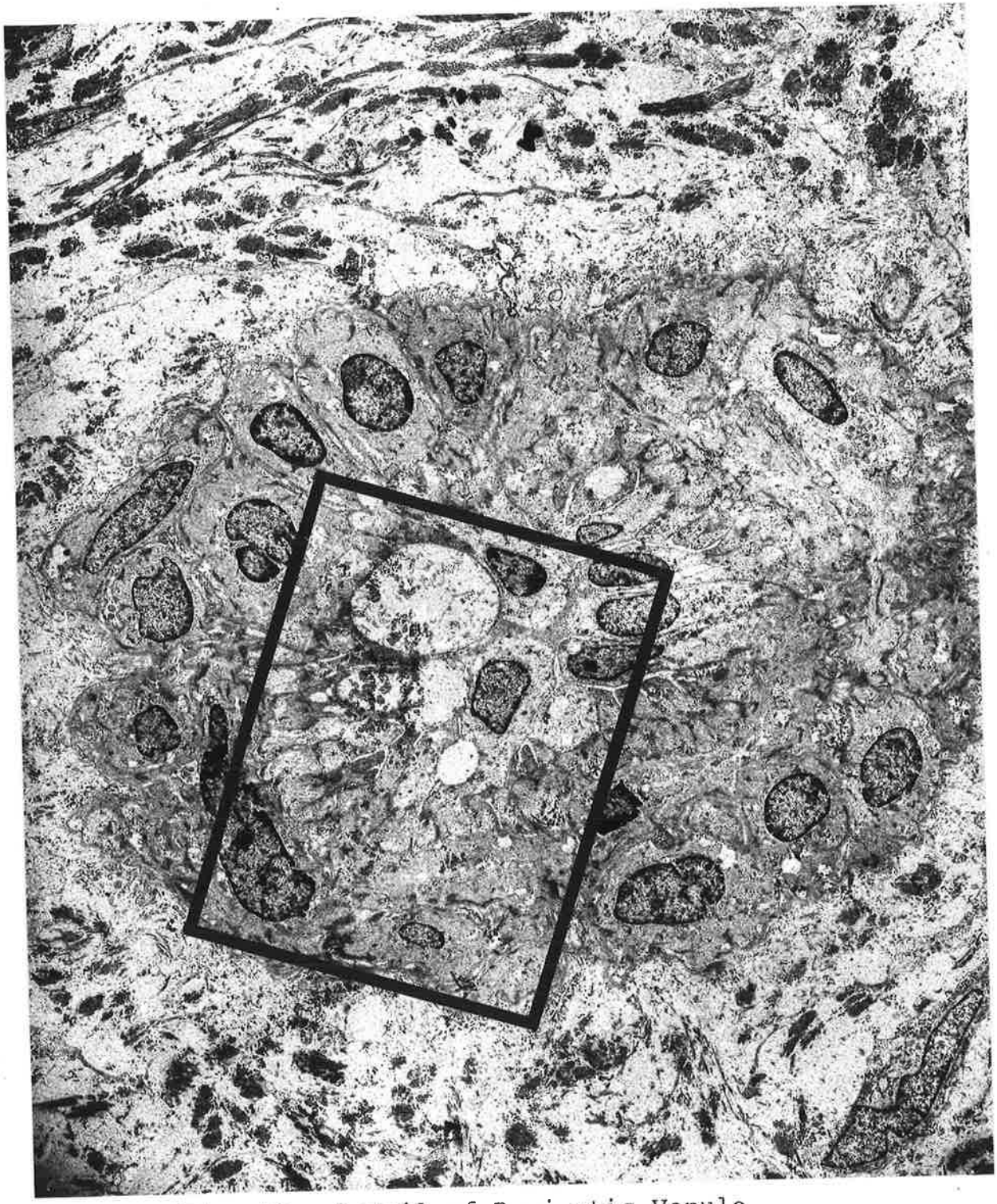


Fig. 5C. Detail of Pericytic Venule Arrowed in Figure 5B - Serial Section:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,500X).

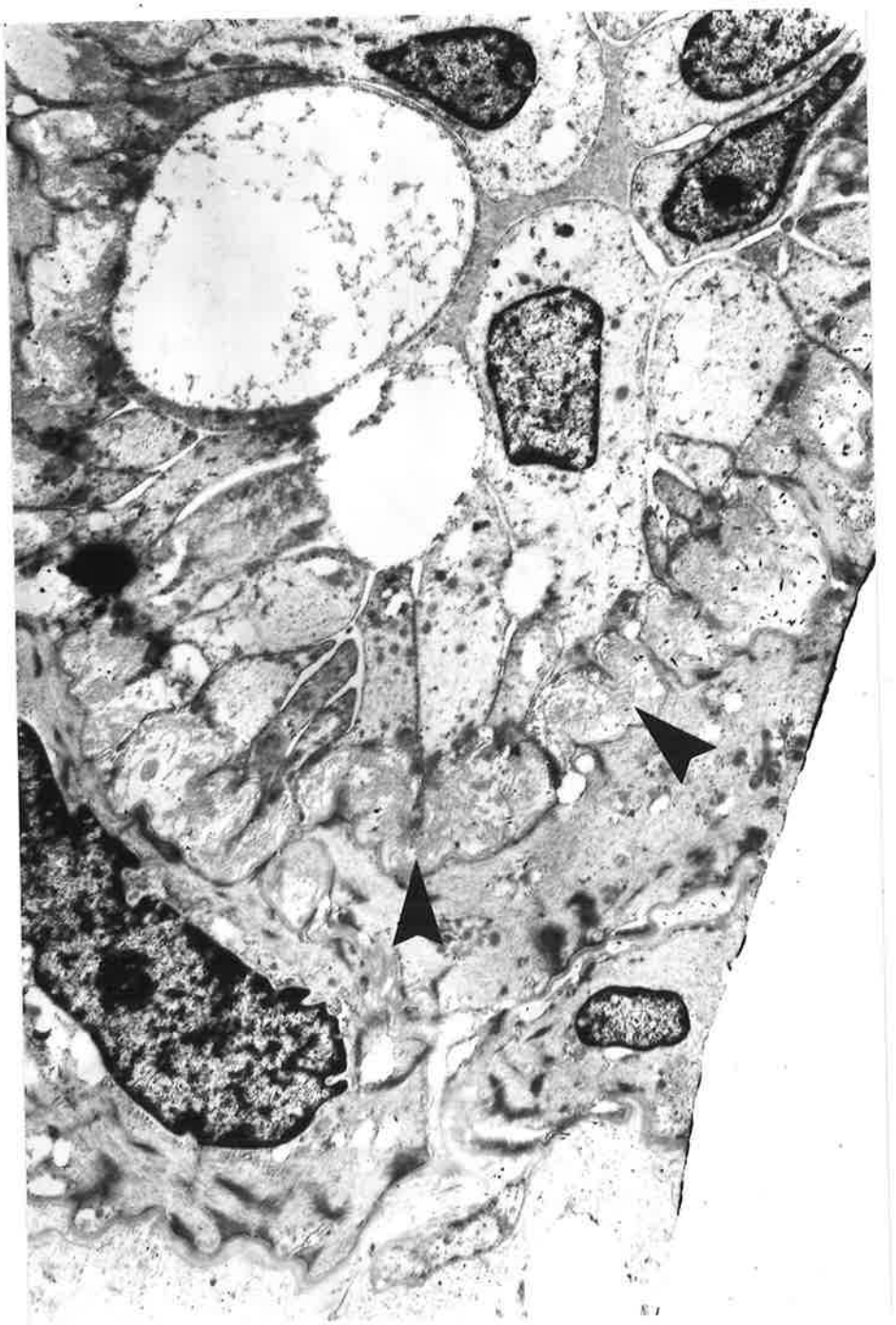


Fig. 5D. Detail of Pericytic Venule in Figure 5C (squared) - Serial Section, basement membrane (arrowed):

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Acetate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

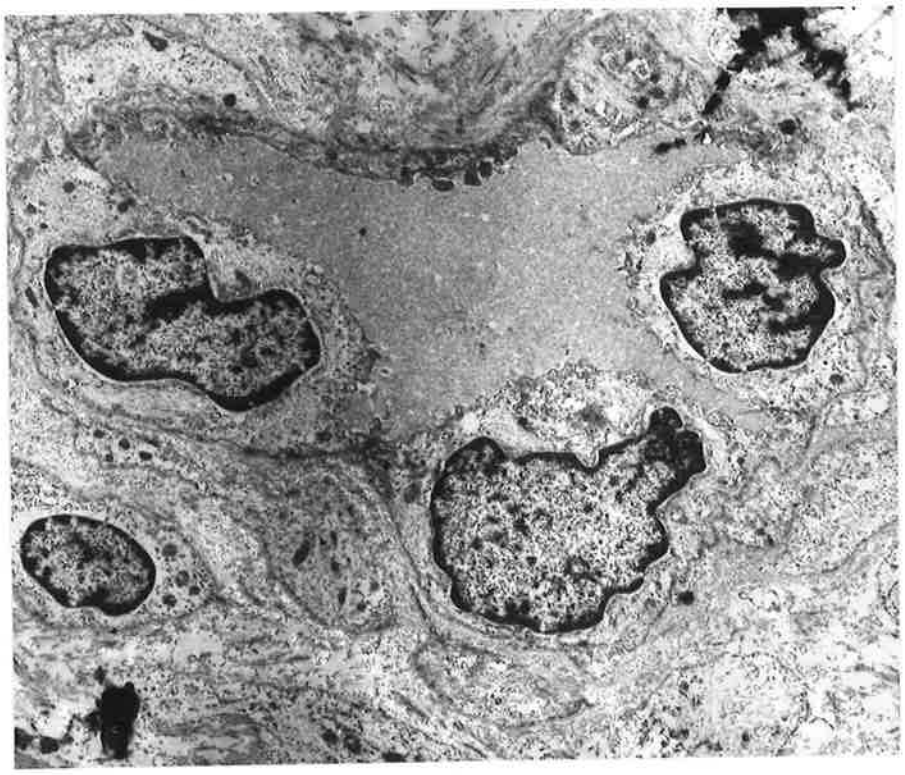


Fig. 6. Ligamentous Pericytic Venule:
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

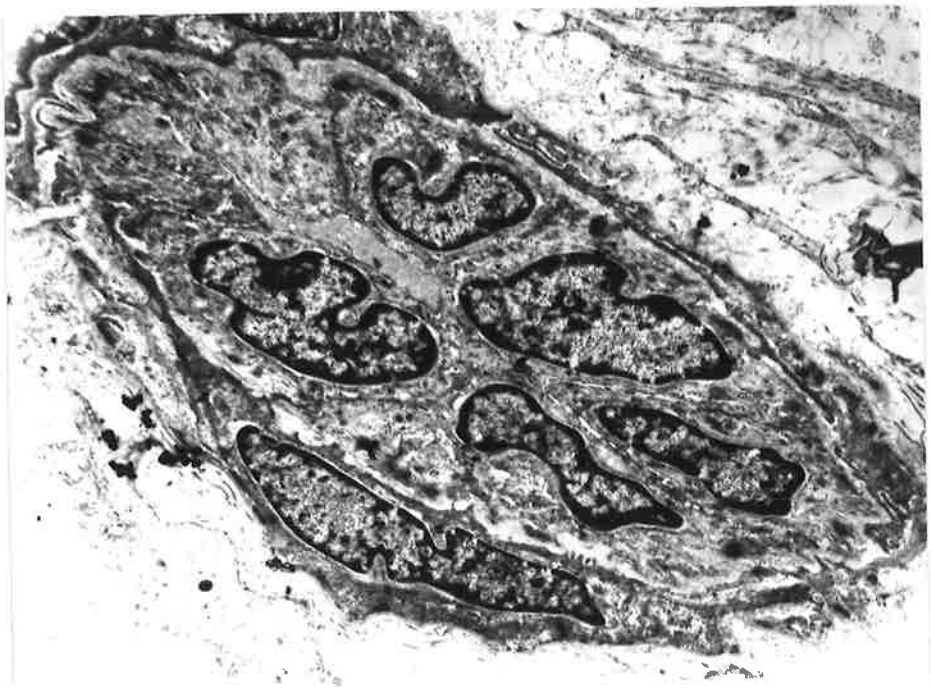


Fig. 7. Ligamentous Pericytic Venule:
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

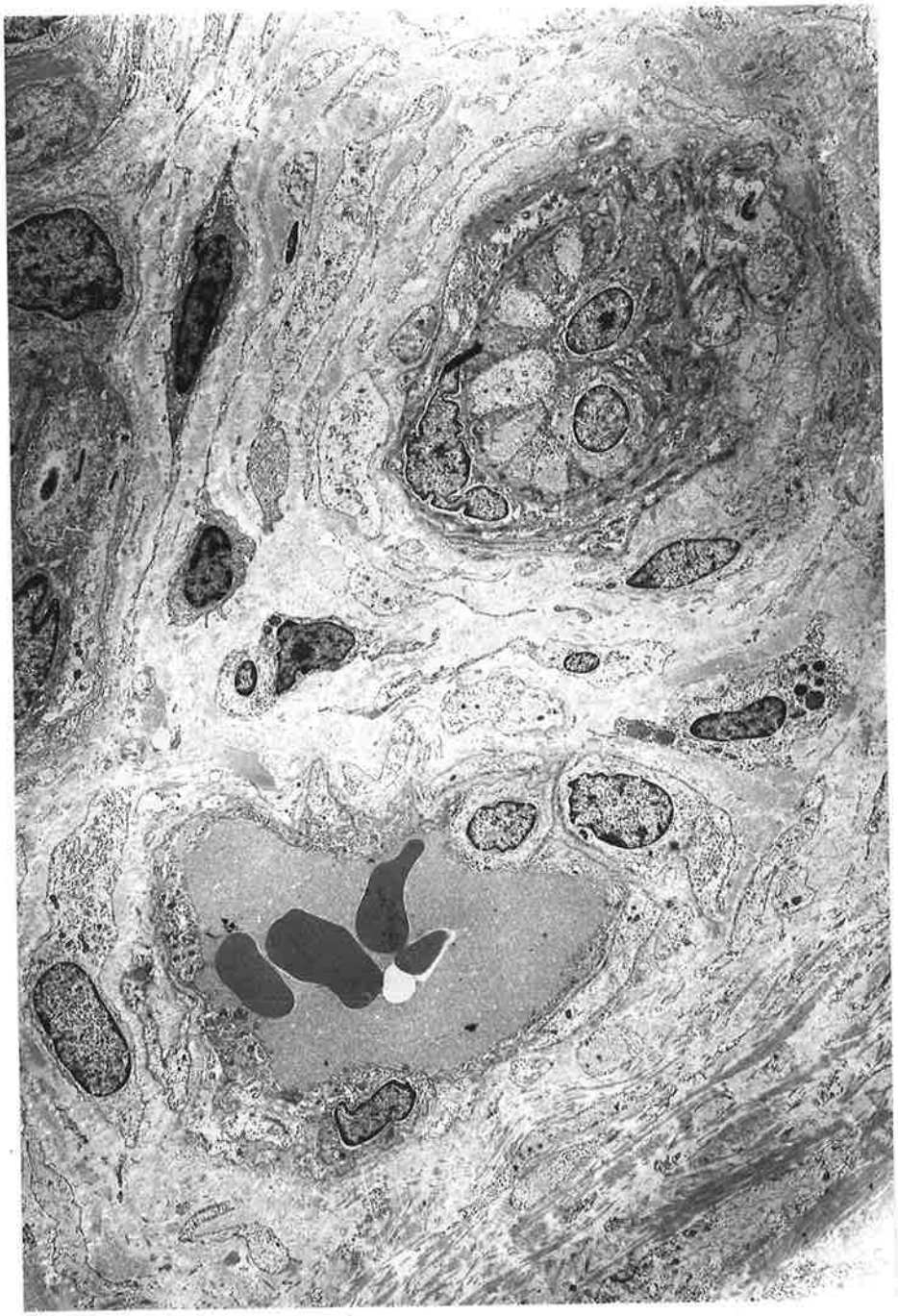


Fig. 8. Ligamentous Pericytic Venules in Loose Connective Tissue:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,000X).

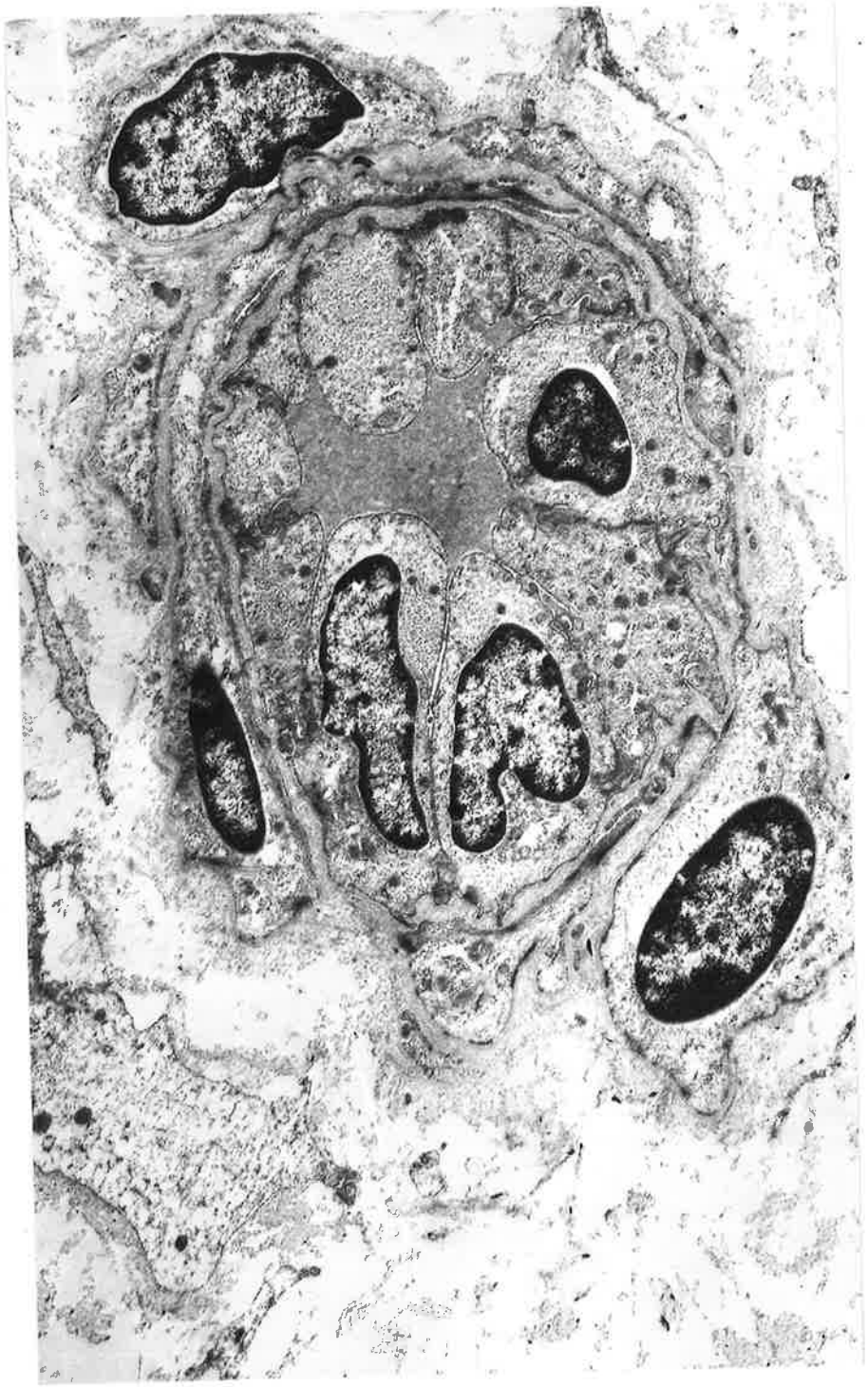


Fig. 9. Ligamentous Pericytic Venule:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

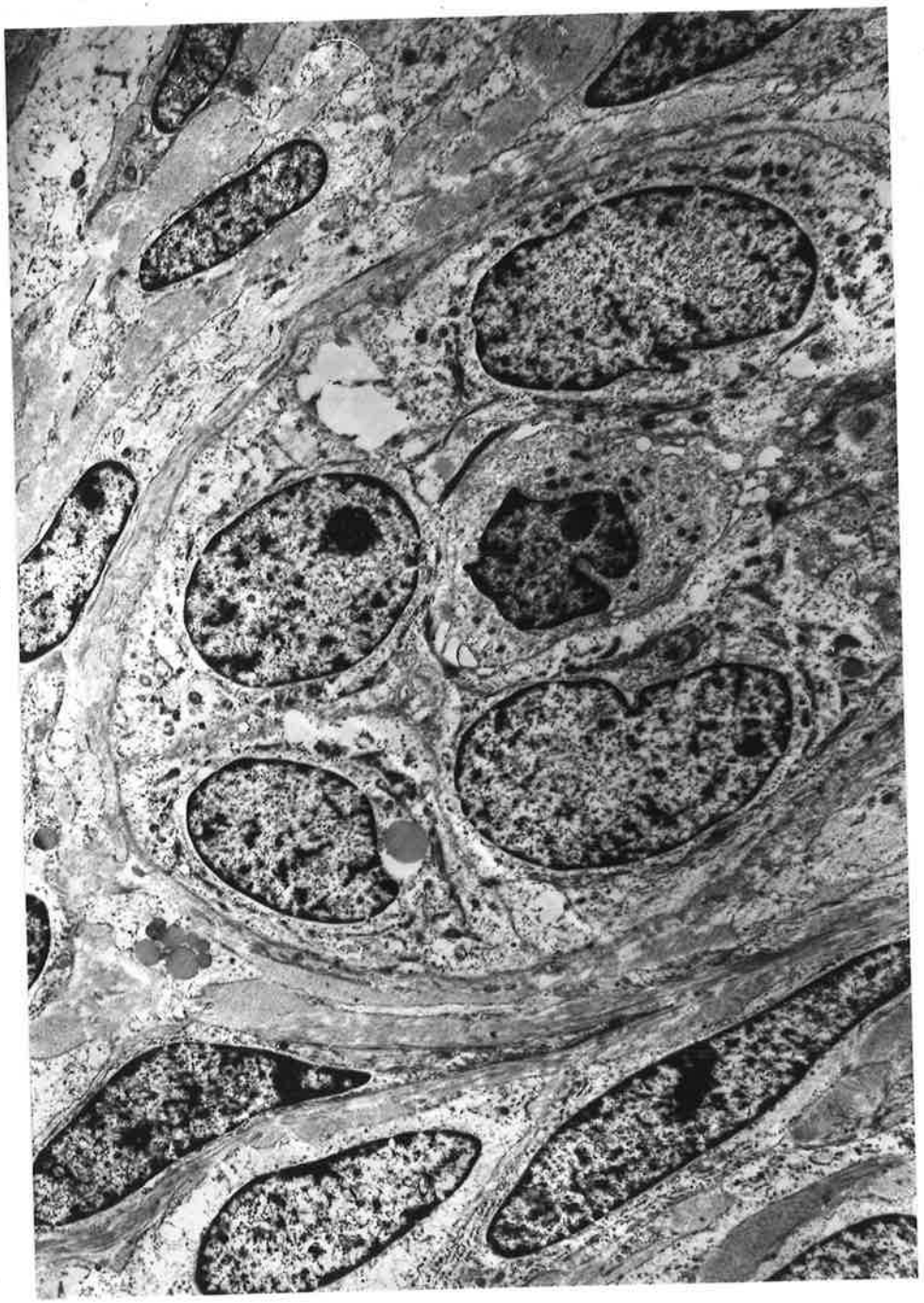


Fig. 10A. Ligamentous Pericytic Venule: Associated fat inclusions.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,000X).

Fig.10B. Ligamentous Pericytic Venule:
Detail of fat inclusions in Figure 10A.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

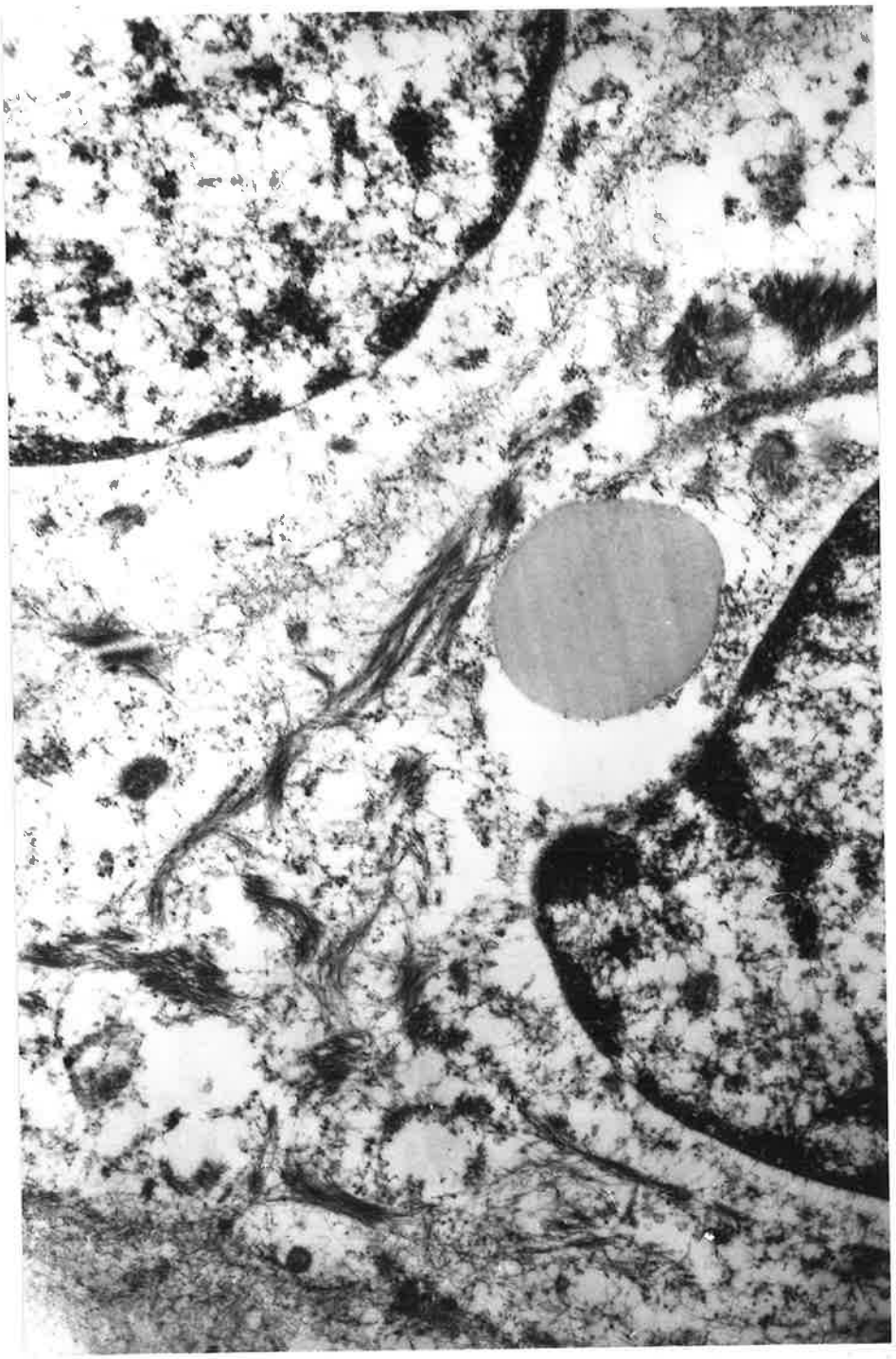


Fig. 10C. Ligamentous Pericytic Venule:
Detail of fat inclusion in Figure 10A.
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

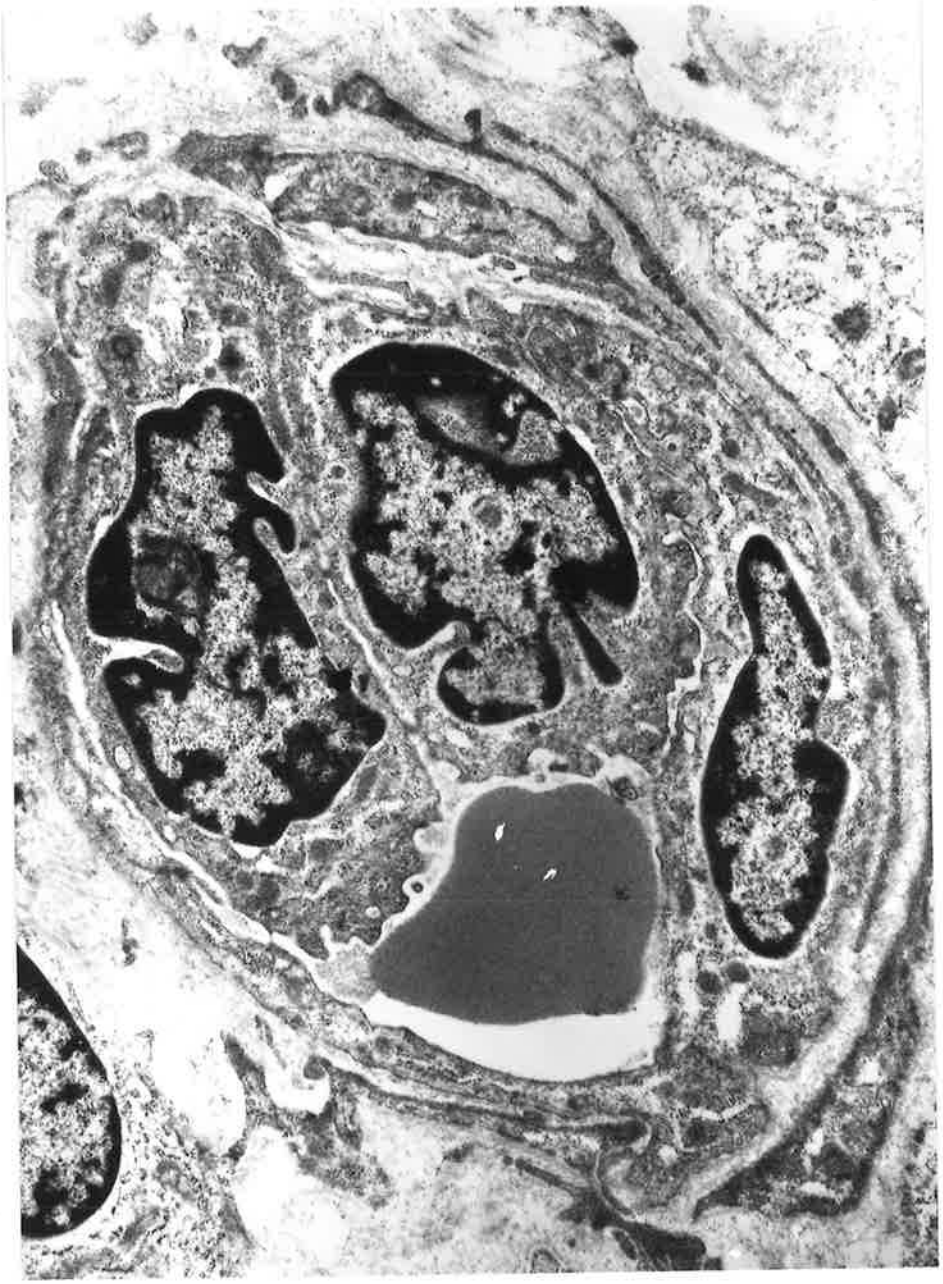


Fig. 11. Alveolar Pericytic Venule:
Enclosed erythrocyte.
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

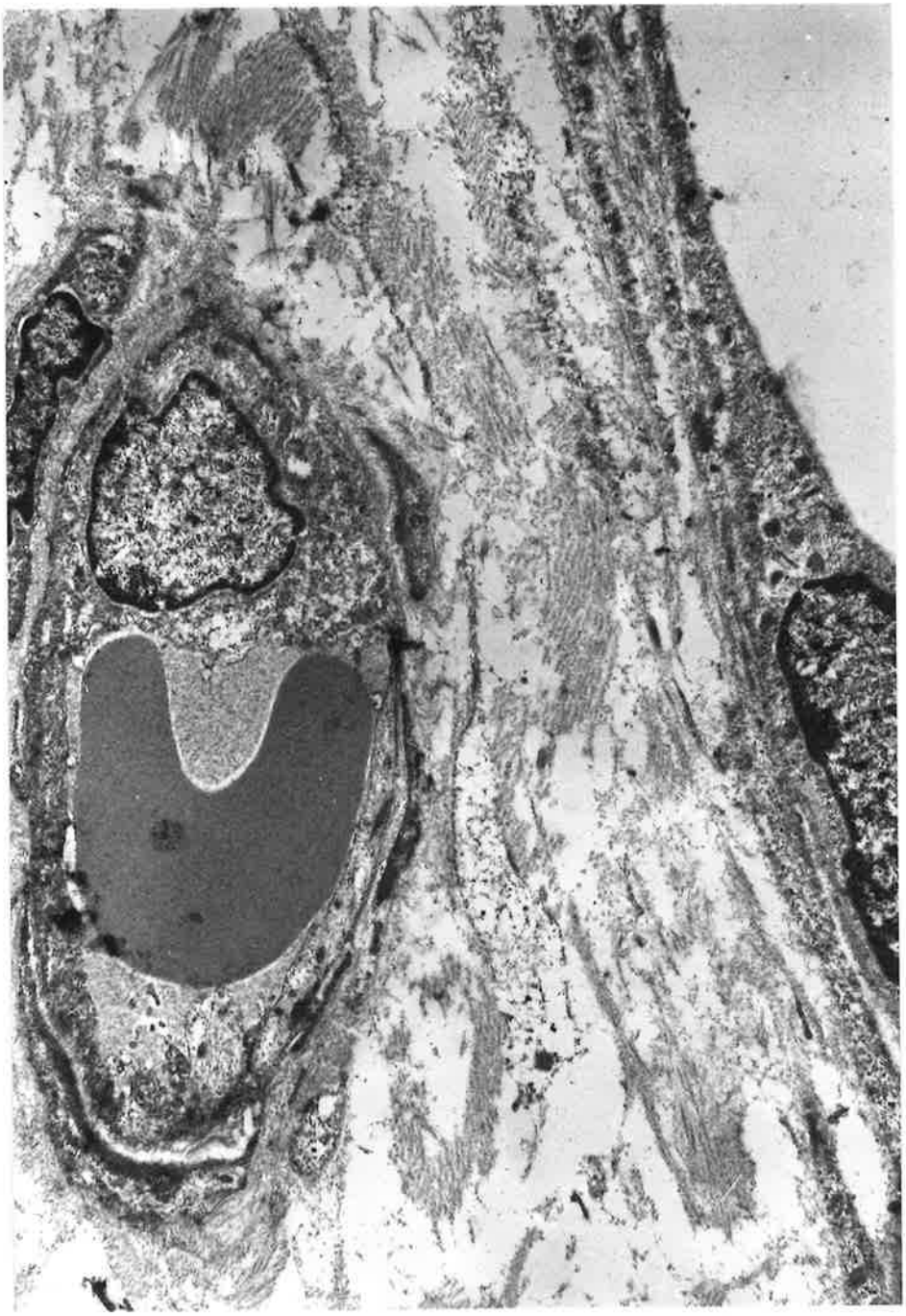


Fig. 12. Alveolar Pericytic Venule and Associated Large Vessel Wall: Enclosed erythrocyte.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

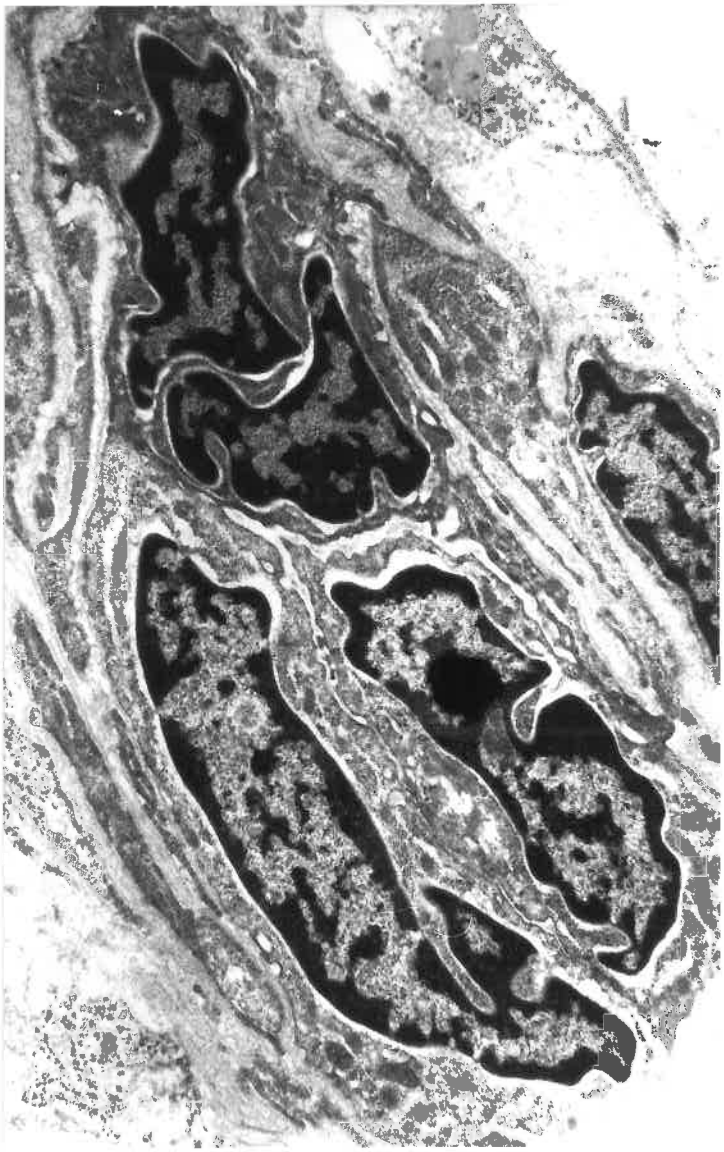


Fig. 13. Alveolar Pericytic Venule:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

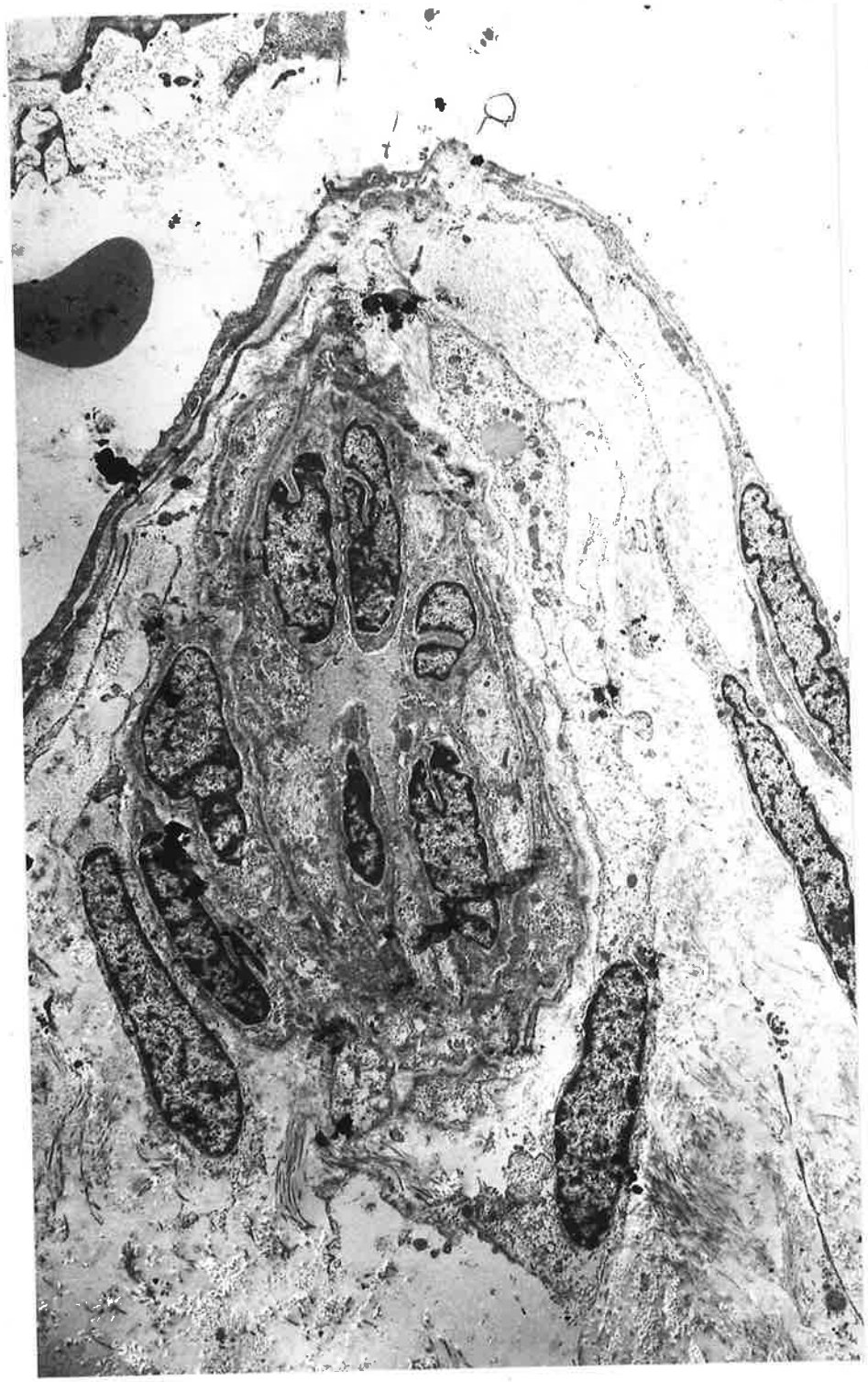


Fig. 14. Alveolar Pericytic Venule and Associated Large Vessel Wall:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

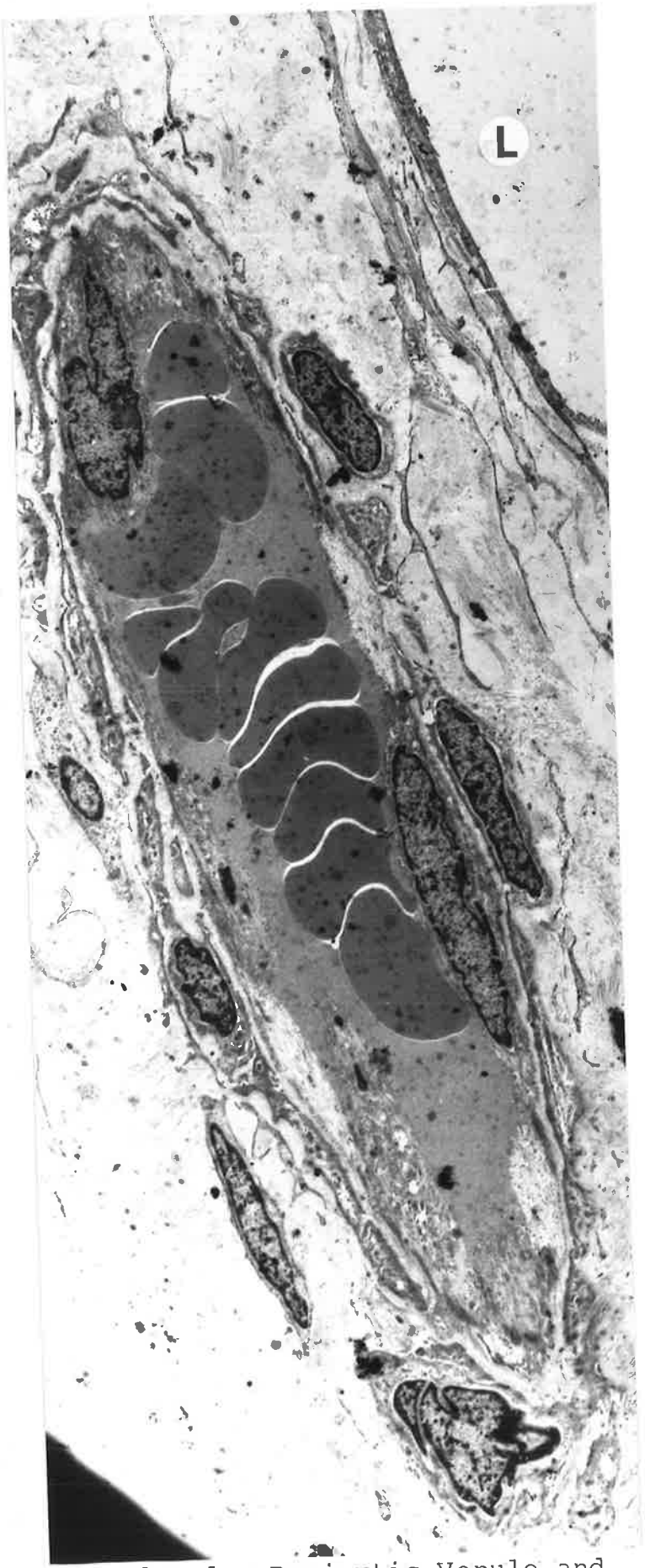


Fig. 15. Alveolar Pericytic Venule and Associated Large Vessel Wall: Contained erythrocytes. Large vessel lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

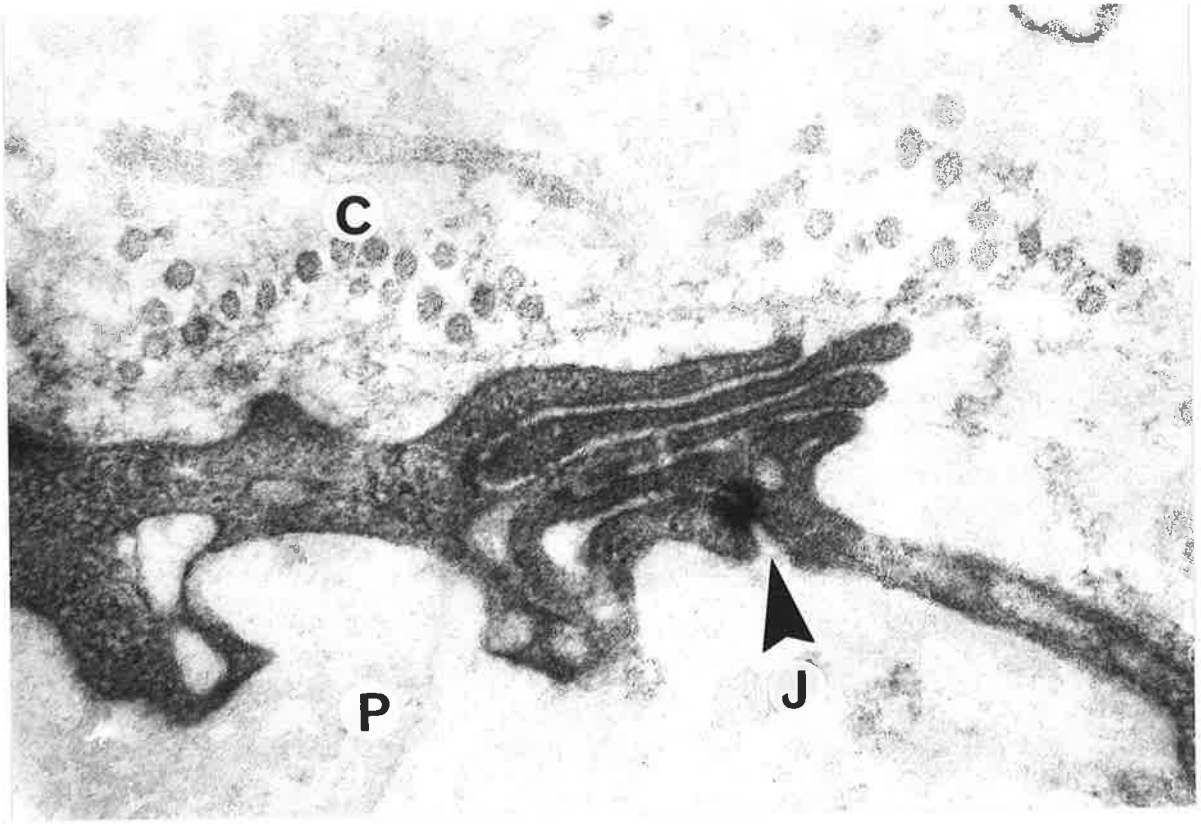


Fig. 16. Collagen Fibres Parallelling Endothelial Wall: Collagen (C), endothelial junction (J), lumen of pericytic venule (P).
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(38,000X).

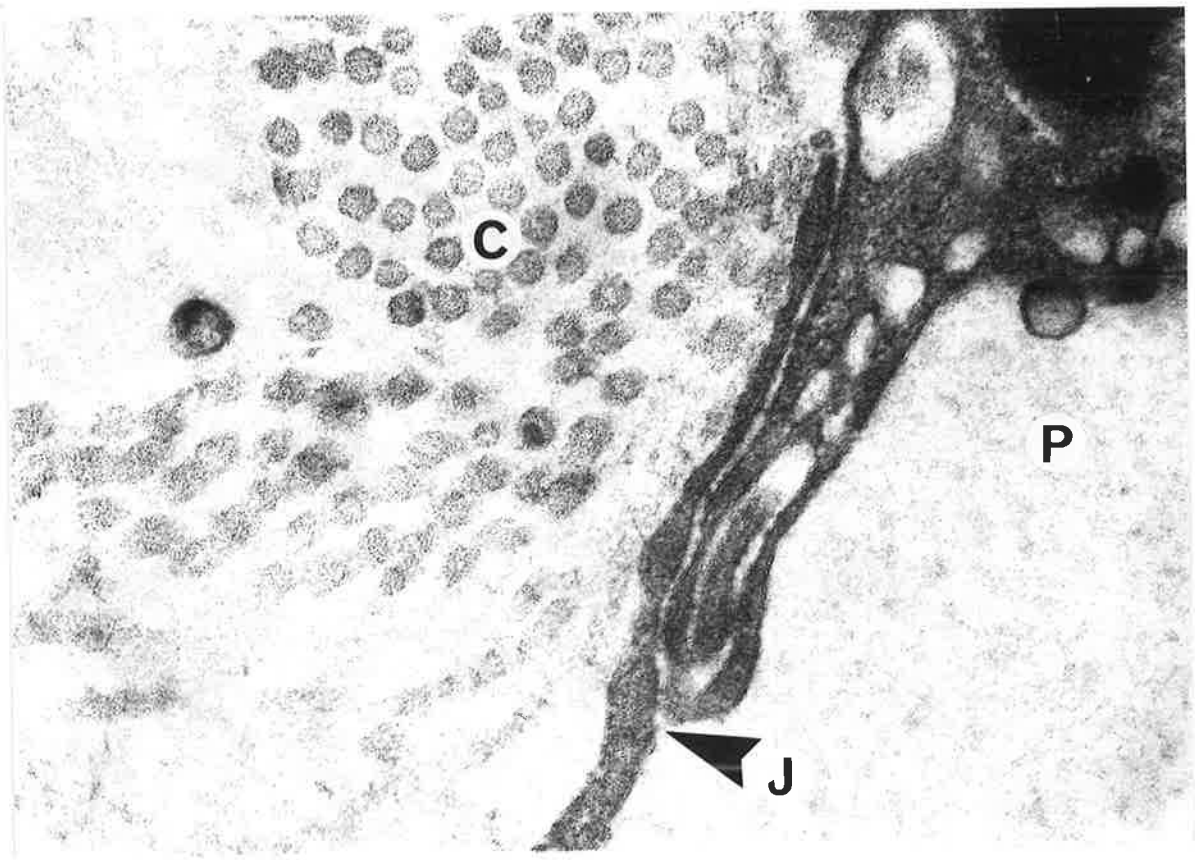


Fig. 17. Collagen Fibres Parallelling Endothelial Wall: Collagen (C), endothelial junction (J), lumen of pericytic venule (P).
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified. (38,000X)

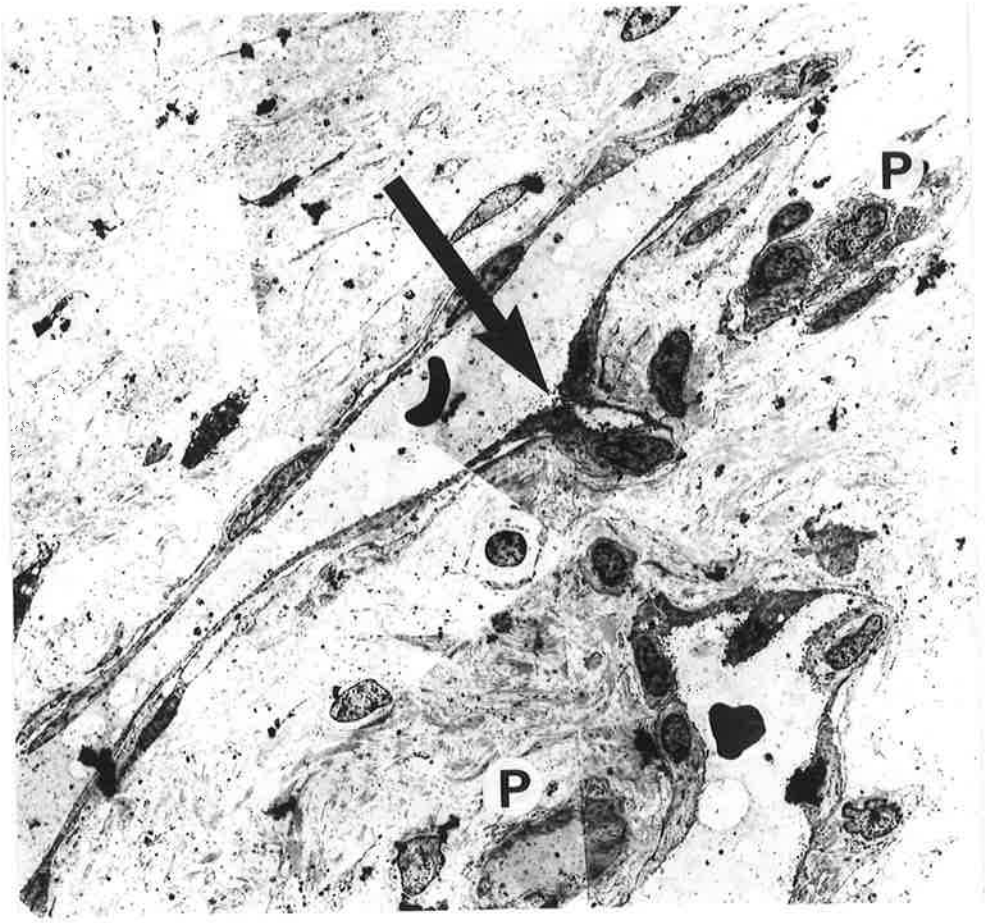


Fig. 18A. Ligamentous Large Vessels:

Pericytic venule (P), junction (arrow).

Ultramicrograph Montage. Osmium Tetroxid
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(800X).

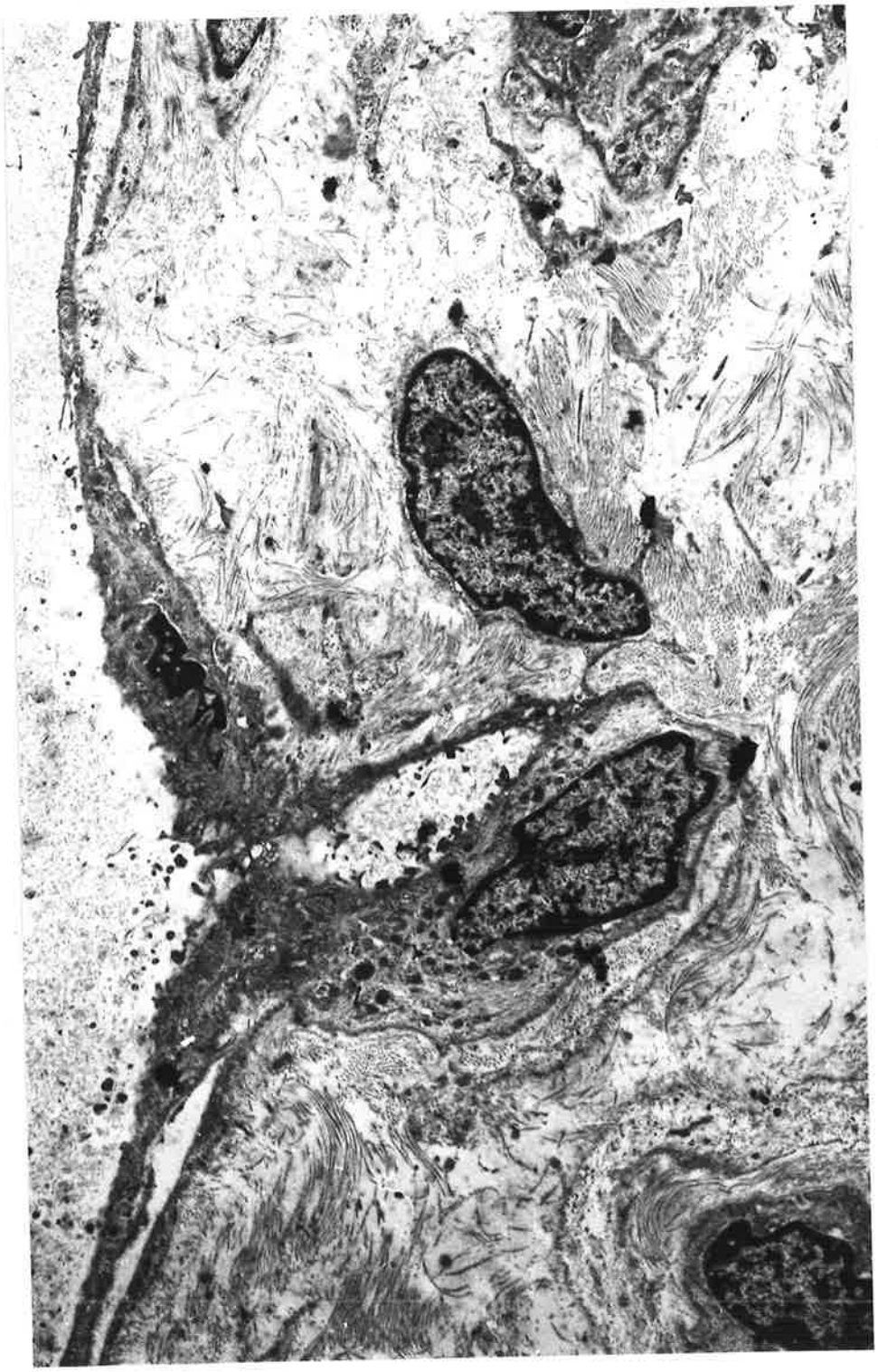


Fig. 18B. Detail of Junction of Ligamentous Large Vessel in Figure 18A. Showing insertion of collagen fibrils.

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(5,000X).

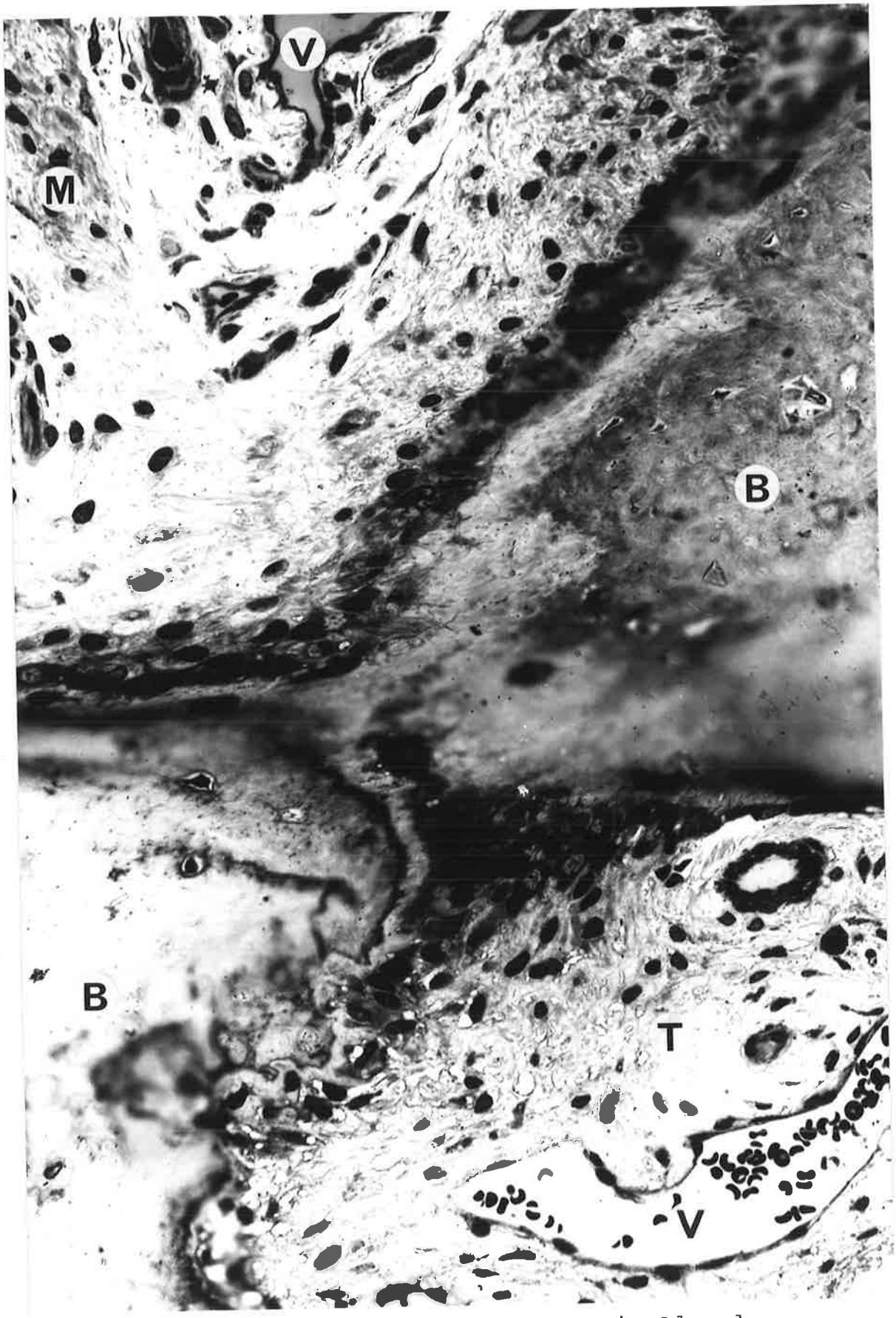


Fig. 19A. Section Through Foramen in Alveolar Socket Wall:
At edge of foramen.

Alveolar bone (B), periodontal ligament (M), trabecular space (T), large vessel (V).

Light micrograph. Toluidine Blue.
Decalcified. (375X).

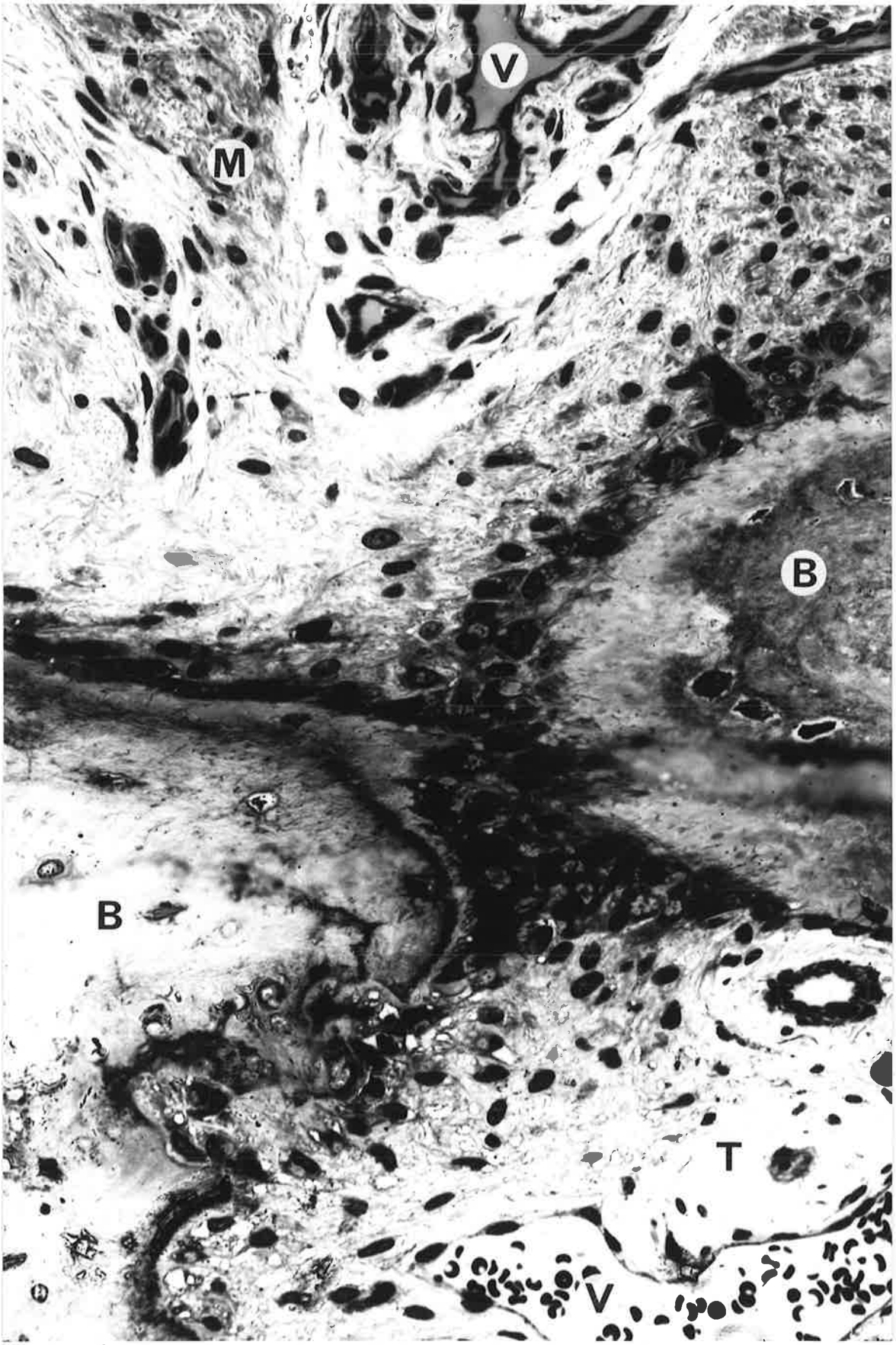


Fig. 19B. Semi Serial Section Through Foramen in Alveolar Socket Wall:
At edge of Foramen.
Alveolar bone (B), periodontal ligament (M), trabecular space (T), large vessel (V).
Light micrograph. Toluidine Blue.
Decalcified. (375X).

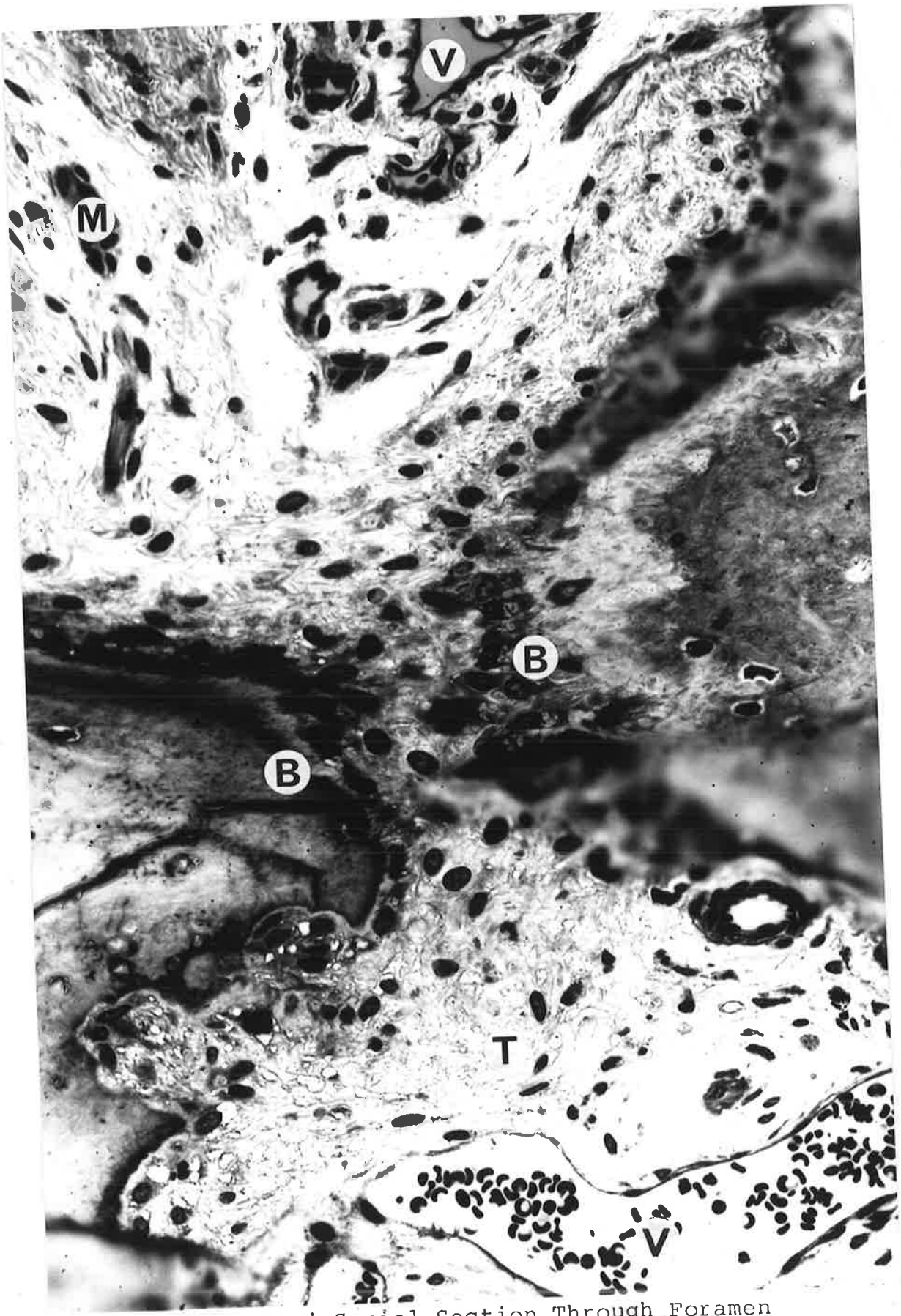


Fig. 19C. Semi Serial Section Through Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), periodontal ligament (M), trabecular space (T), large vessel (V).

Light micrograph. Decalcified. Toluidine Blue. (375X).

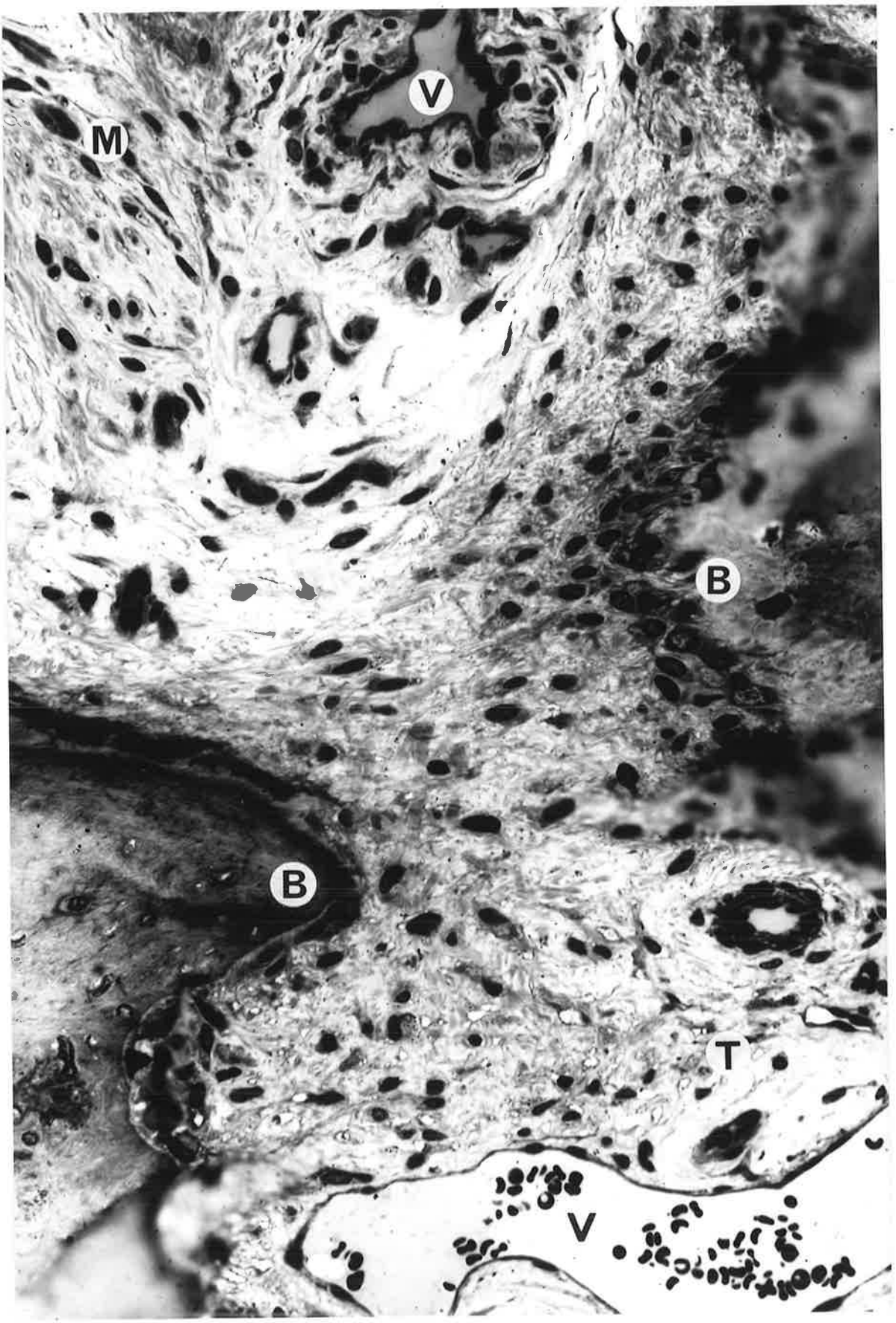


Fig. 19D. Semi Serial Section Through Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), trabecular space (T), periodontal ligament (M), large vessel (V).

Light micrograph. Toluidine Blue.
Decalcified. (375X).

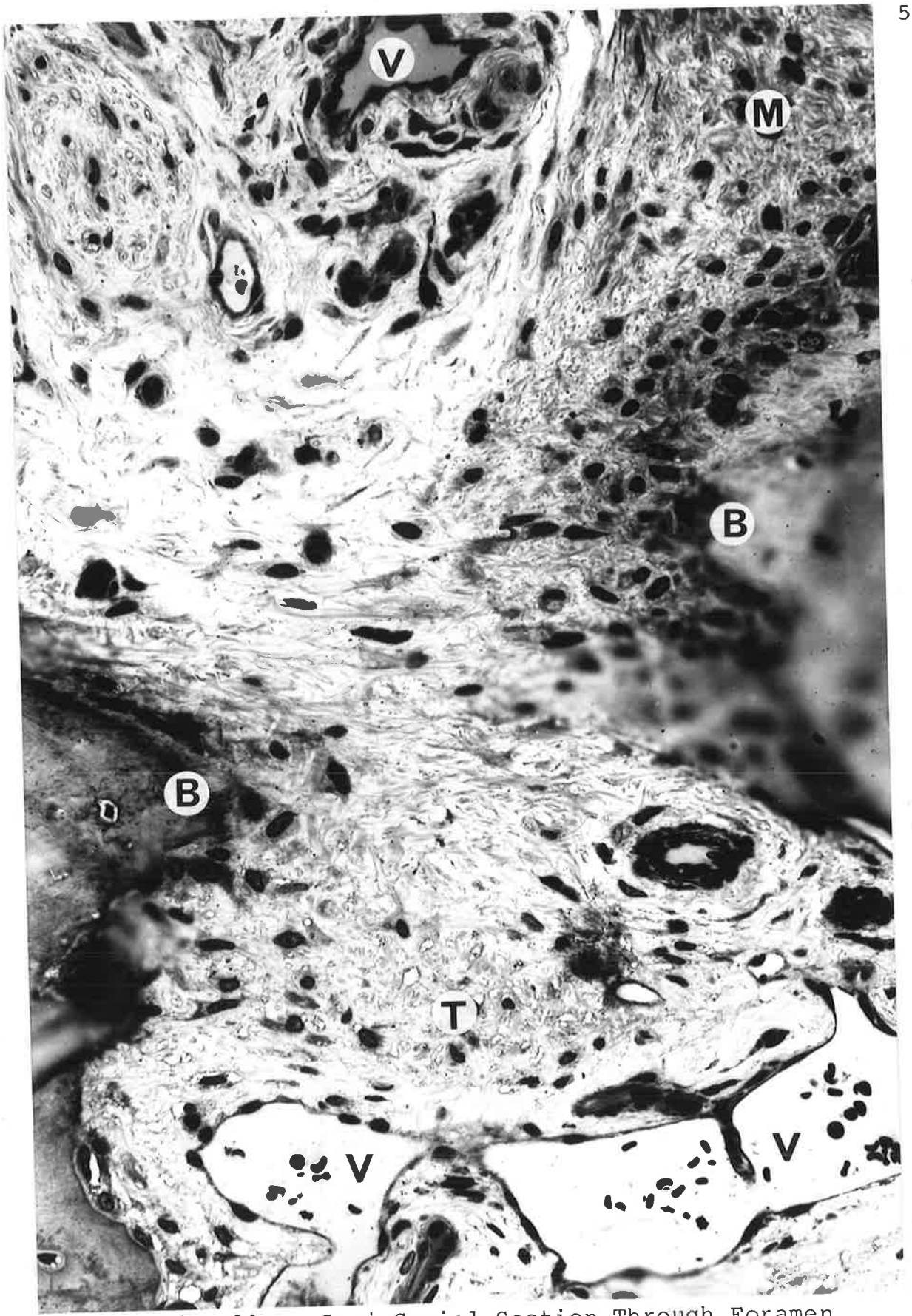


Fig. 19E. Semi Serial Section Through Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), periodontal ligament (M), trabecular space (T), large vessel (V).
Light micrograph. Toluidine Blue.
Decalcified. (375X).

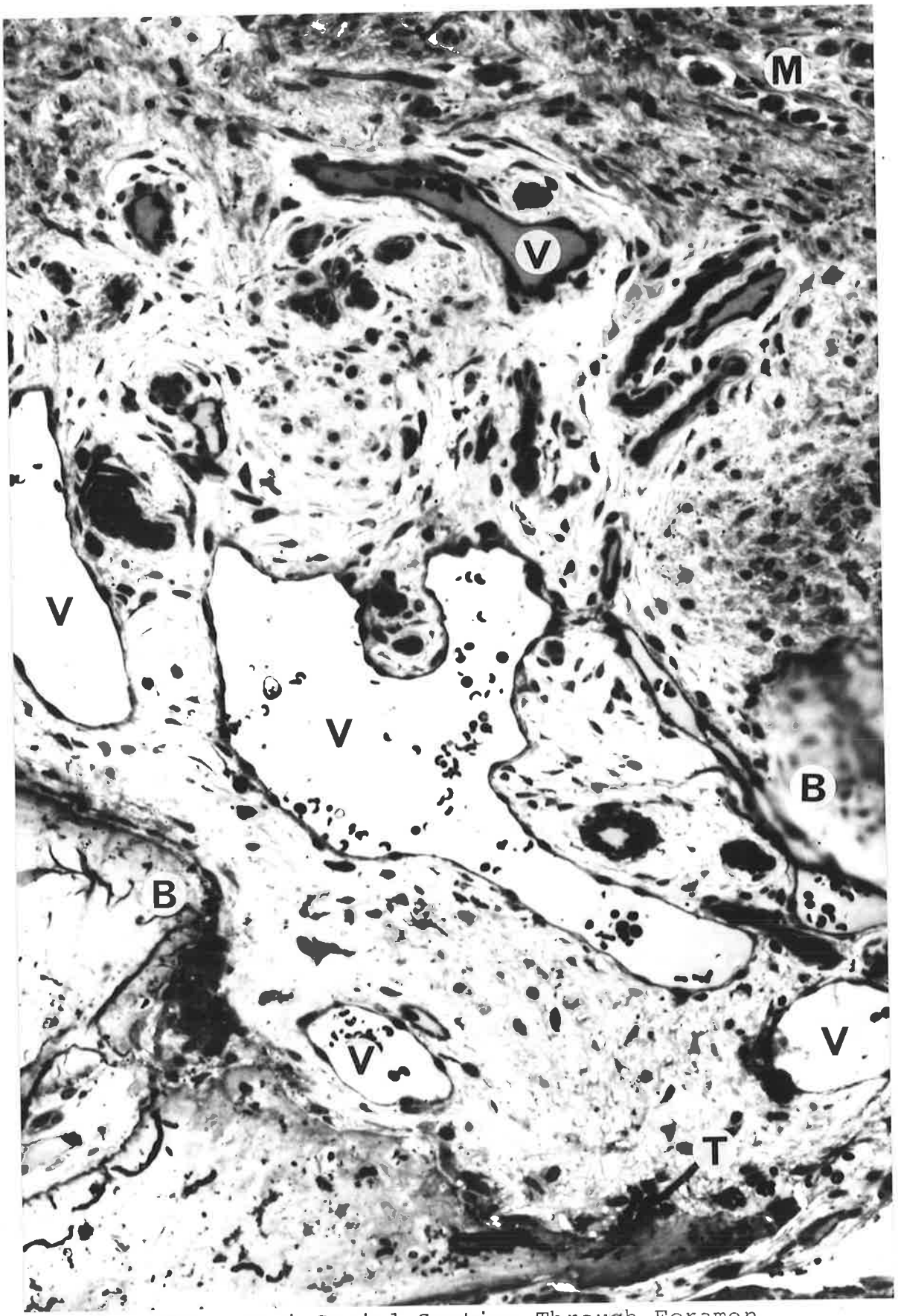


Fig. 19F. Semi Serial Section Through Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), periodontal ligament (M), trabecular space (T), large vessel (V).

Light micrograph. Toluidine Blue.
Decalcified. (375X).

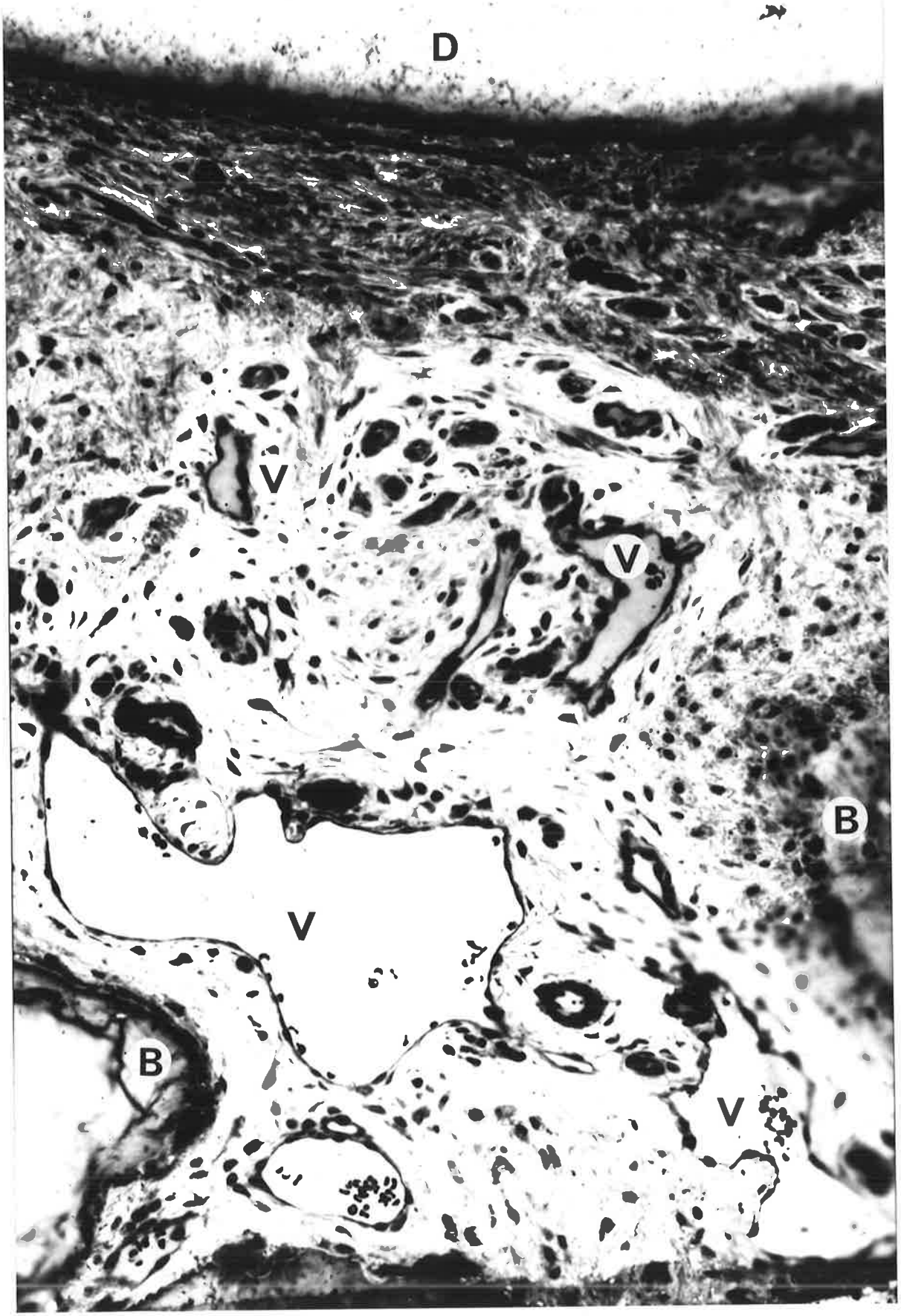


Fig. 19G. Semi Serial Section of Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), dentine (D), large vessel (V).
Light micrograph. Toluidine Blue.
Decalcified. (375X).

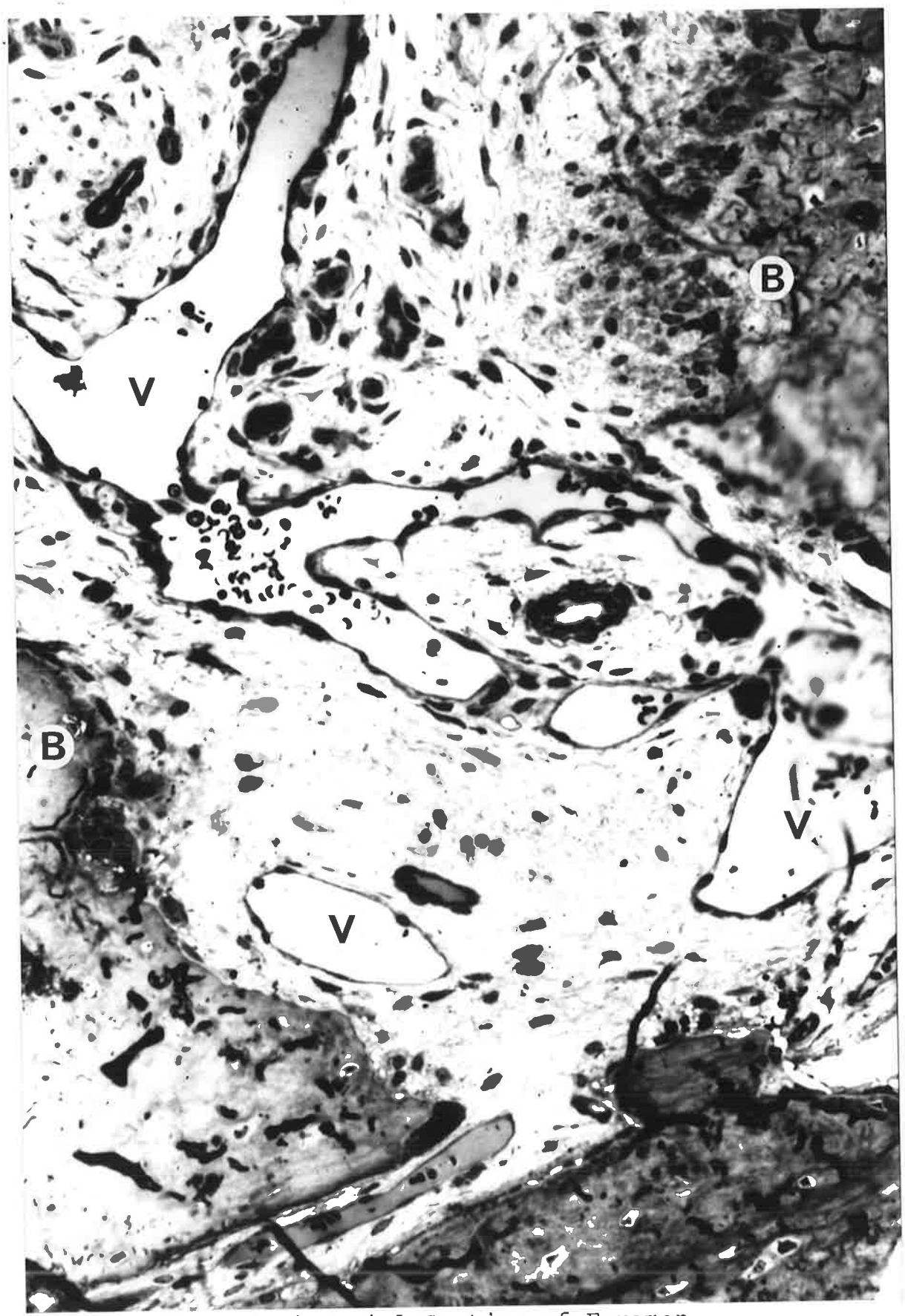


Fig. 19H. Semi Serial Section of Foramen in Alveolar Socket Wall:

Foramen between walls of alveolar bone (B,B), large vessel (V).

Light micrograph. Decalcified. Toluidine Blue. (375X).

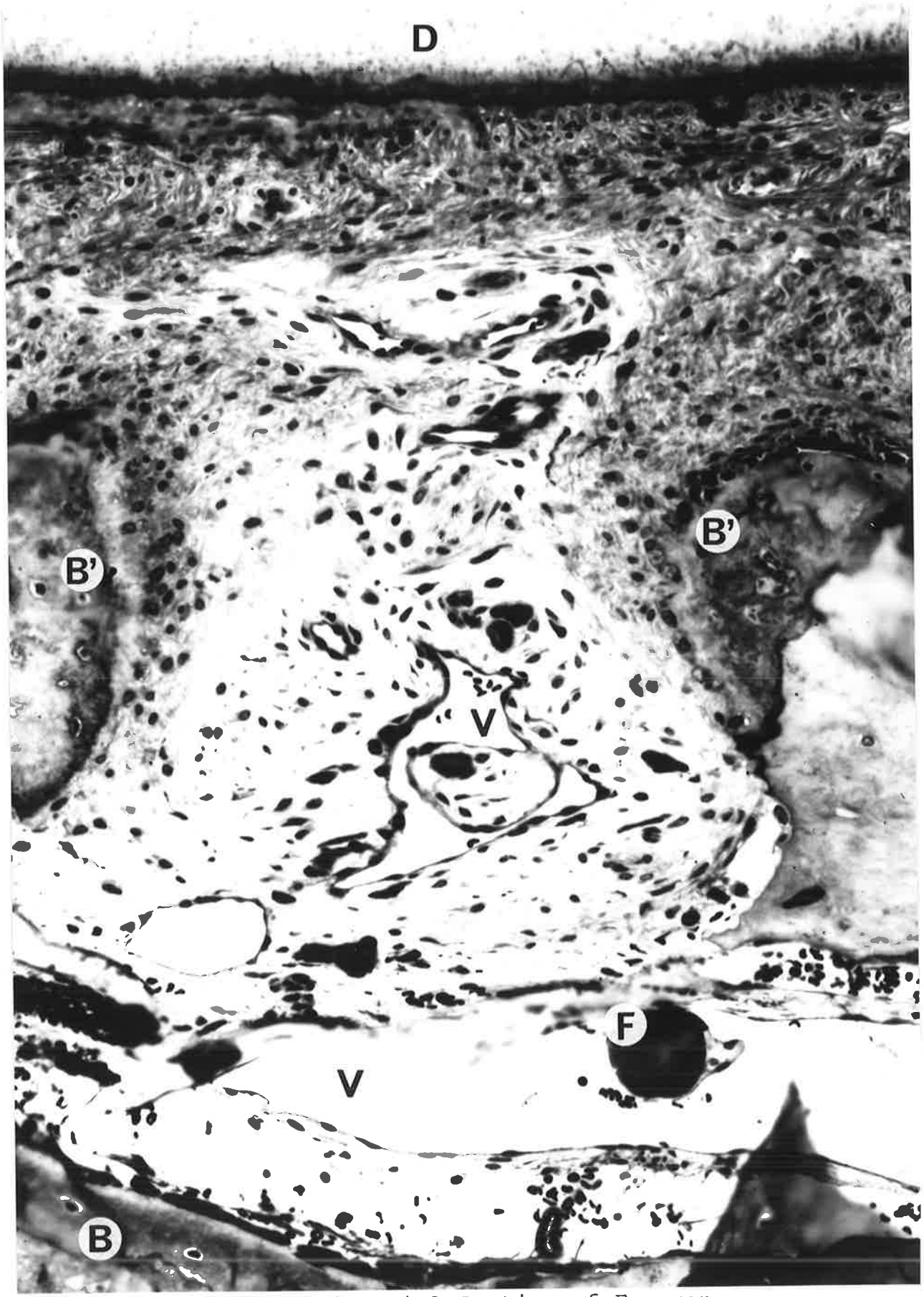


Fig. 19I. Semi Serial Section of Foramen in Alveolar Socket Wall:
Foramen between walls of alveolar bone (B',B'), dentine (D), large vessel (V), extravascular fat body (F).
Light micrograph. Toluidine Blue.
Decalcified. (375X).

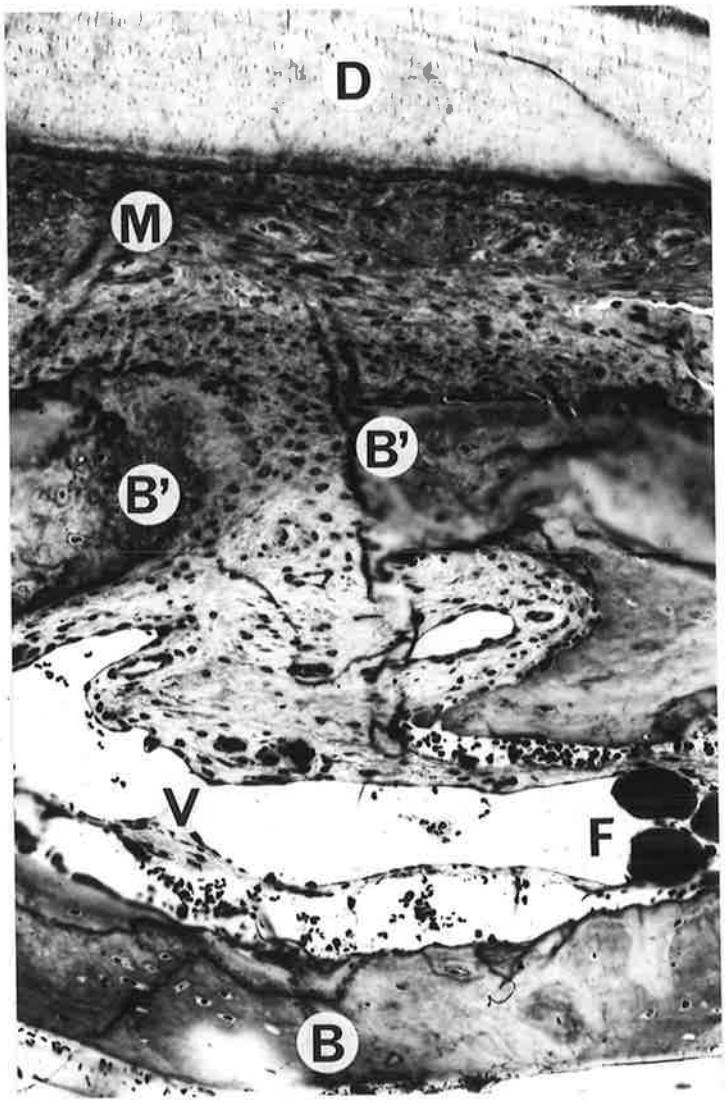


Fig. 20A. Alveolar Large Vessel with Fat Bodies:

Continuation of serial sectioning from Figure 19:

Dentine (D), periodontal ligament (M), alveolar bone (B), large vessel (V), foramen (between B',B'), fat bodies (F).

Light micrograph. Toluidine Blue.

Decalcified. (300X).

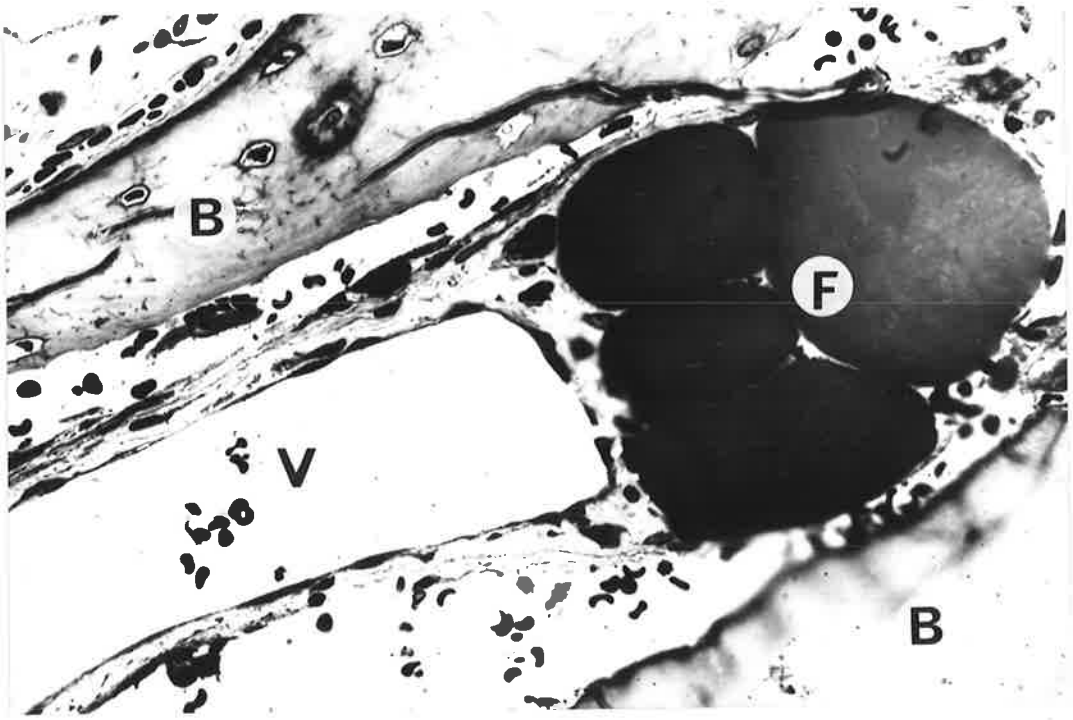


Fig. 20B. Semi Serial Section of Fat Bodies in Figure 20A.

Alveolar bone (B), fat bodies (F), large vessel (V).

Light micrograph. Toluidine Blue.

Decalcified. (375X).

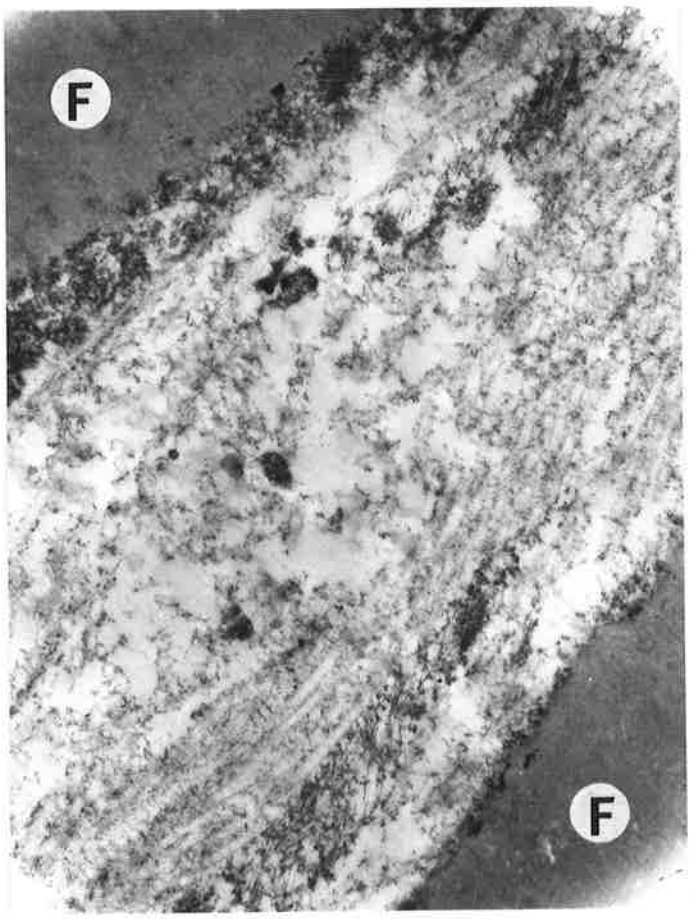


Fig. 20C. Detail of Tissue Separating Two Fat Bodies in Figure 20A.

Fat body (F).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

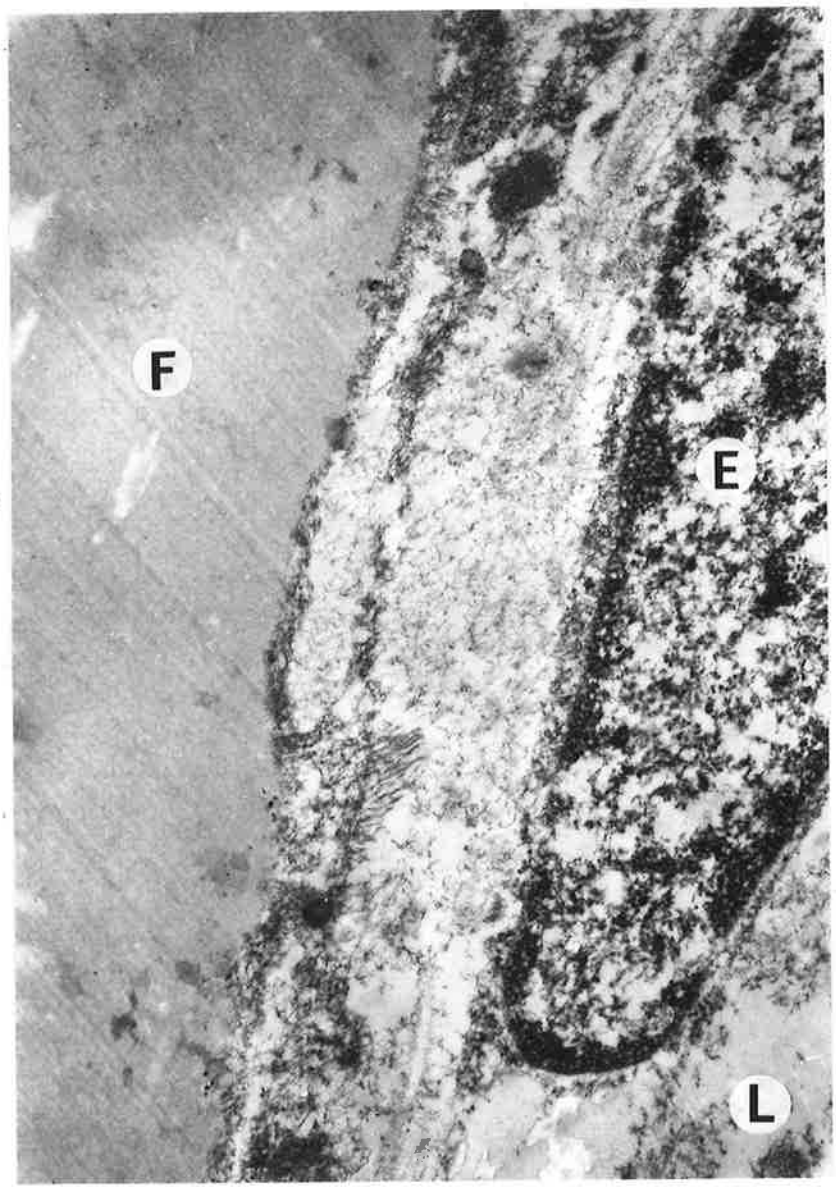


Fig. 20D. Detail of Tissue Separating Fat Body from Lumen of Alveolar Large Vessel in Figure 20A.

Fat body (F), lumen (L), endothelial nucleus (E).

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

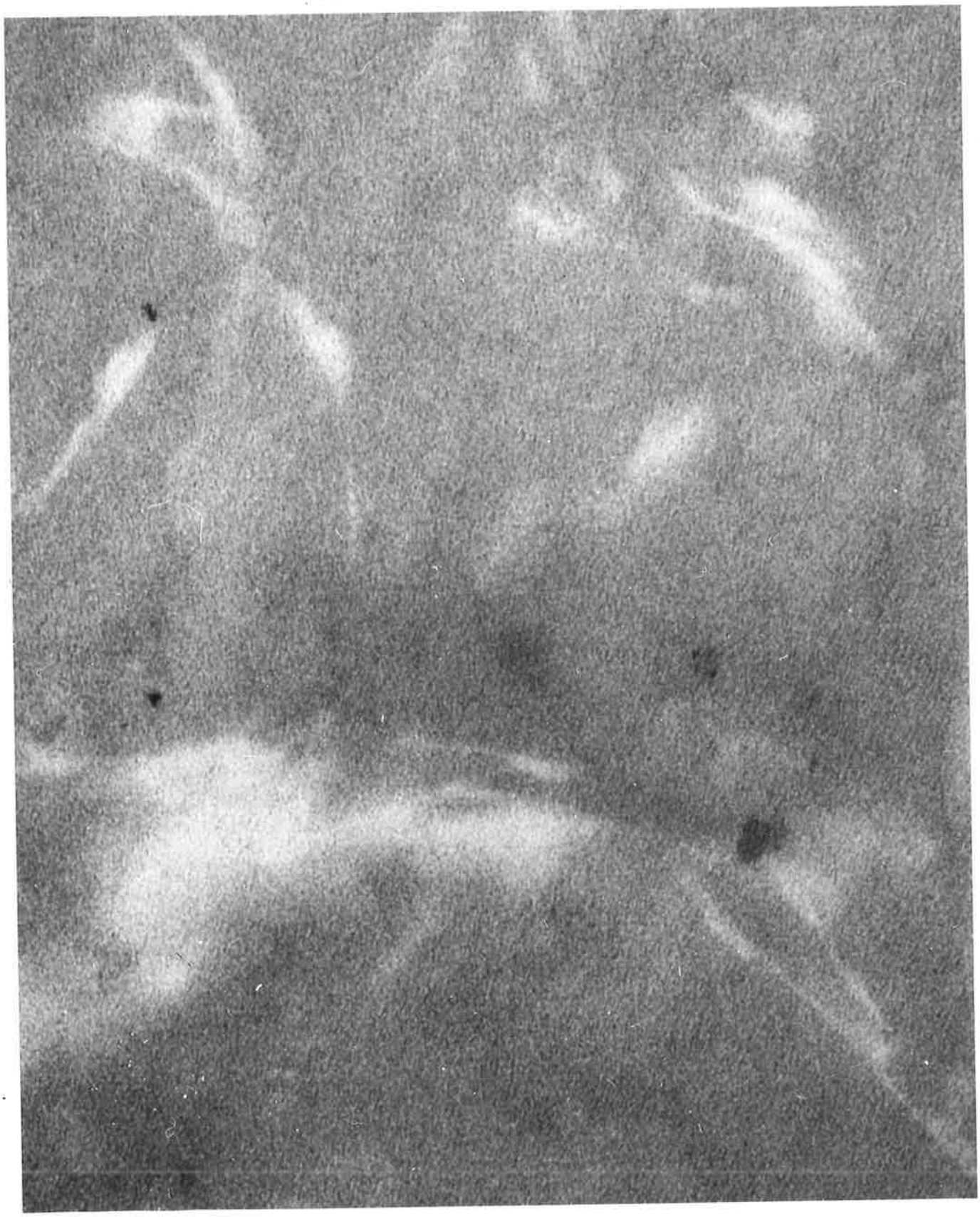


Fig. 20E. Detail of Fat Body in Figure 20A.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

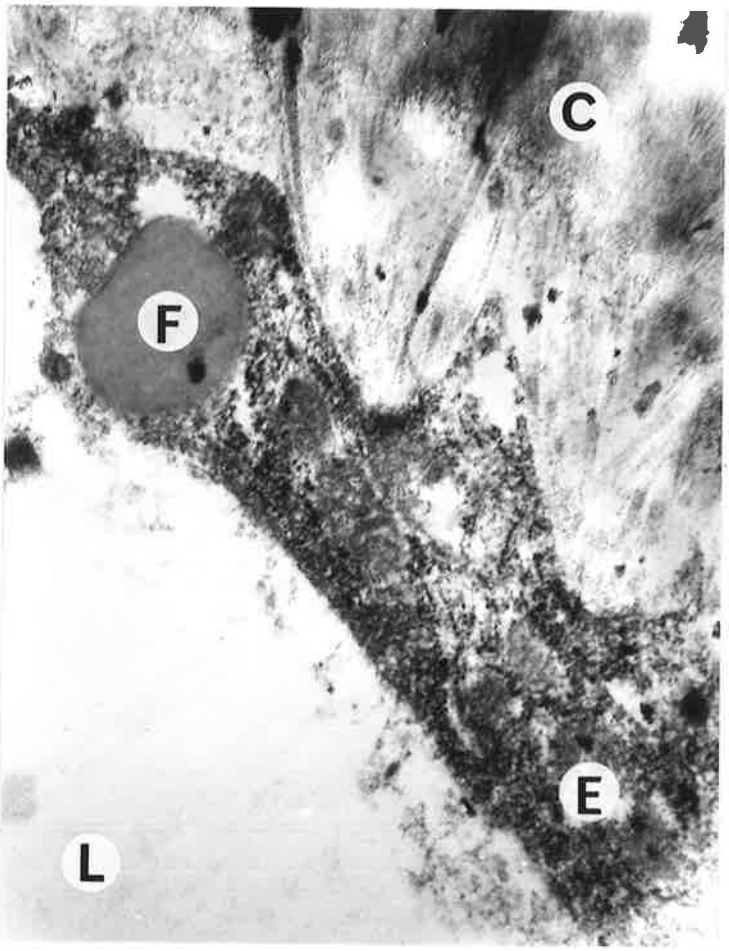


Fig. 20F. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Inserting collagen fibres (C), fat vesicle (F), endothelium (E), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

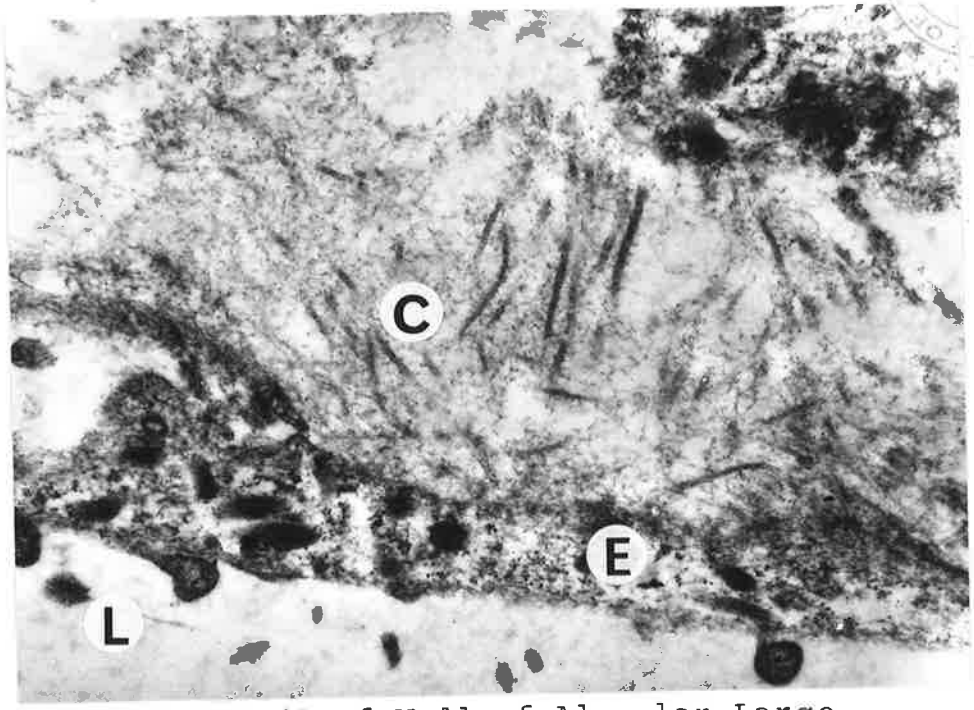


Fig. 20G. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Fibrils (C) inserting into endothelium (E), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified. (30,000X).

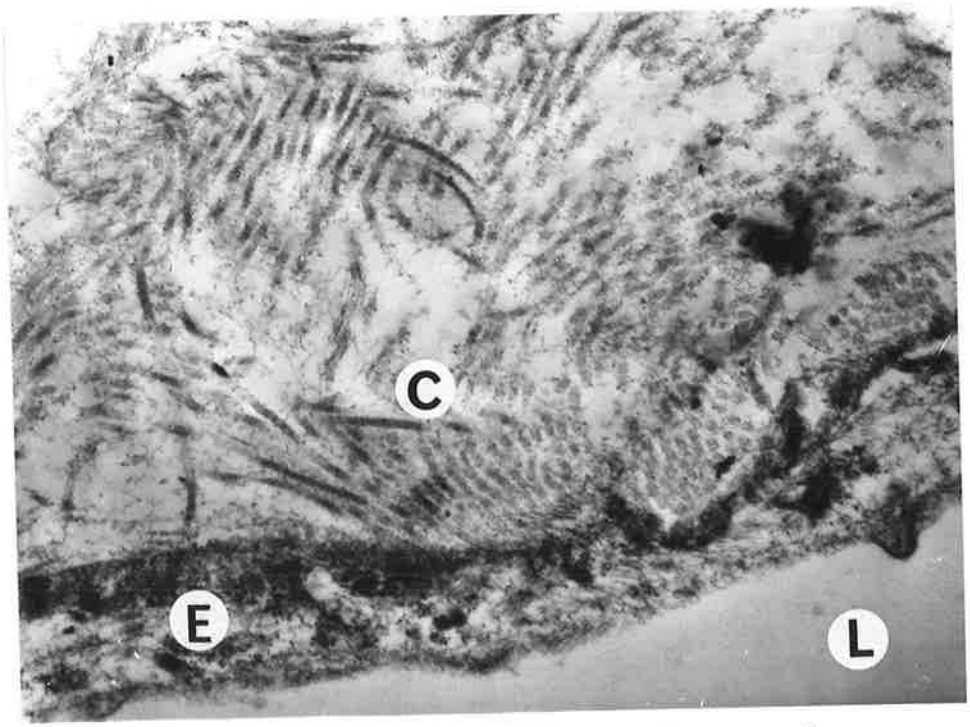


Fig. 20H. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Fibrils (C) inserting into endothelium (E), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified. (10,000X).

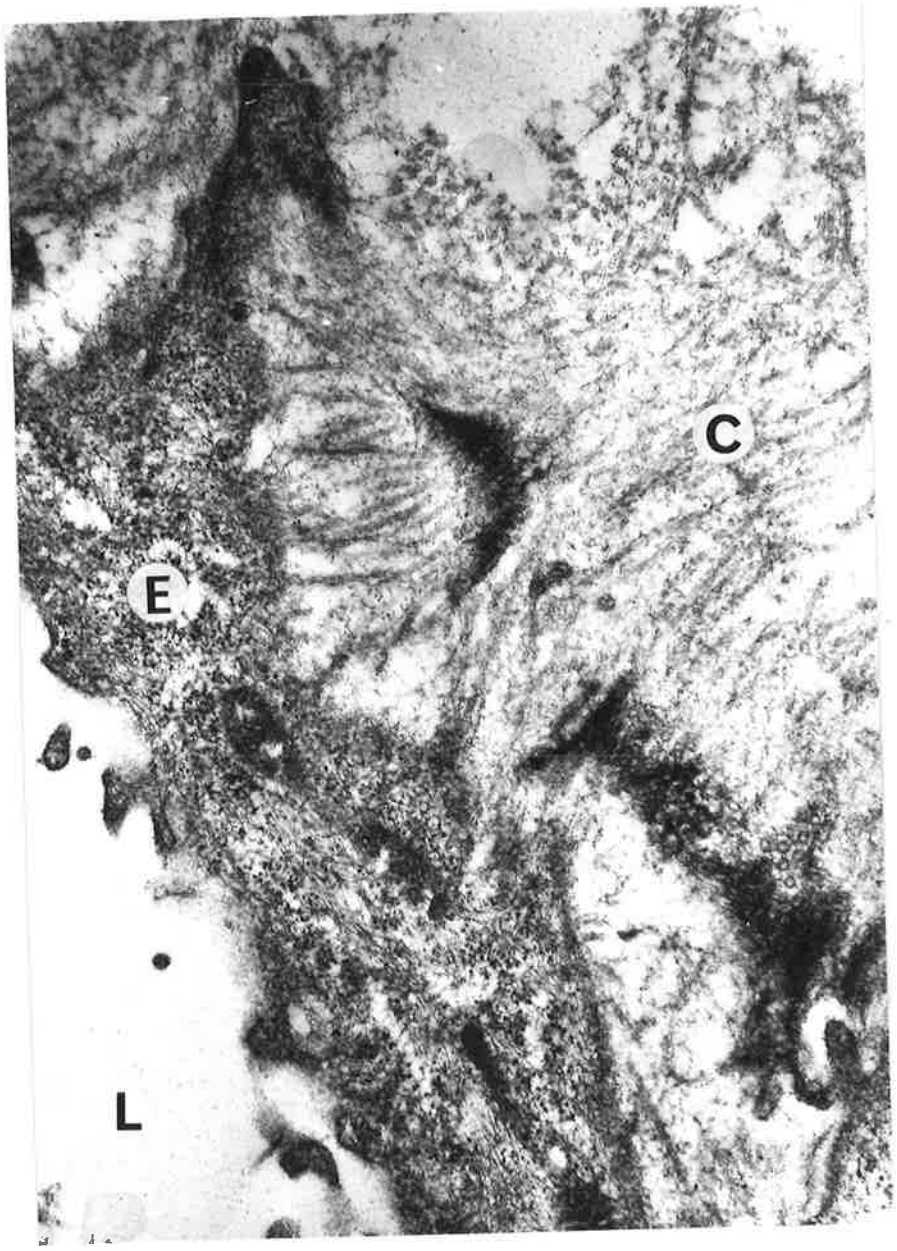


Fig. 20I. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Fibrils (C) inserting into endothelium (E), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

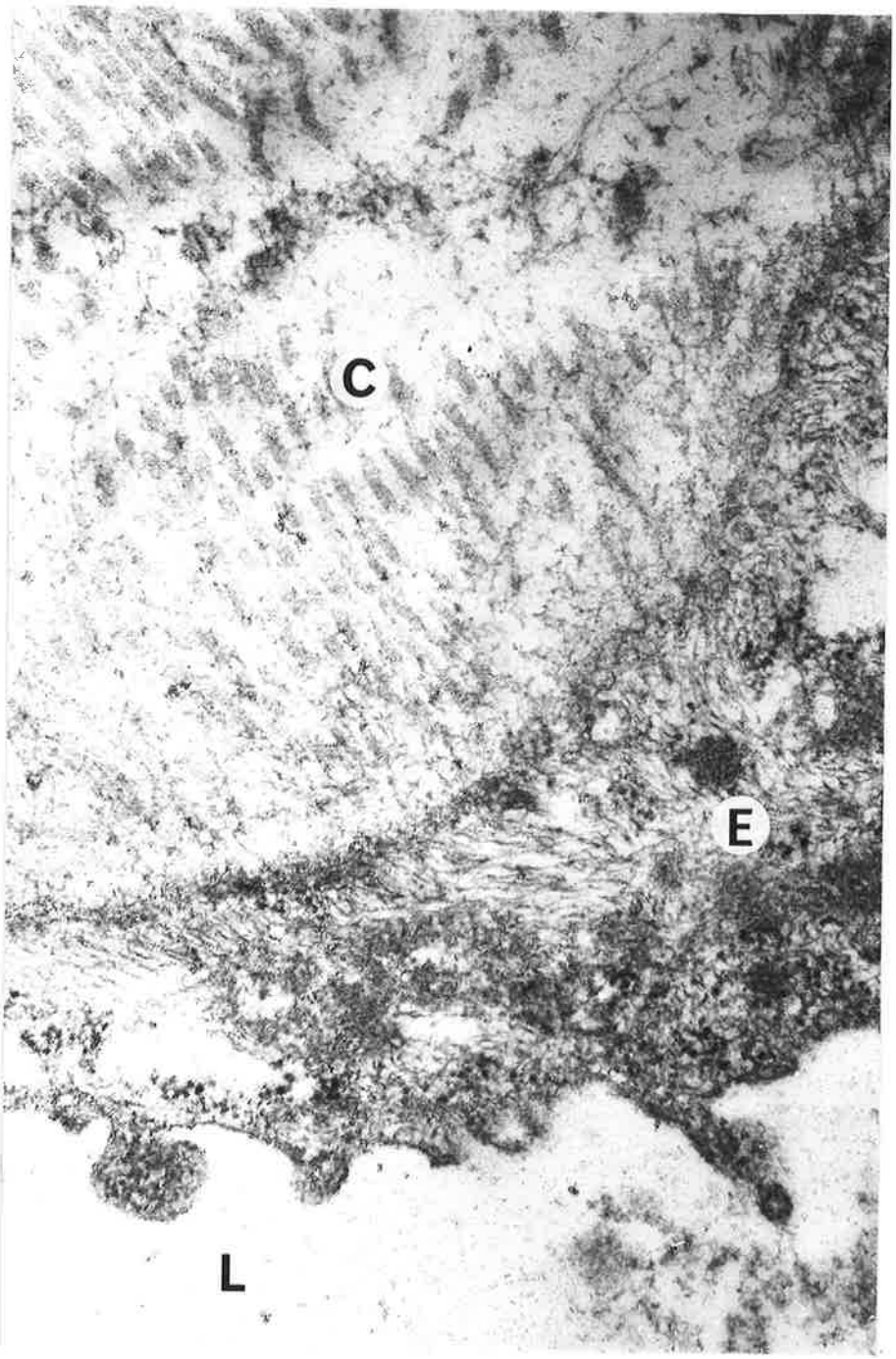


Fig. 20J. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Fibrils (C) inserting into endothelium (E), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(19,000X).

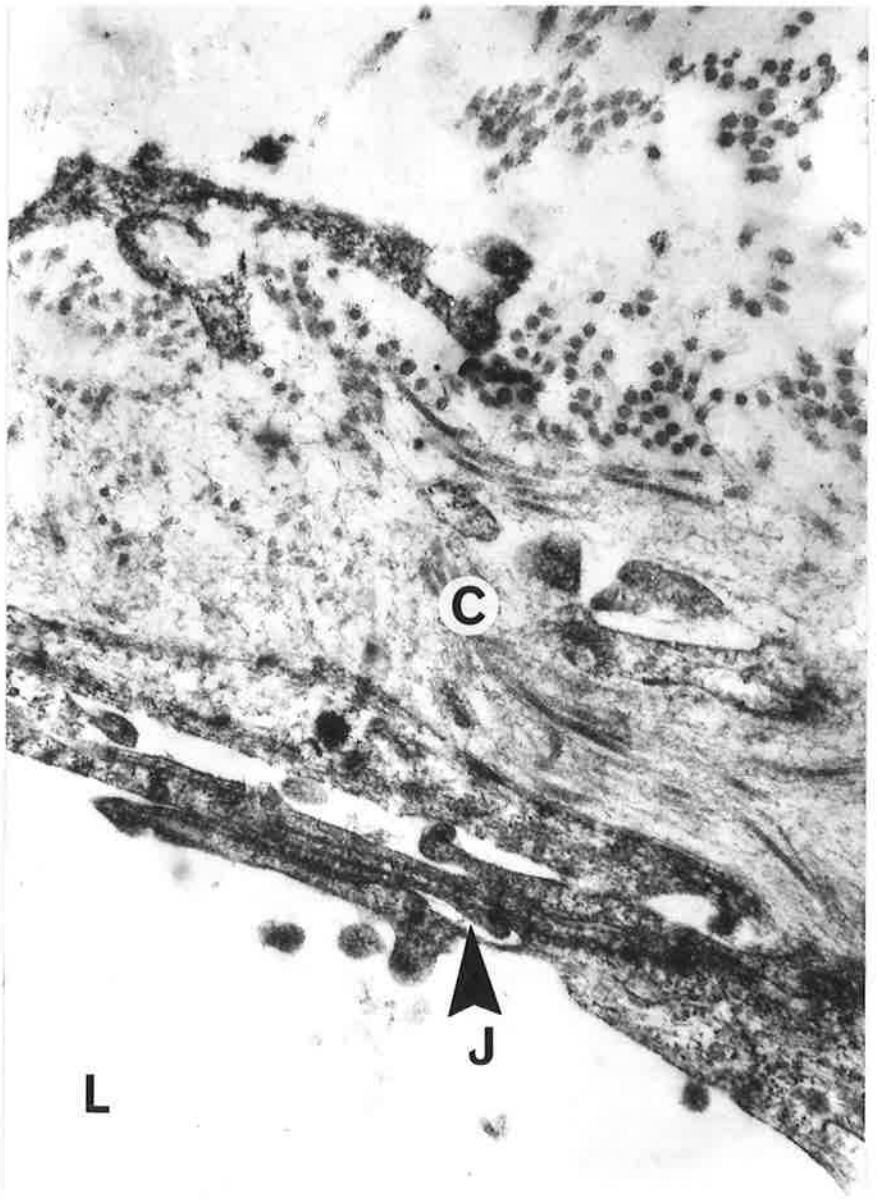


Fig. 20K. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Lumen (L), junction (J), collagen fibrils (C) inserting into endothelium.

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

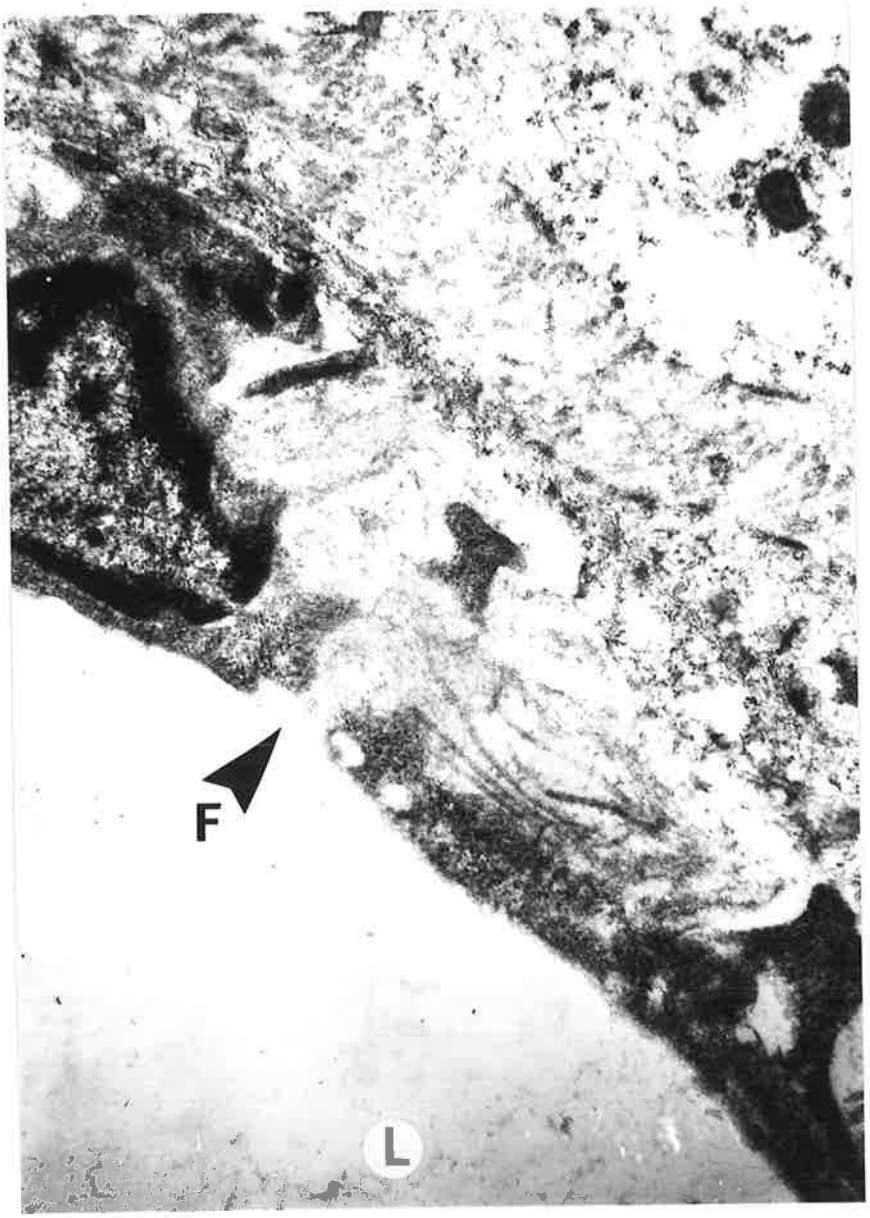


Fig. 20L. Detail of Wall of Alveolar Large Vessel in Figure 20A.

Fenestra (F), lumen (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(42,000X).

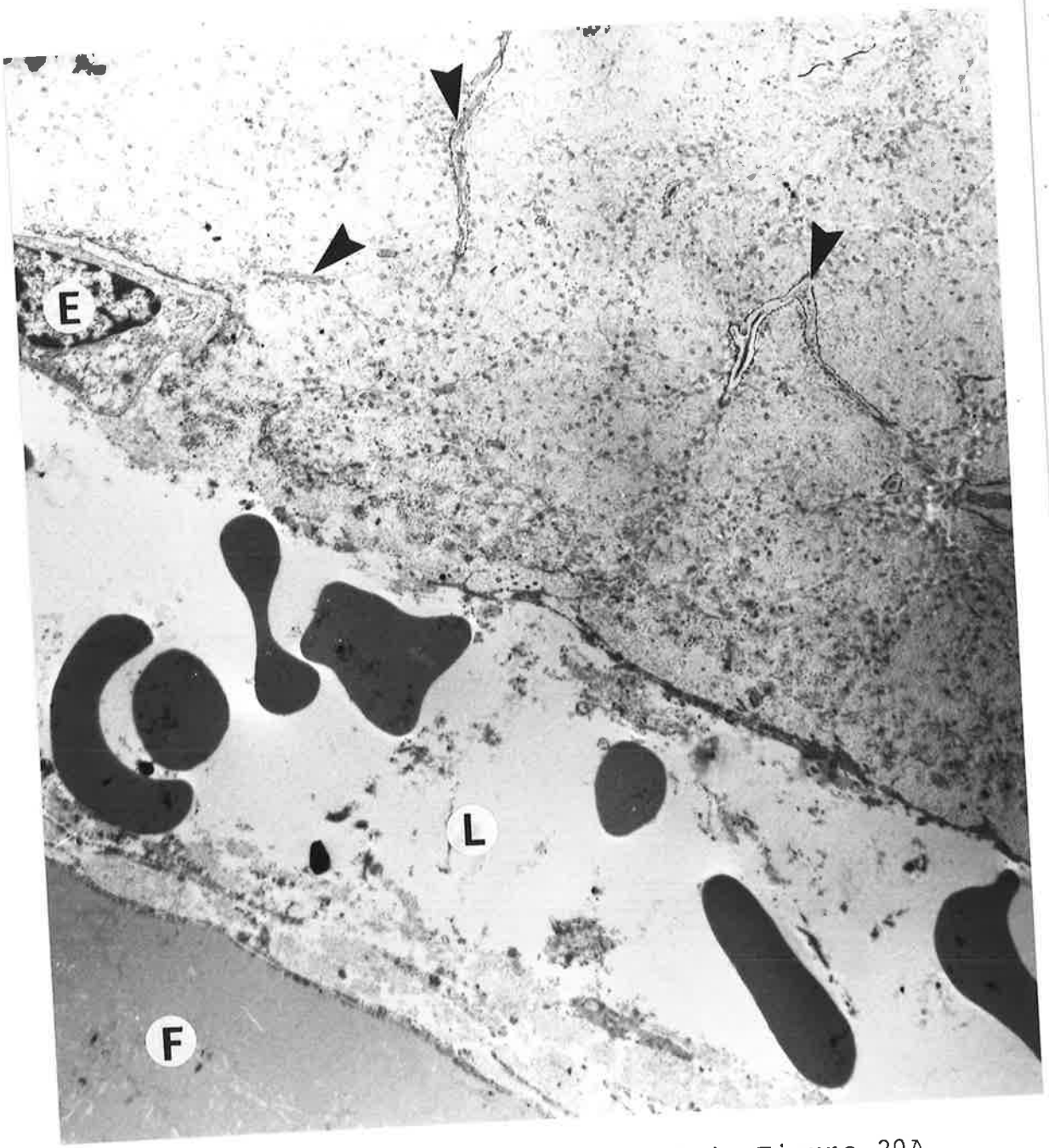


Fig. 20M. Detail of Alveolar Wall in Figure 20A.

Fat body (F), vessel (L), endosteal cell (E), canaliculi (arrowed).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,500X).

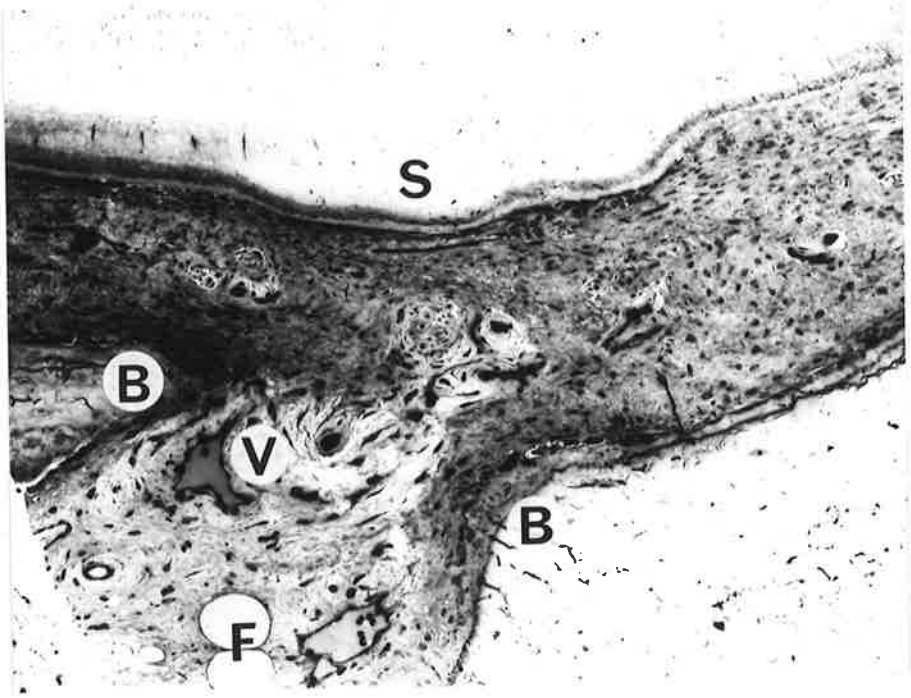


Fig. 21A. Foramen in Alveolar Wall of Tooth Socket:

Cemental pearl (S), foramen between (B,B) in alveolar bone, fat spaces (F), large vessel (V).

Light micrograph. Toluidine Blue.

Decalcified. (150X).

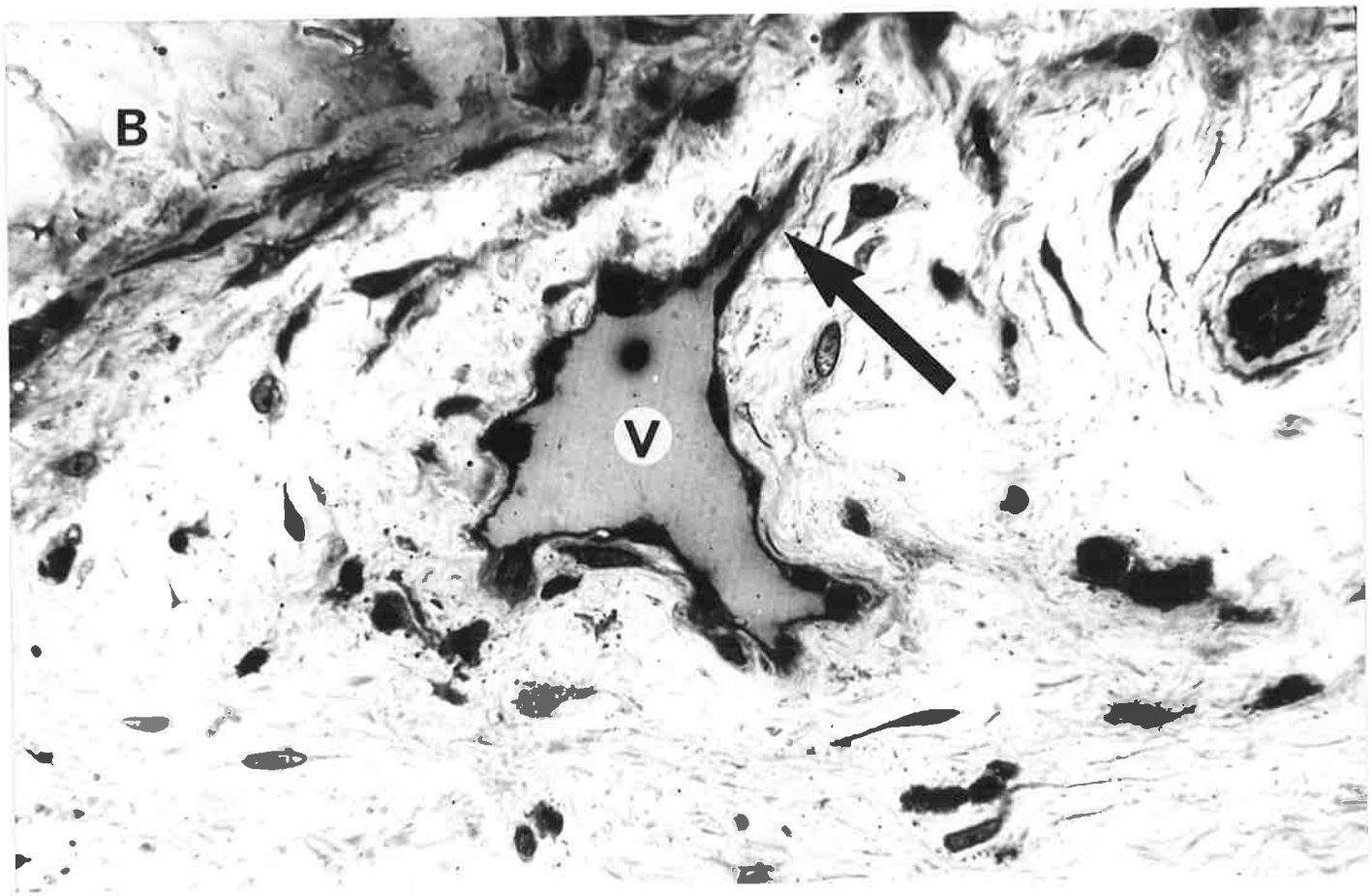


Fig. 21B. Foramen in Alveolar Wall of Tooth Socket in Figure 21A.

Alveolar bone (B), detail of large vessel (V).

Light micrograph. Toluidine Blue.

Decalcified. (750X).

Fig. 21C. Detail of Large Vessel Wall Arrowed in Figure 21B.

Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

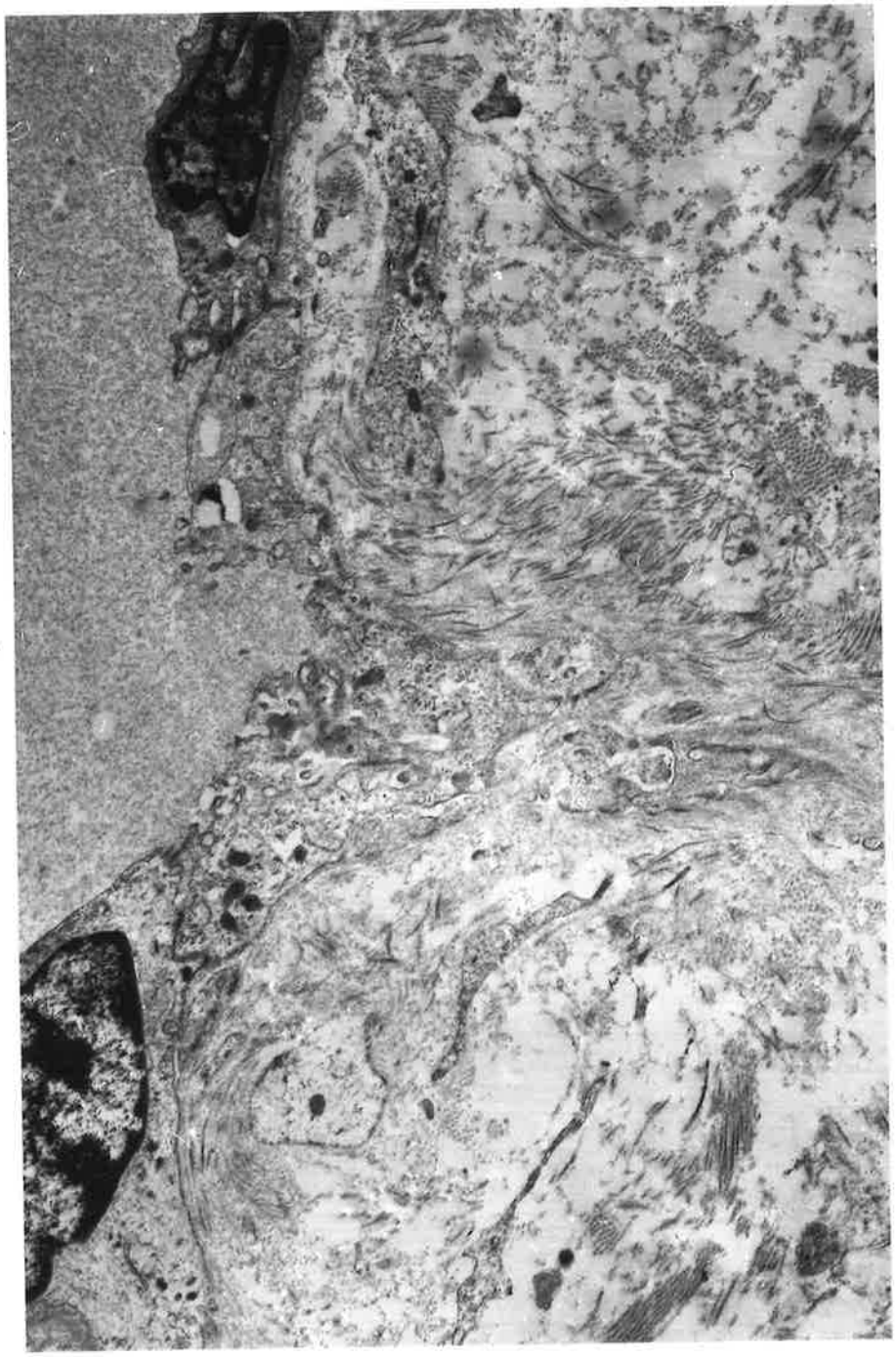


Fig. 21D. Detail of Arrowed Endothelial Junction of Large Vessel Wall in Figure 21C.

Endothelial junction with inserting fibrils.

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

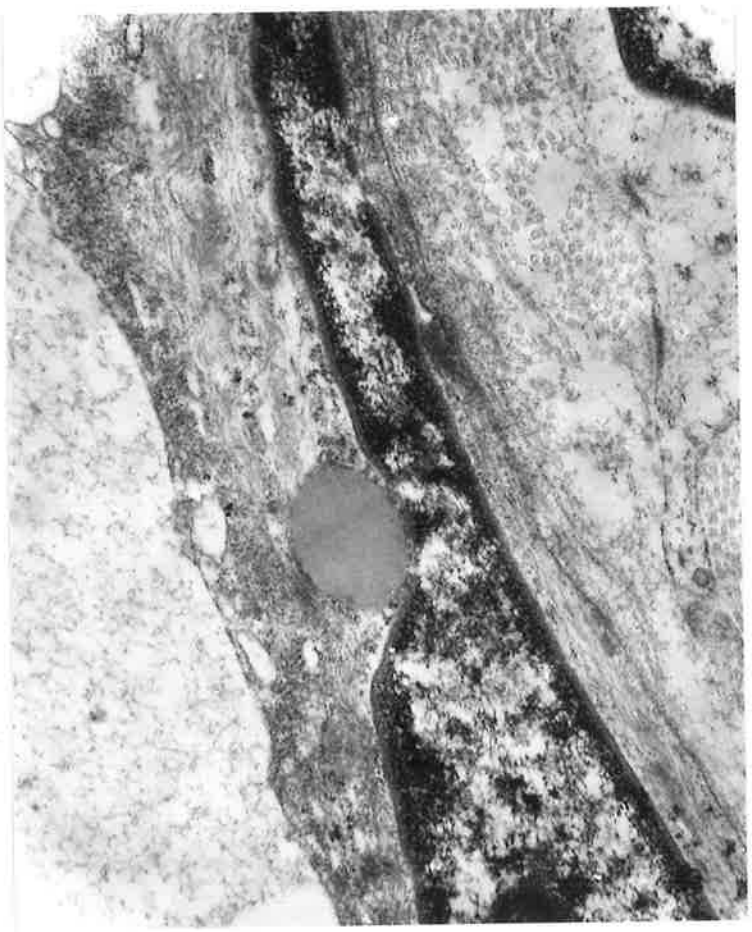


Fig. 21E. Detail of Arrowed Endothelial Cell of Large Vessel Wall in Figure 21C. Showing fat vesicle.

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

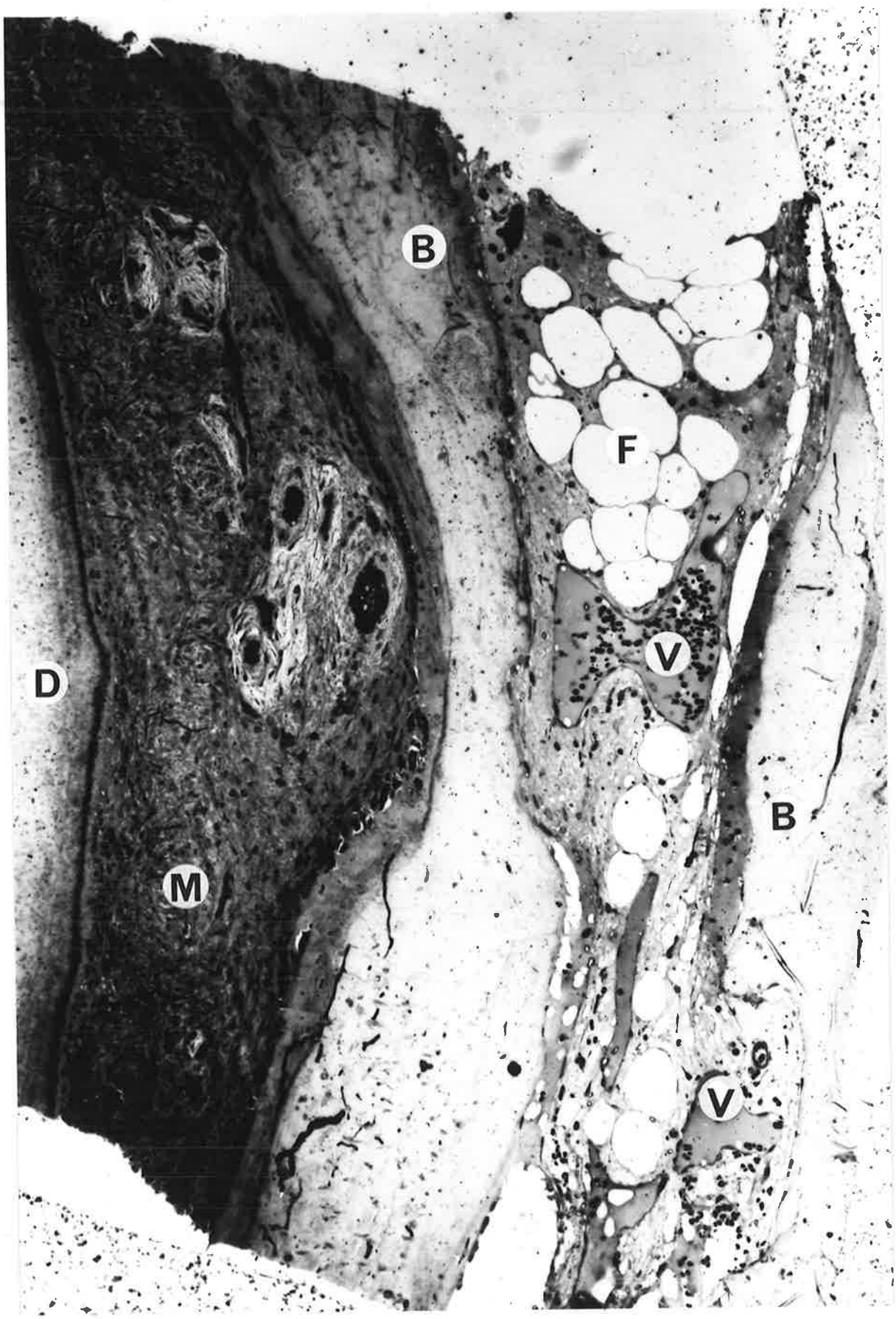


Fig. 22A. Section of Periodontium:

Dentine (D), periodontal ligament (M), alveolar bone (B), alveolar large vessels (V), fat spaces (F).

Light micrograph. Toluidine Blue.
Decalcified. (125X).

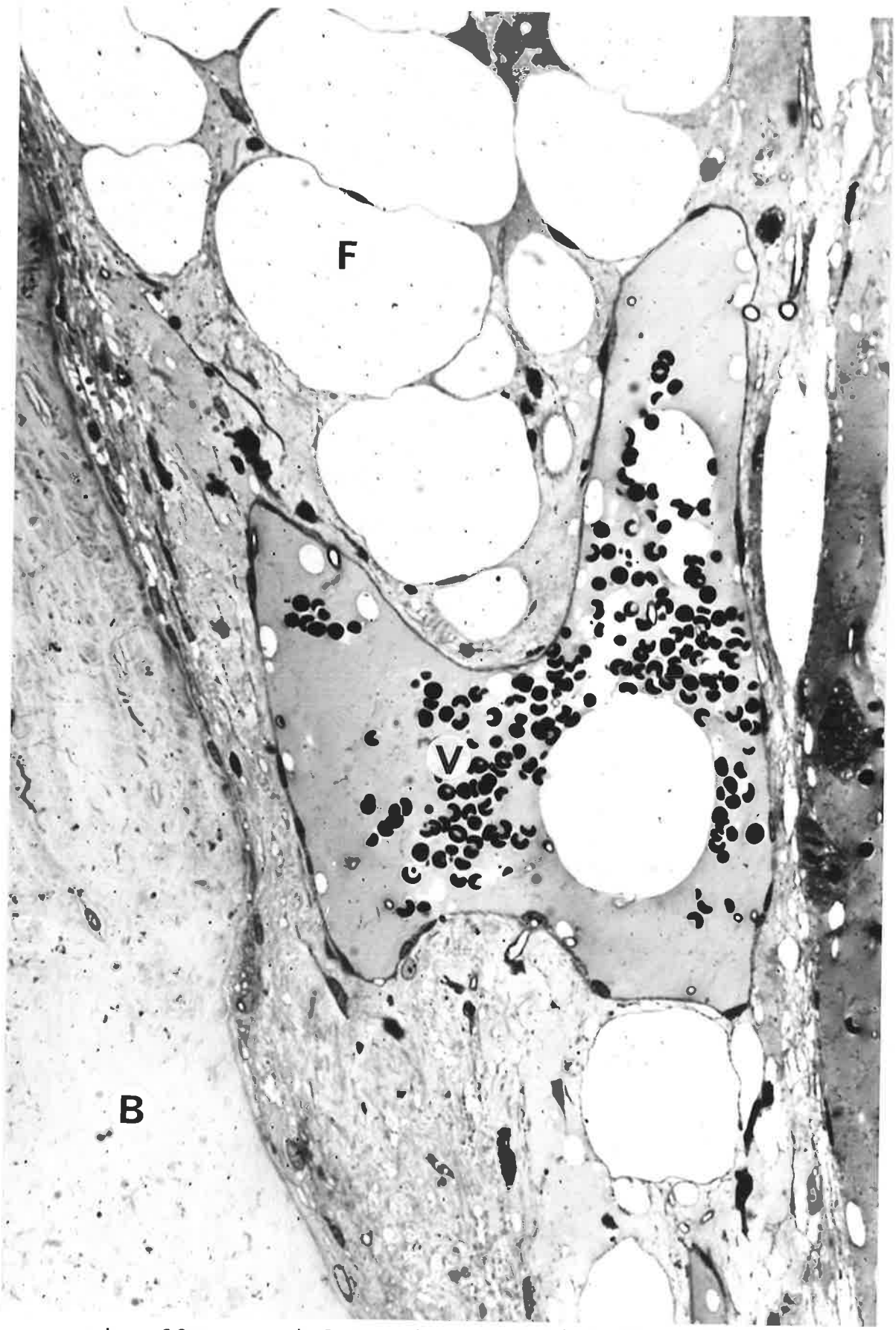


Fig. 22B. Serial Section of Periodontium in Figure 22A.

Alveolar large vessel with approximating fat spaces.

Vessel (V), fat spaces (F), alveolar bone (B).

Light micrograph. Decalcified. Toluidine Blue. (450X).

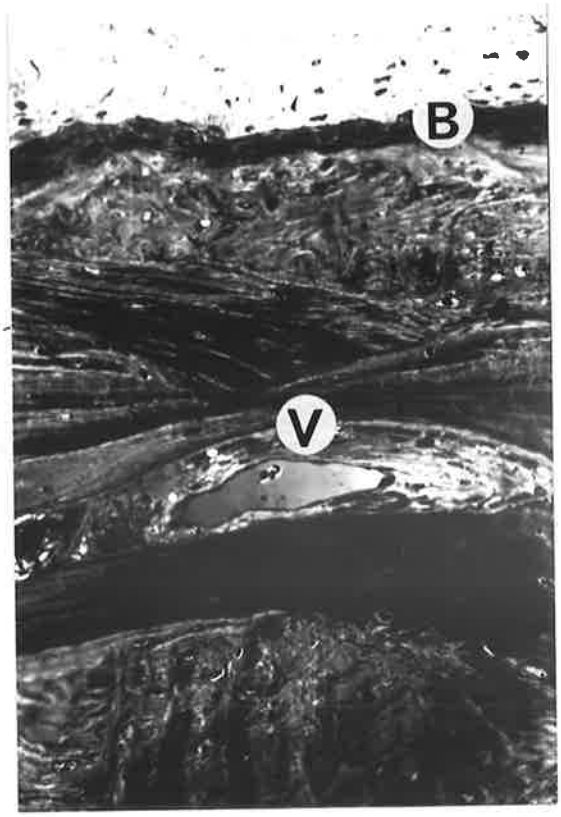


Fig. 23A. Section of Buccal Plate of Alveolar Bone:

Alveolar large vessel (V), buccal plate (B).

Light micrograph. Toluidine Blue.

Decalcified. (125X).

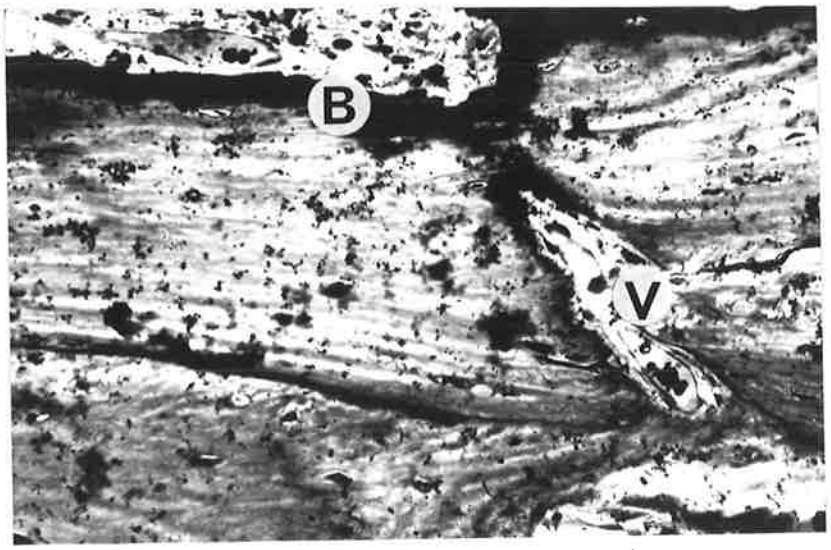


Fig. 23B. Semi Serial Section of Buccal Plate of Alveolar Bone in Figure 23A.

Alveolar large vessel (V) approximating buccal plate (B).

Light micrograph. Toluidine Blue.

Decalcified. (125X).

Fig. 23C. Semi Serial Section of Buccal Plate of Alveolar Bone in Figure 23B.

Alveolar large vessel (V) about to communicate with superficial tissues through foramen in buccal plate (B).

Light micrograph. Toluidine Blue.
Decalcified. (125X).

Fig. 23D. Semi Serial Section of Buccal Plate of Alveolar Bone in Figure 23C.

Extra-bony ramification of alveolar large vessel (V).

Light micrograph. Toluidine Blue.
Decalcified. (125X).

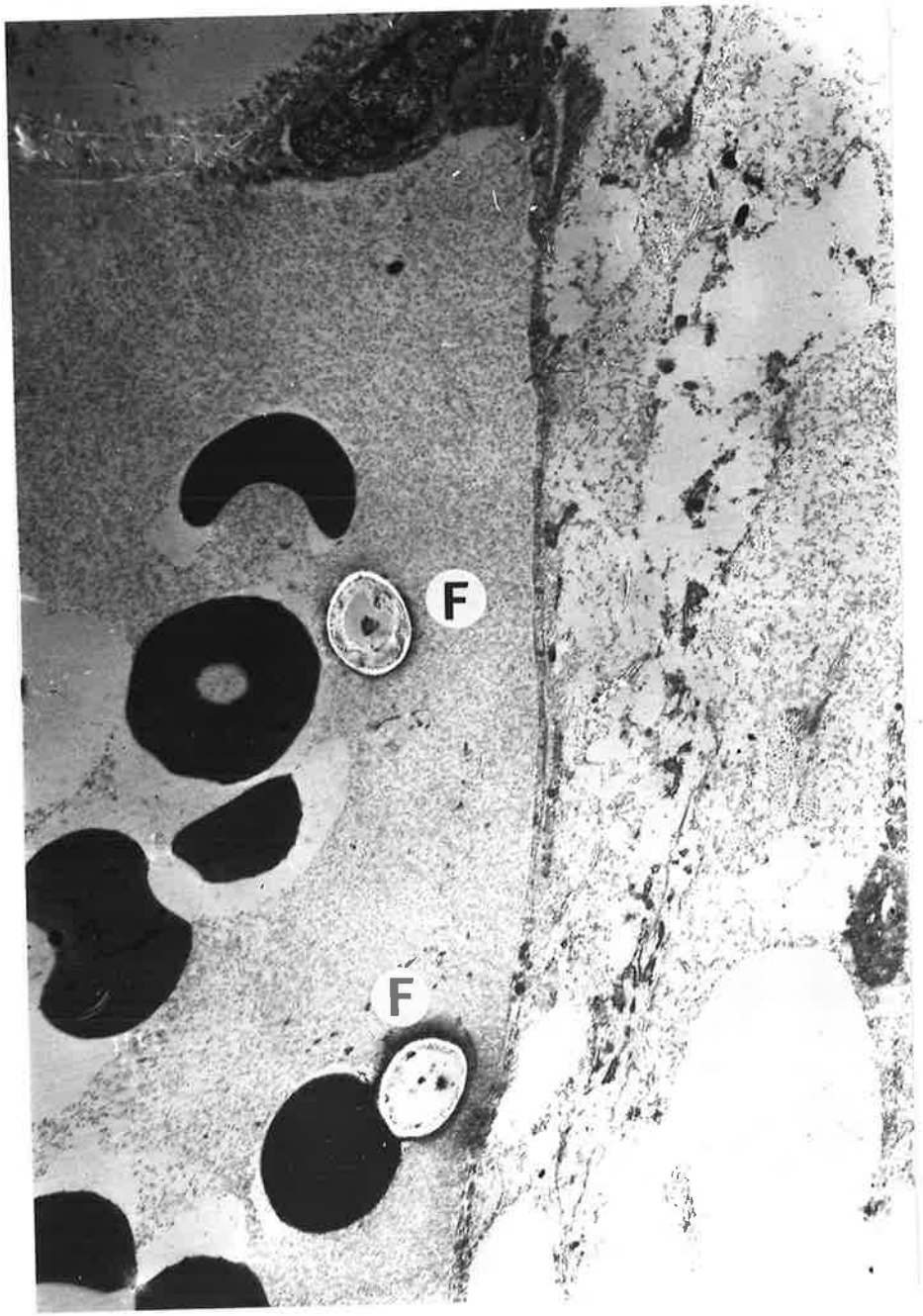


Fig. 24. Alveolar Large Vessel:
Showing intra vascular fat inclusions (F), thin endothelium and flimsy interstitial tissue.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

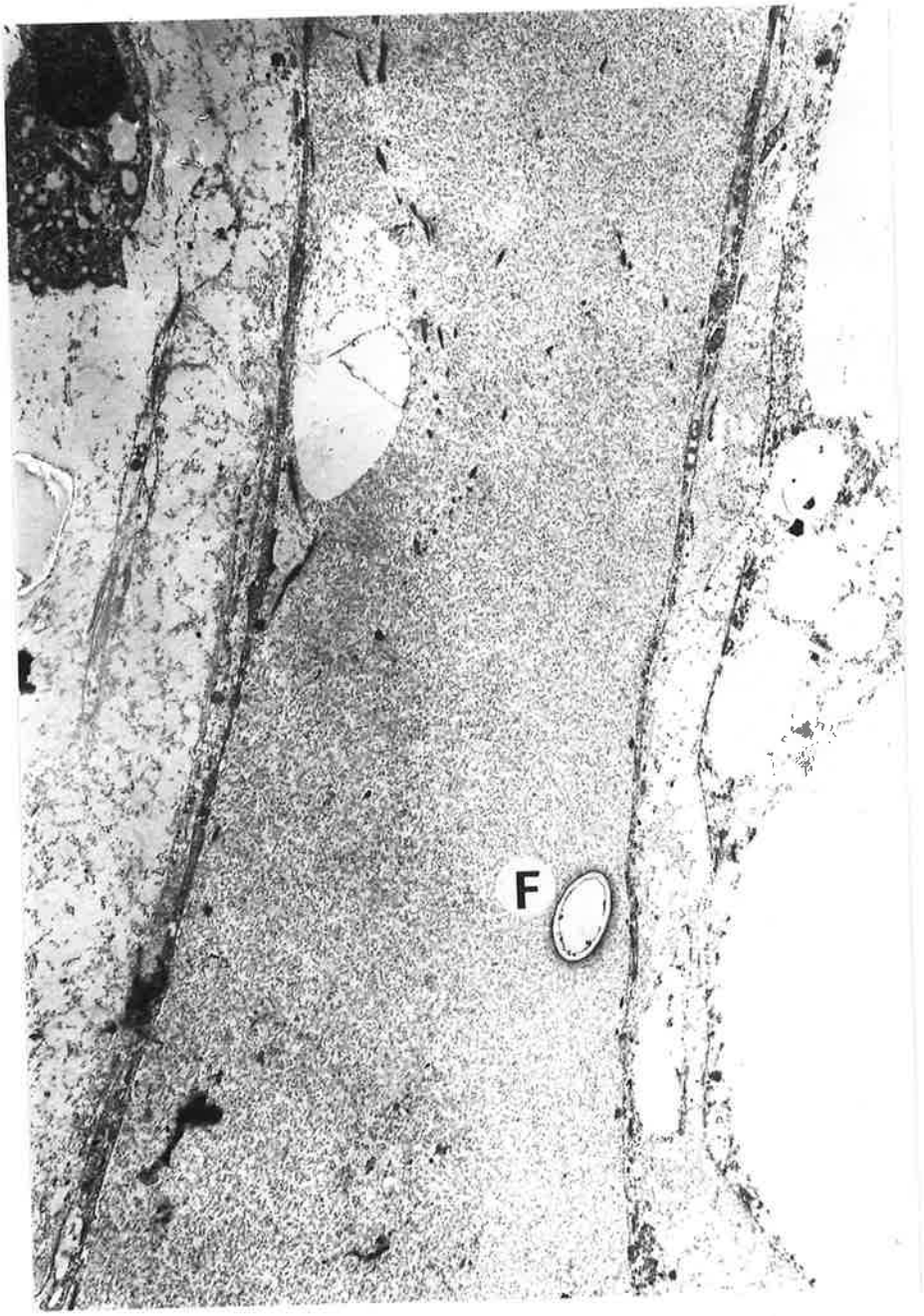


Fig. 25. Alveolar Large Vessel:
Showing intra vascular fat inclusion (F), thin endothelium and flimsy interstitial tissue.

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

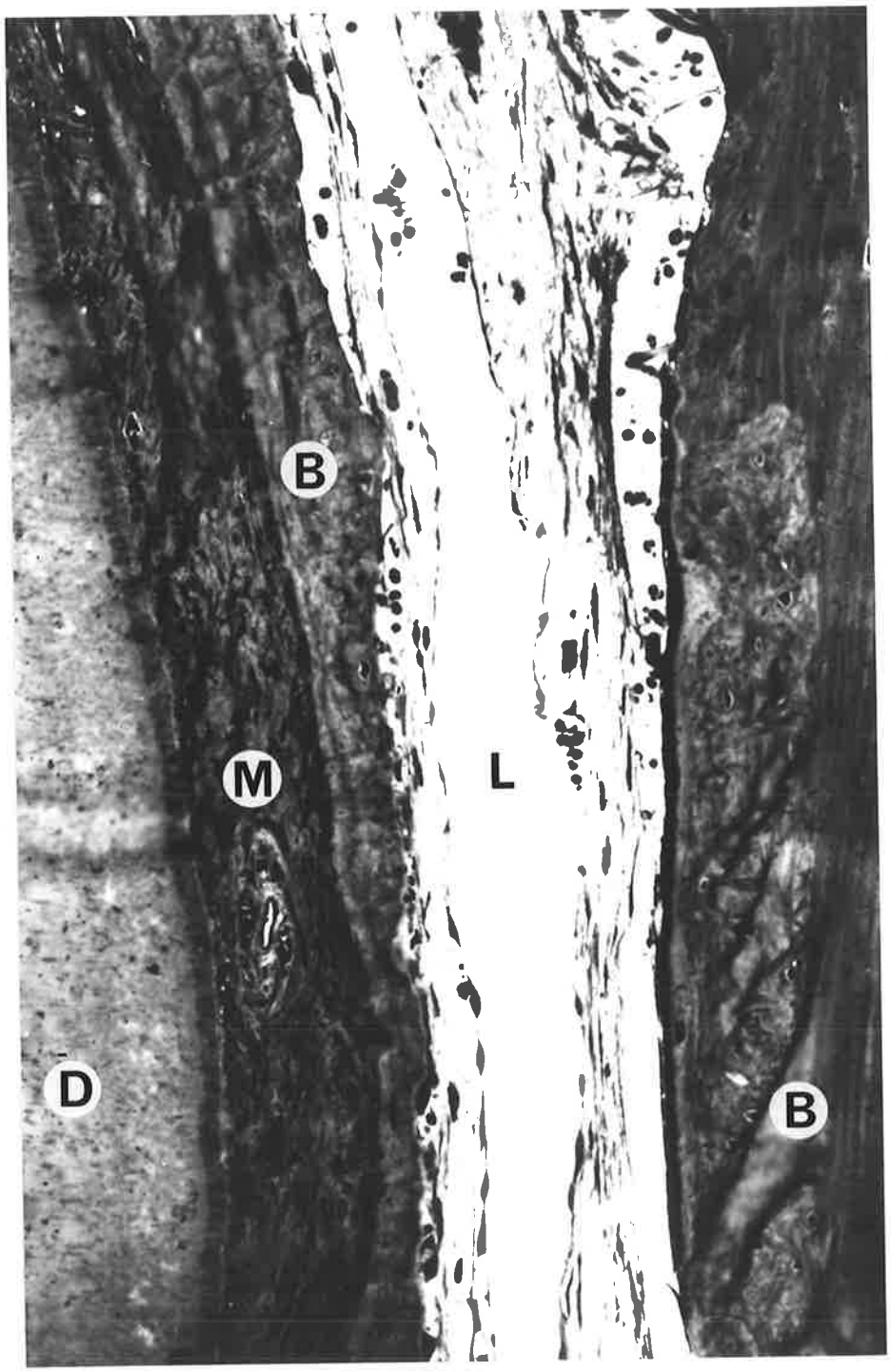


Fig. 26A. Alveolar Large Vessel:

Large vessel lumen (L), alveolar bone (B), periodontal ligament (M), dentine (D).

Light micrograph. Toluidine Blue.

Decalcified. (250X).

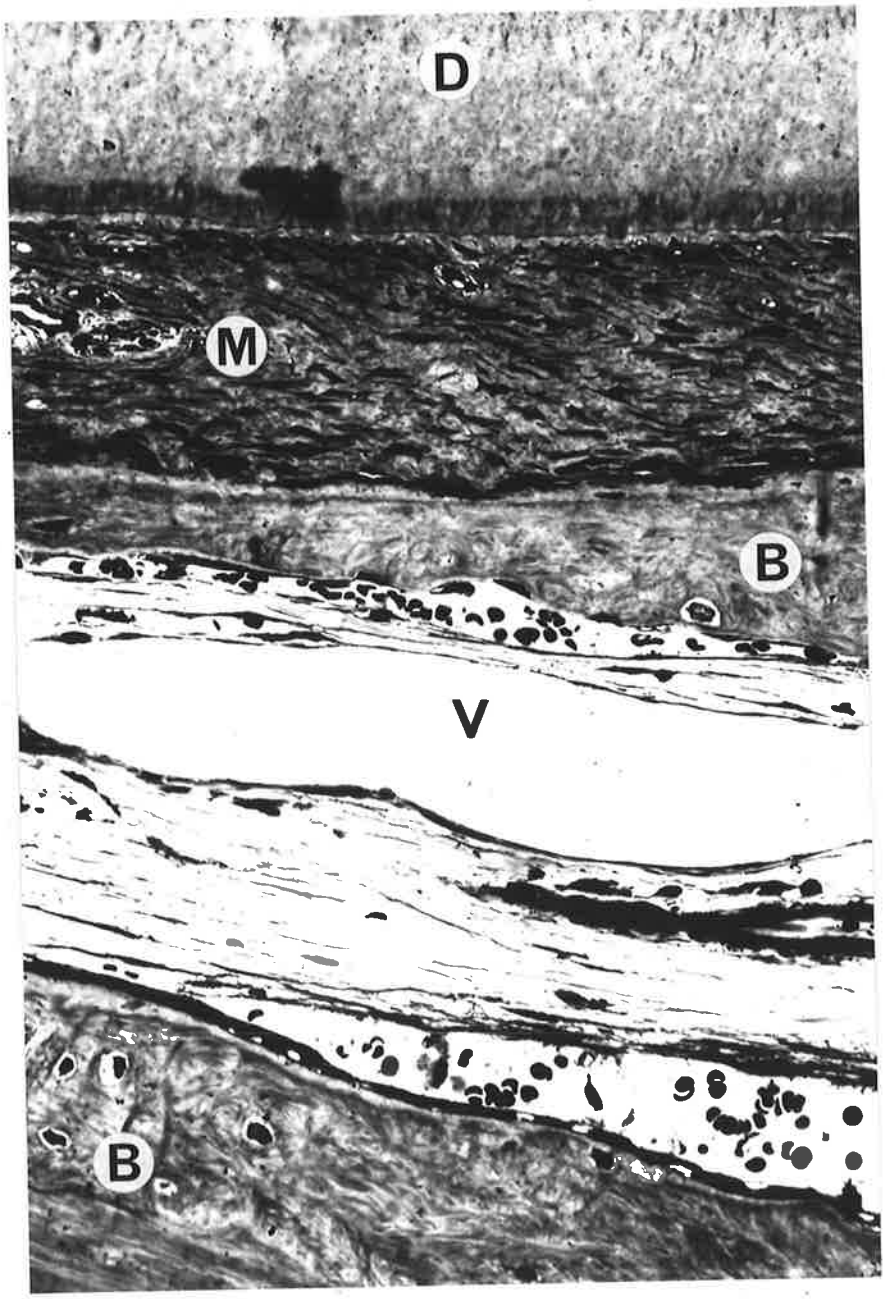


Fig. 26B. Semi Serial Section of Alveolar Large Vessel in Figure 26A.

Dentine (D), periodontal ligament (M), alveolar bone (B), alveolar large vessel (V).

Light micrograph. Toluidine Blue.

Decalcified. (350X).

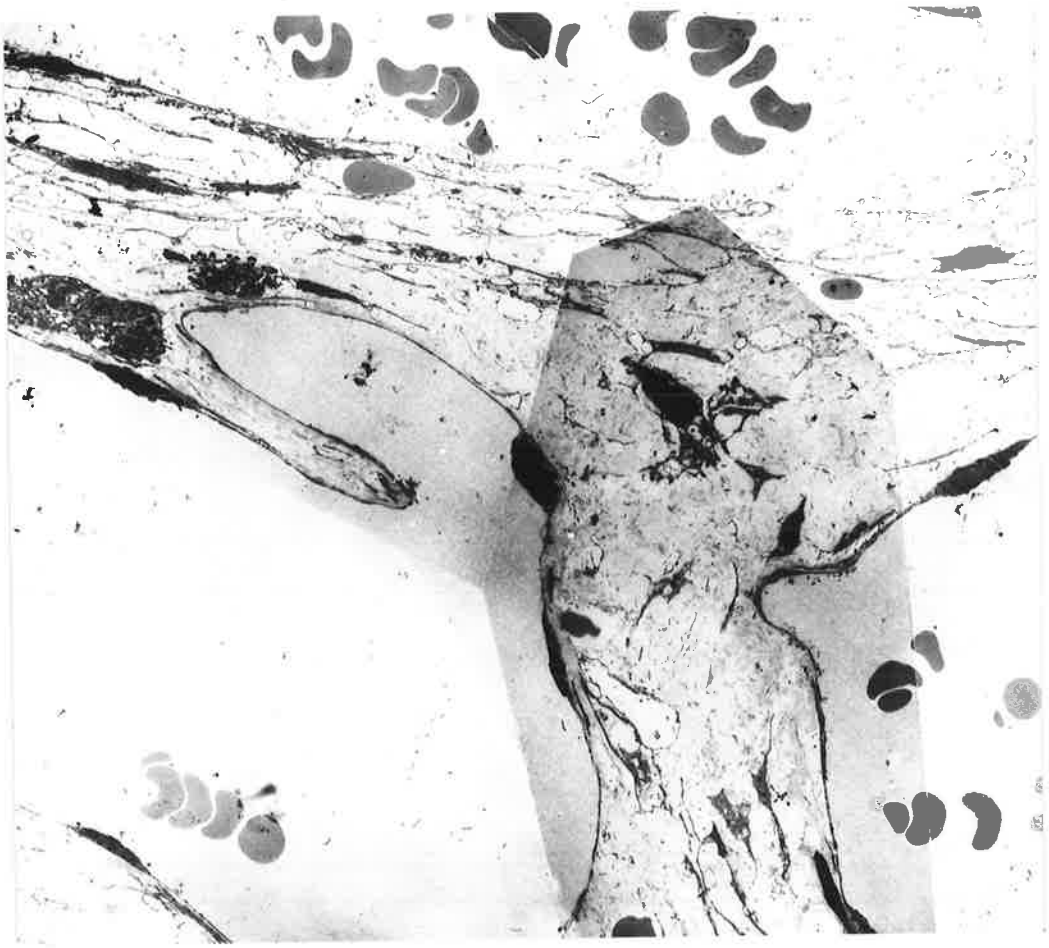


Fig. 26C. Semi Serial Section of Alveolar Large Vessel in Figure 26B.

Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,000X).

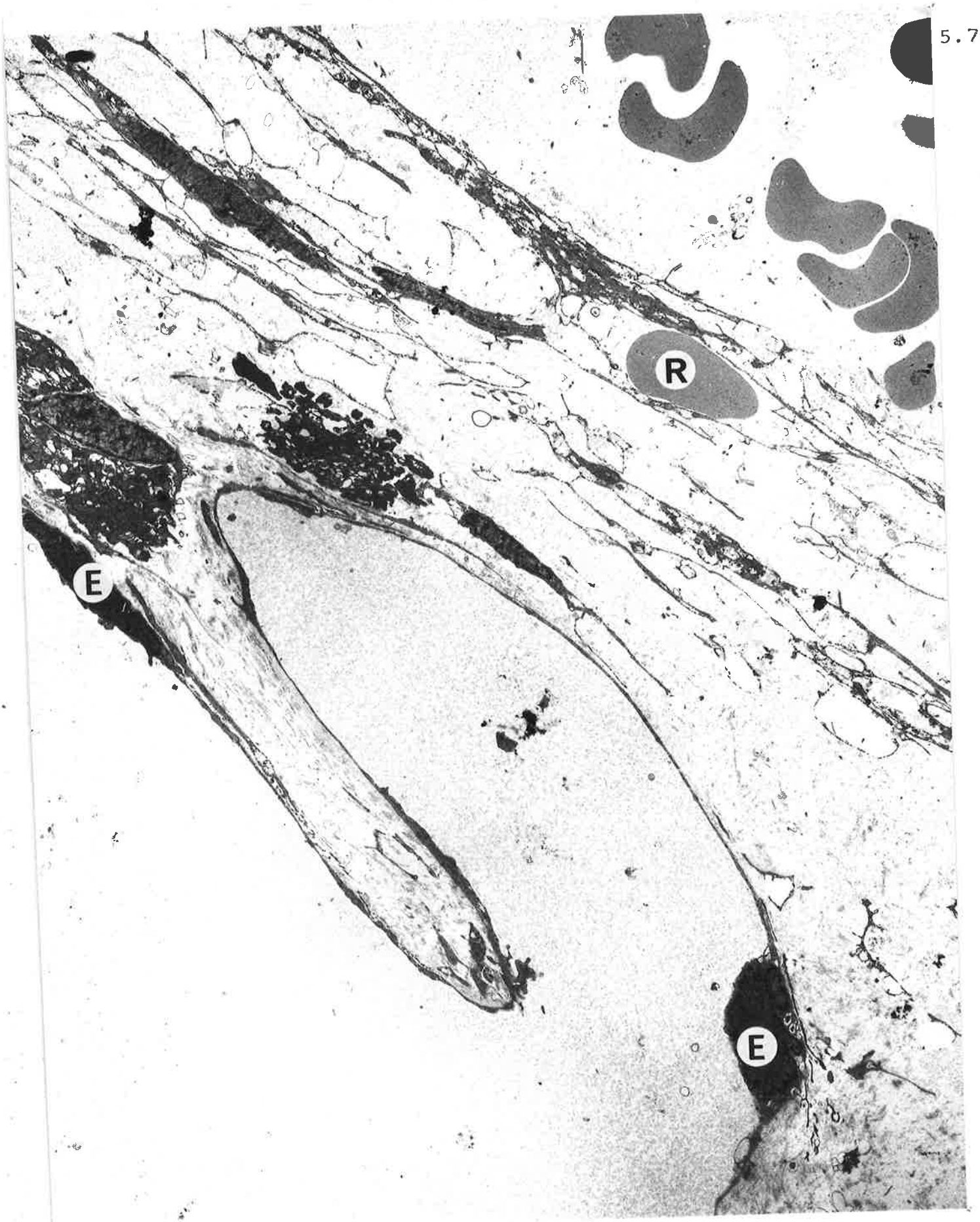


Fig. 26D. Detail of Alveolar Large Vessel in Figure 26C.
Erythrocyte trapped in interstitial tissue (R), endothelial nucleus (E).
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,000X).

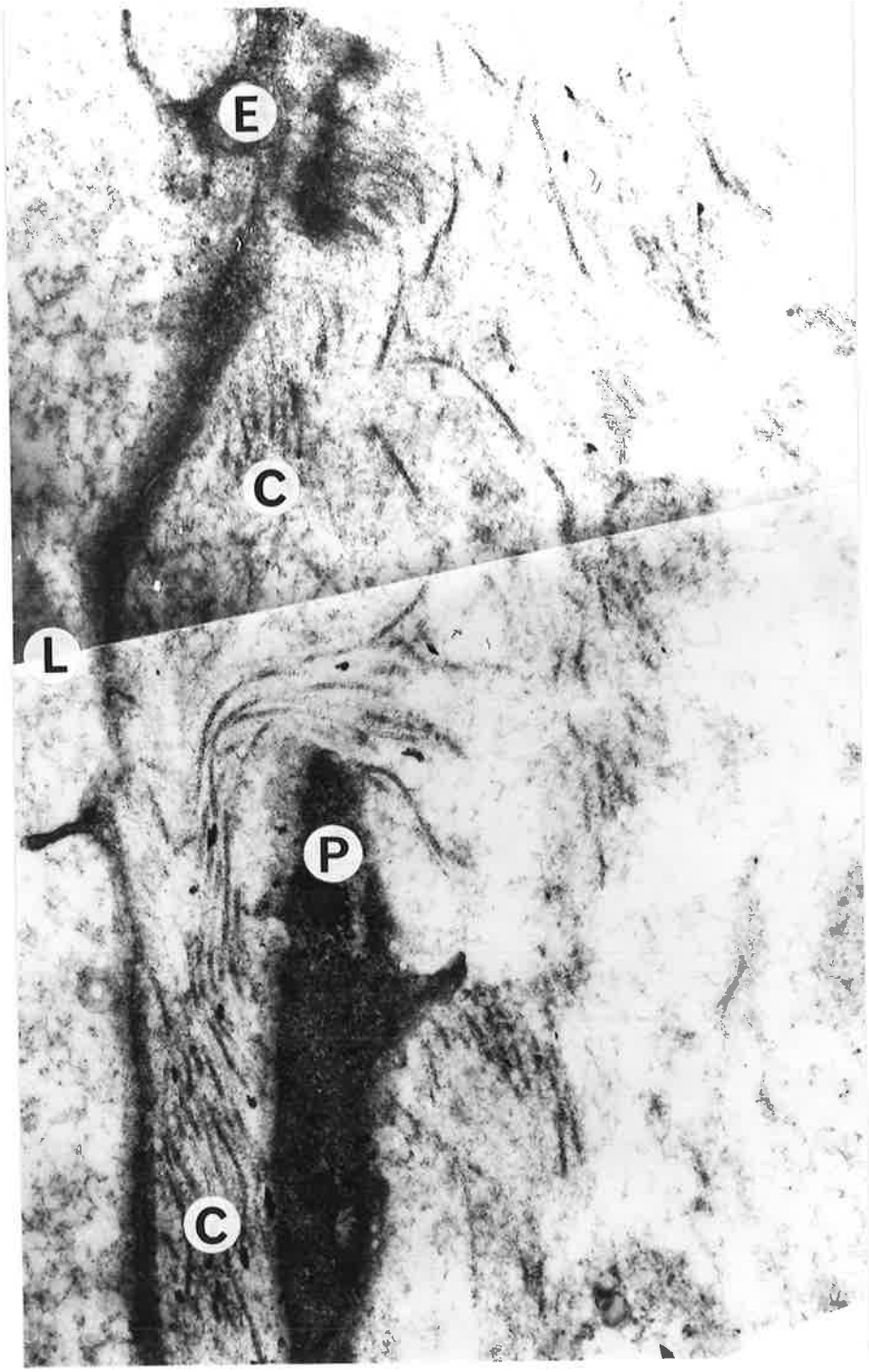


Fig. 26E. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), endothelial cytoplasm (E), pericyte (P), inserting collagen fibrils (C).

Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

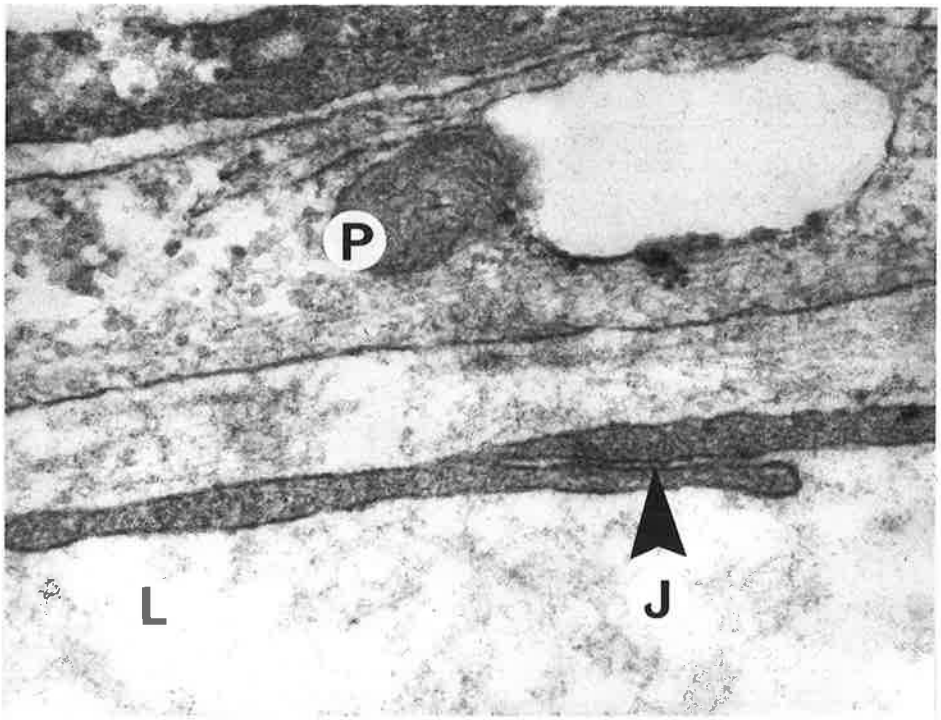


Fig. 26F. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), junction (J), pericyte (P).
Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

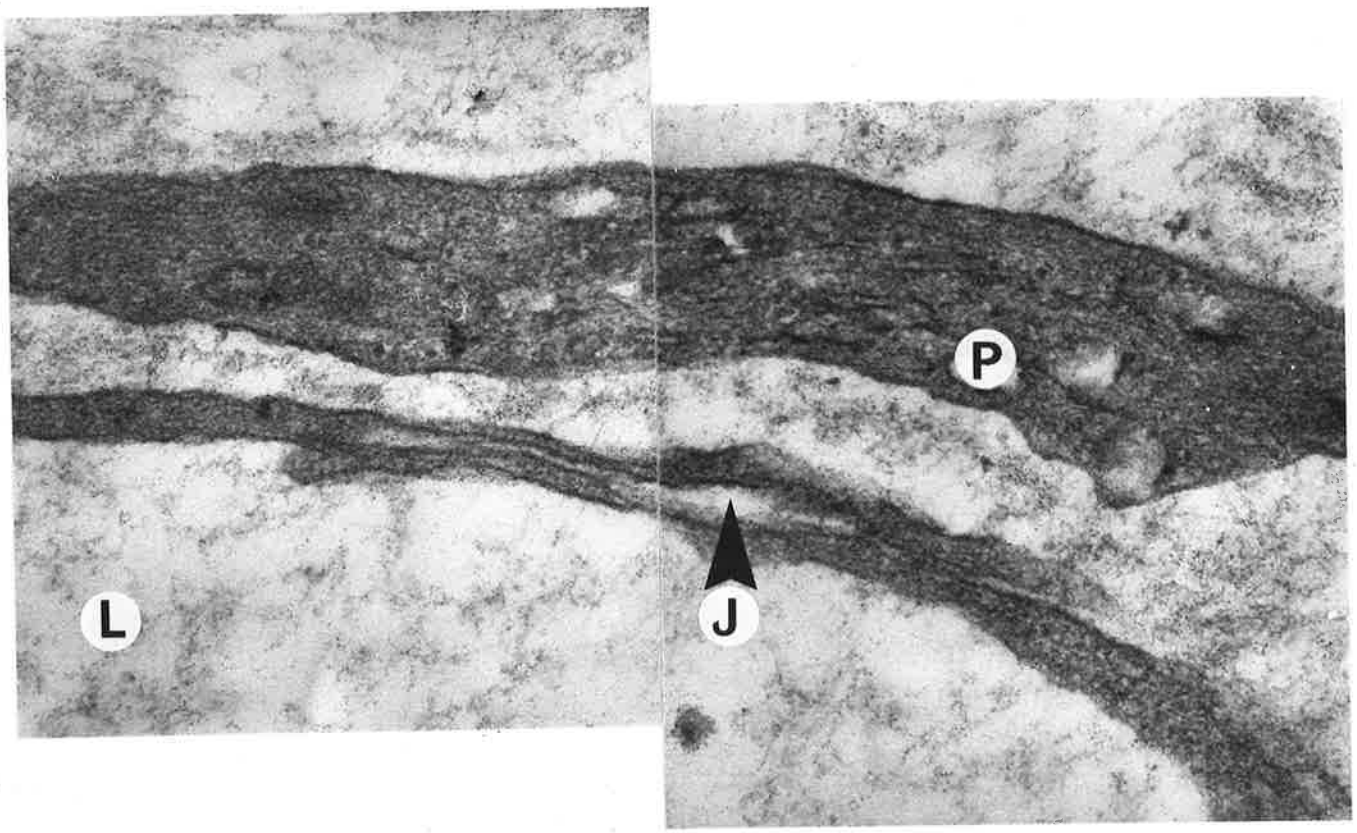


Fig. 26G. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), junction (J), pericyte (P).

Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

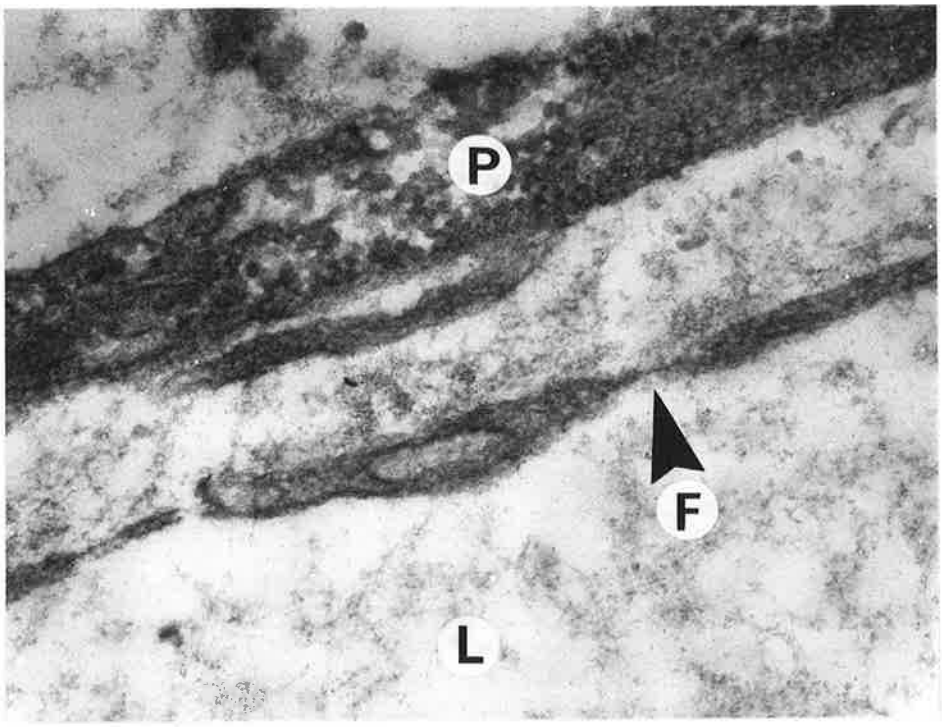


Fig. 26H. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), fenestra (F), pericyte (P).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

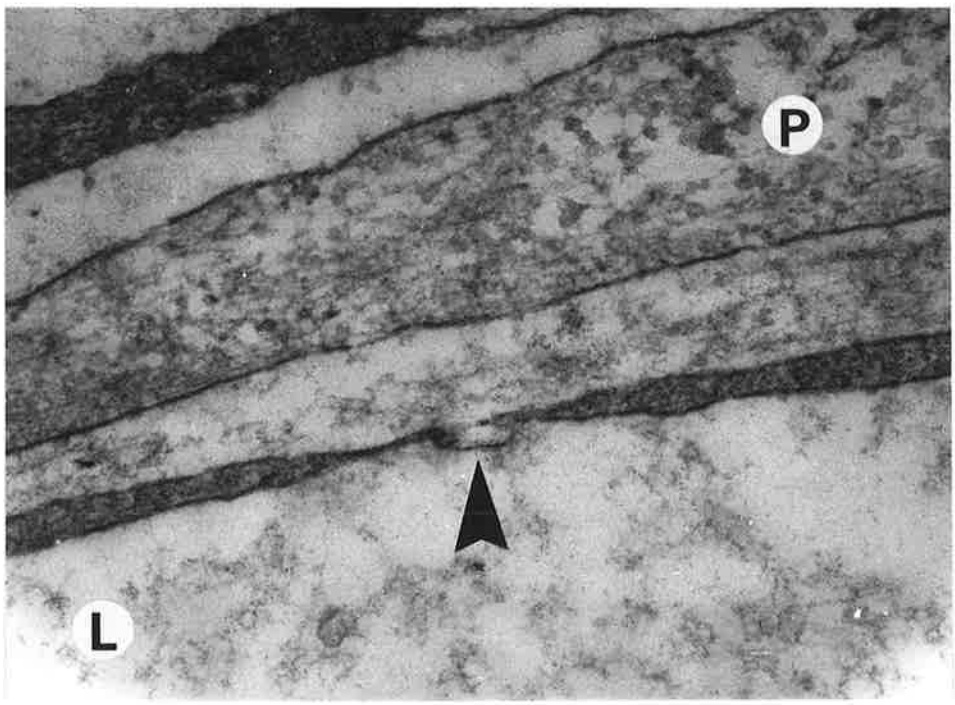


Fig. 26I. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), vesicle (arrowed), pericyte (P).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

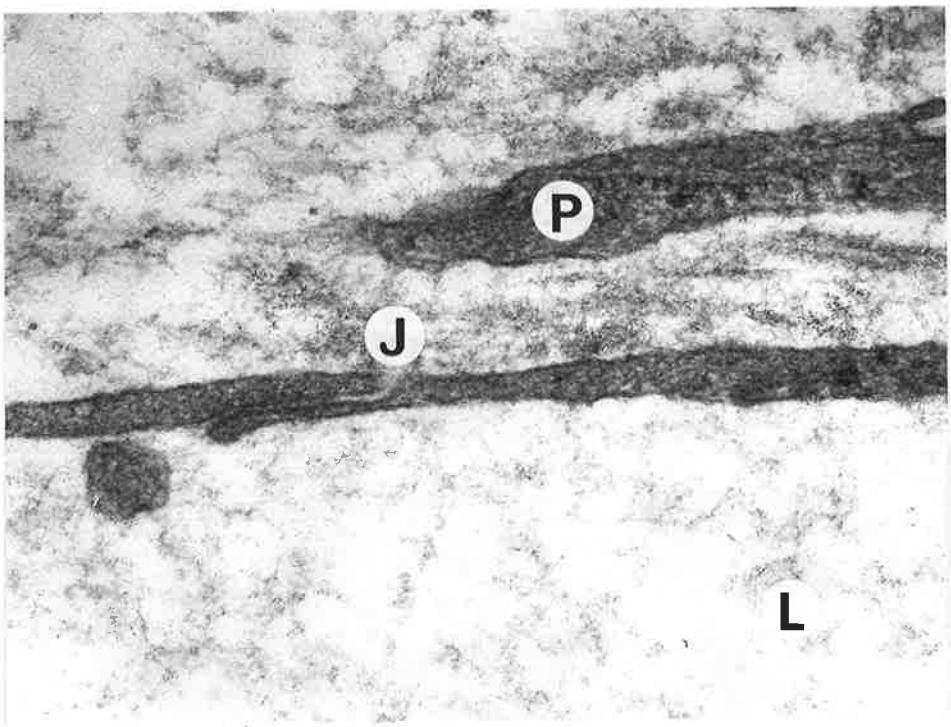


Fig. 26J. Detail of Wall of Alveolar Large Vessel in Figure 26C.
Lumen (L), junction (J), pericyte (P).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(40,000X).

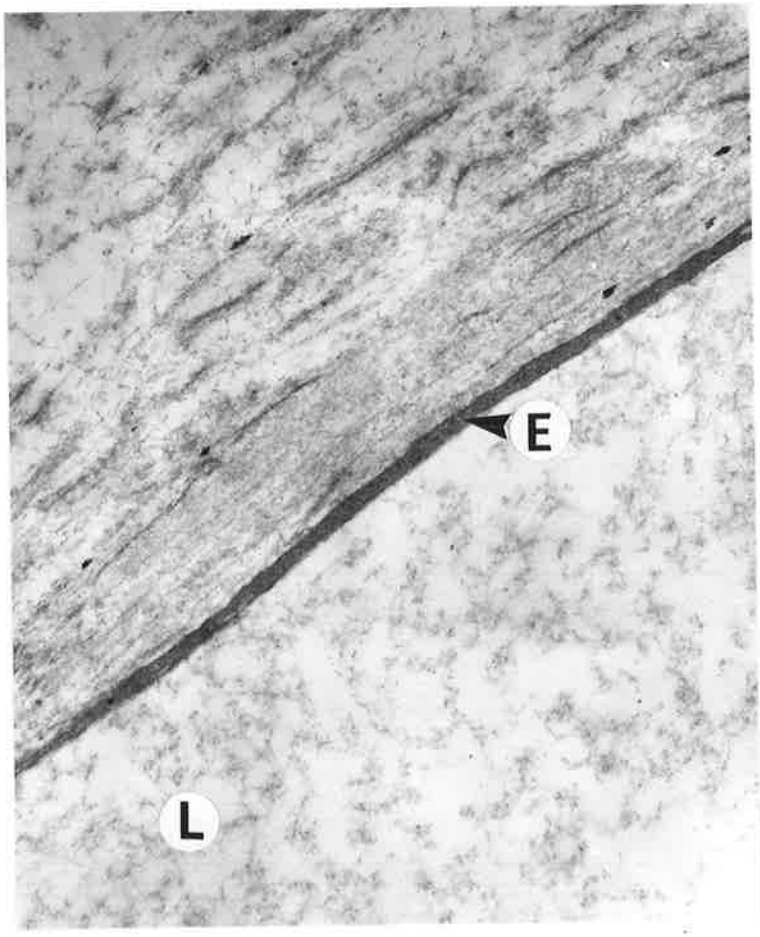


Fig. 26K. Detail of Wall of Alveolar Large Vessel in Figure 26C.

Lumen (L), endothelial cytoplasm (E).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(10,000X).

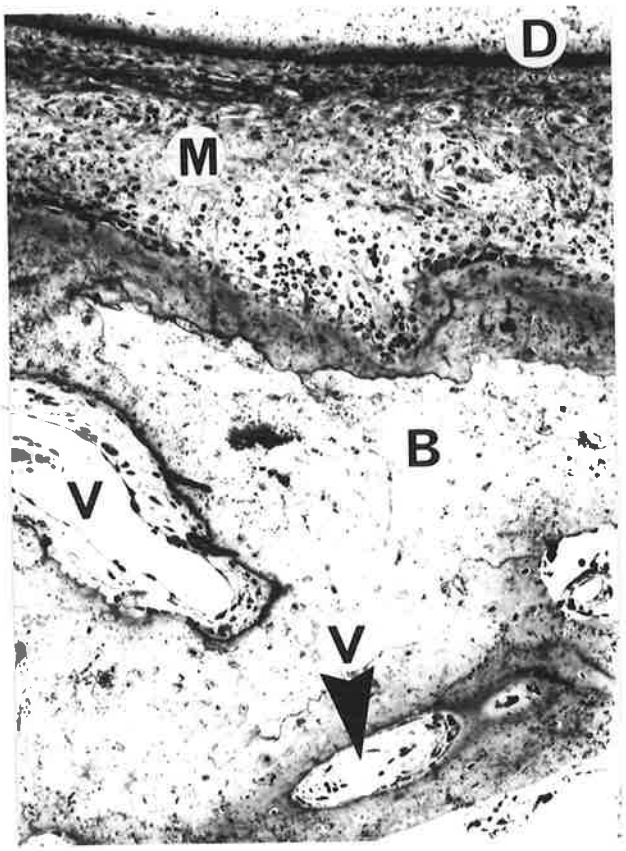


Fig. 27A. Section of Periodontium:

Dentine (D), periodontal ligament (M), alveolar bone (B), alveolar large vessels (V).

Light micrograph. Toluidine Blue.
Decalcified. (100X).

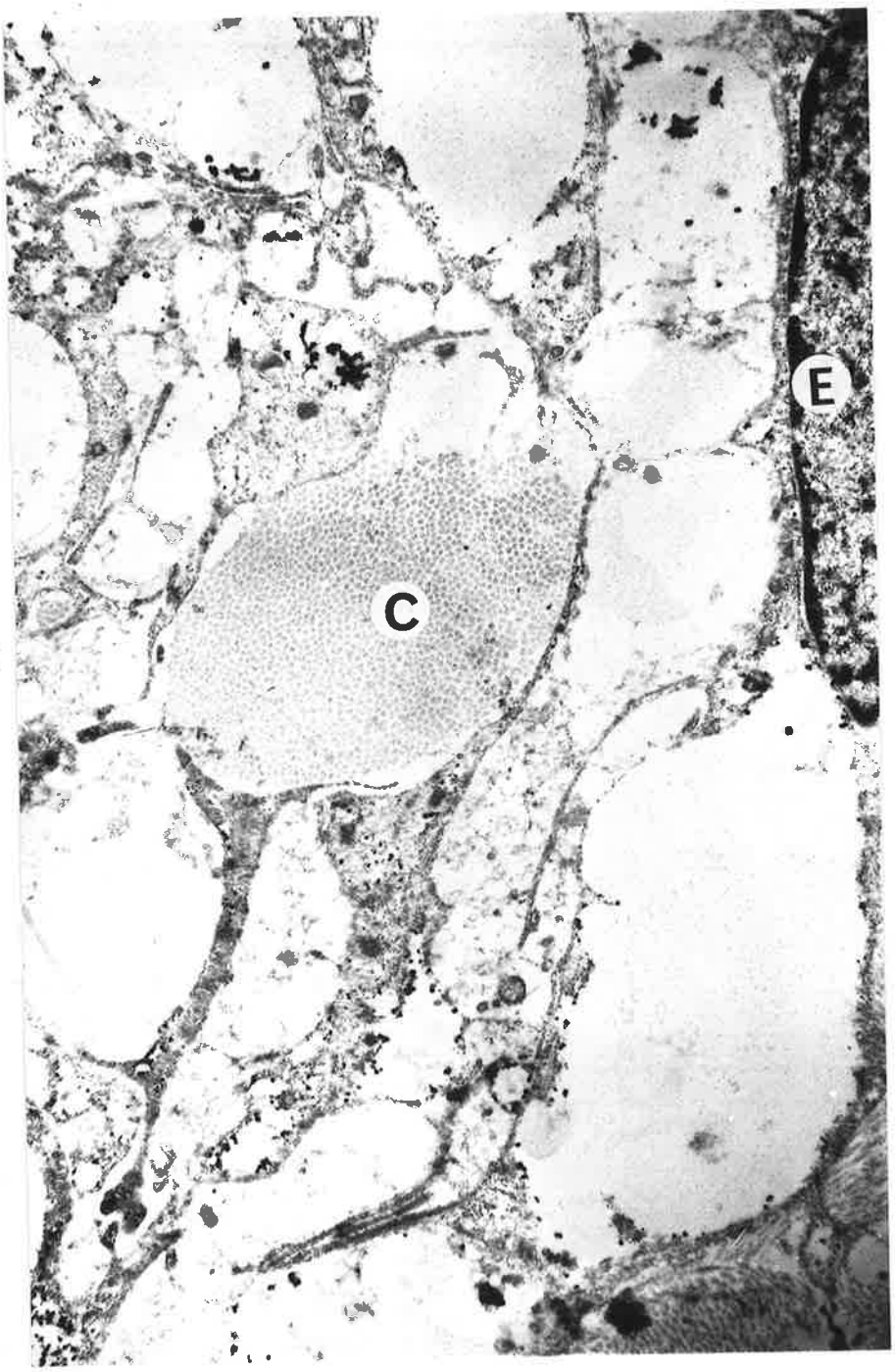


Fig. 27B. Detail of Tissue Supporting Alveolar Large Vessel Arrowed in Figure 27A.

Collagen fibres (C), endothelial nucleus (E).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,500X).

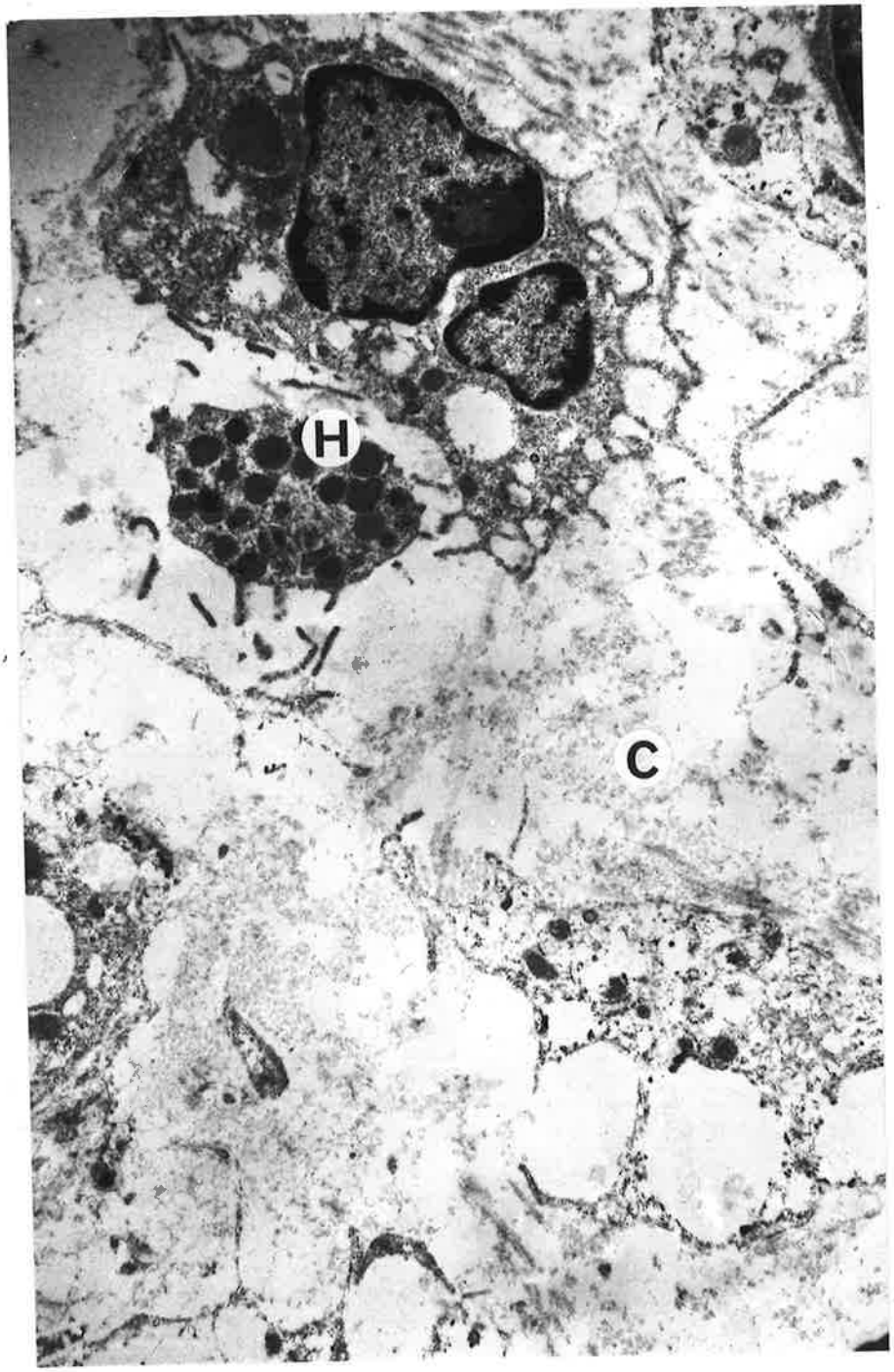


Fig. 27C. Detail of Tissue Supporting Alveolar Large Vessel Arrowed in Figure 27A.

Collagen fibres (C), macrophage (H).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,500X).

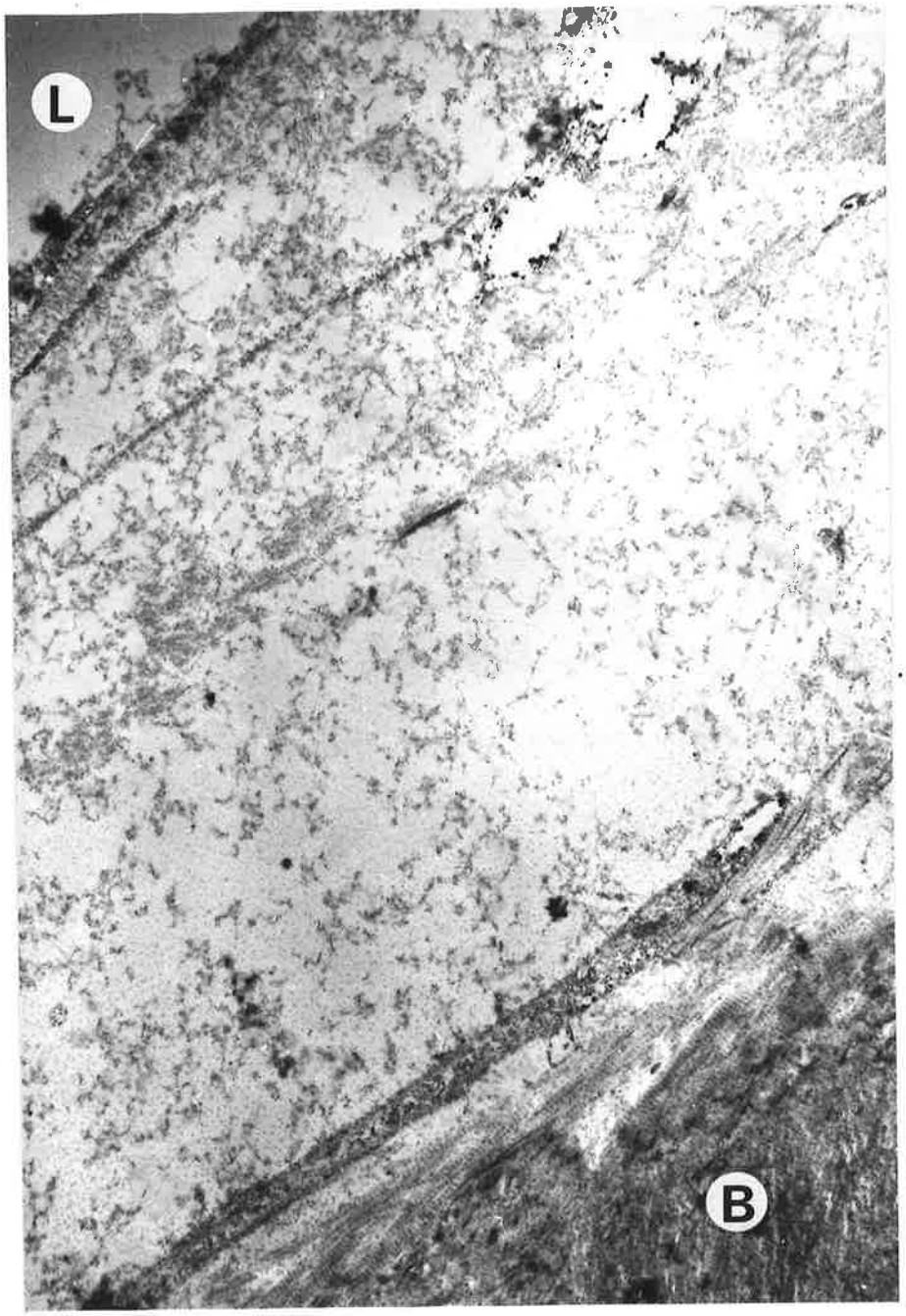


Fig. 27D. Detail of Tissue Supporting Alveolar Large Vessel Arrowed in Figure 27A.

Alveolar bone (B), vessel (L).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(2,500X).

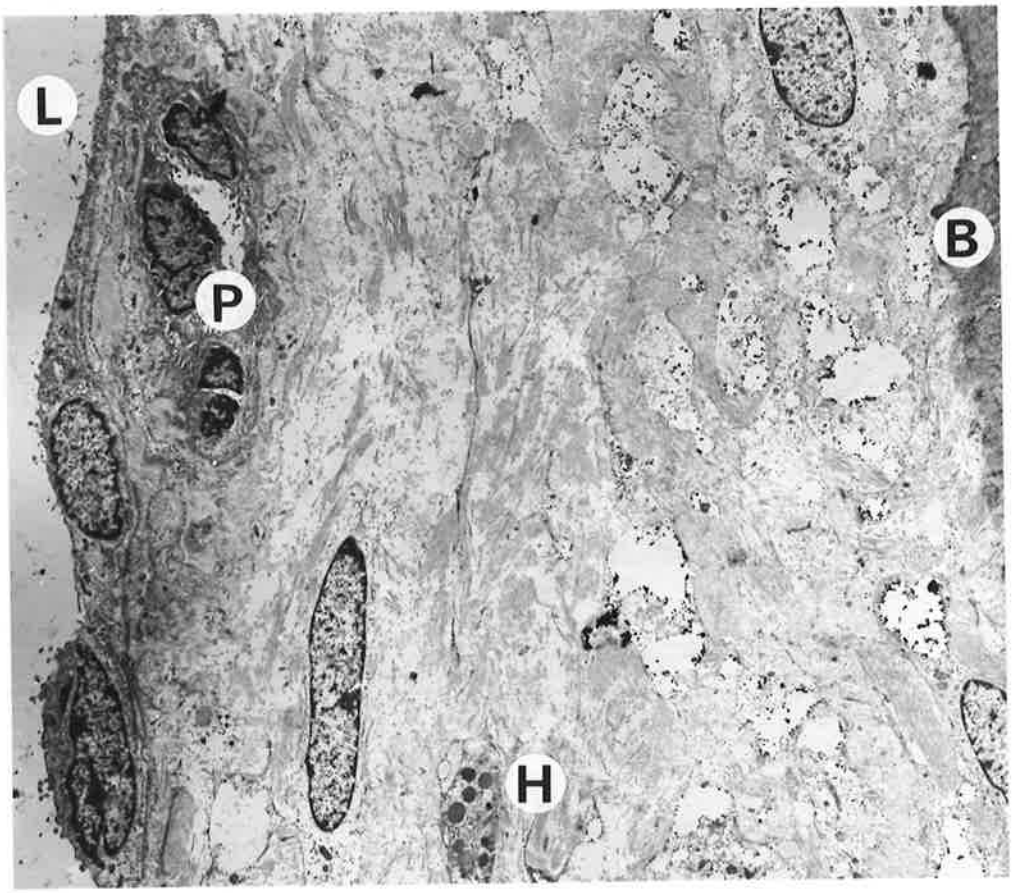


Fig. 27E. Detail of Tissue Supporting Alveolar Large Vessel Arrowed in Figure 27A.

Alveolar bone (B), alveolar large vessel (L), pericytic venule (P), macrophage (H).

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,500X).

Fig. 28A. Alveolar Bone Showing Relationship Between Osteocyte, Canaliculi, and Endosteal Cell:

Ultramicrograph. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(1,500X).

Fig. 28B. Detail of Figure 28A.
Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(3,000X).

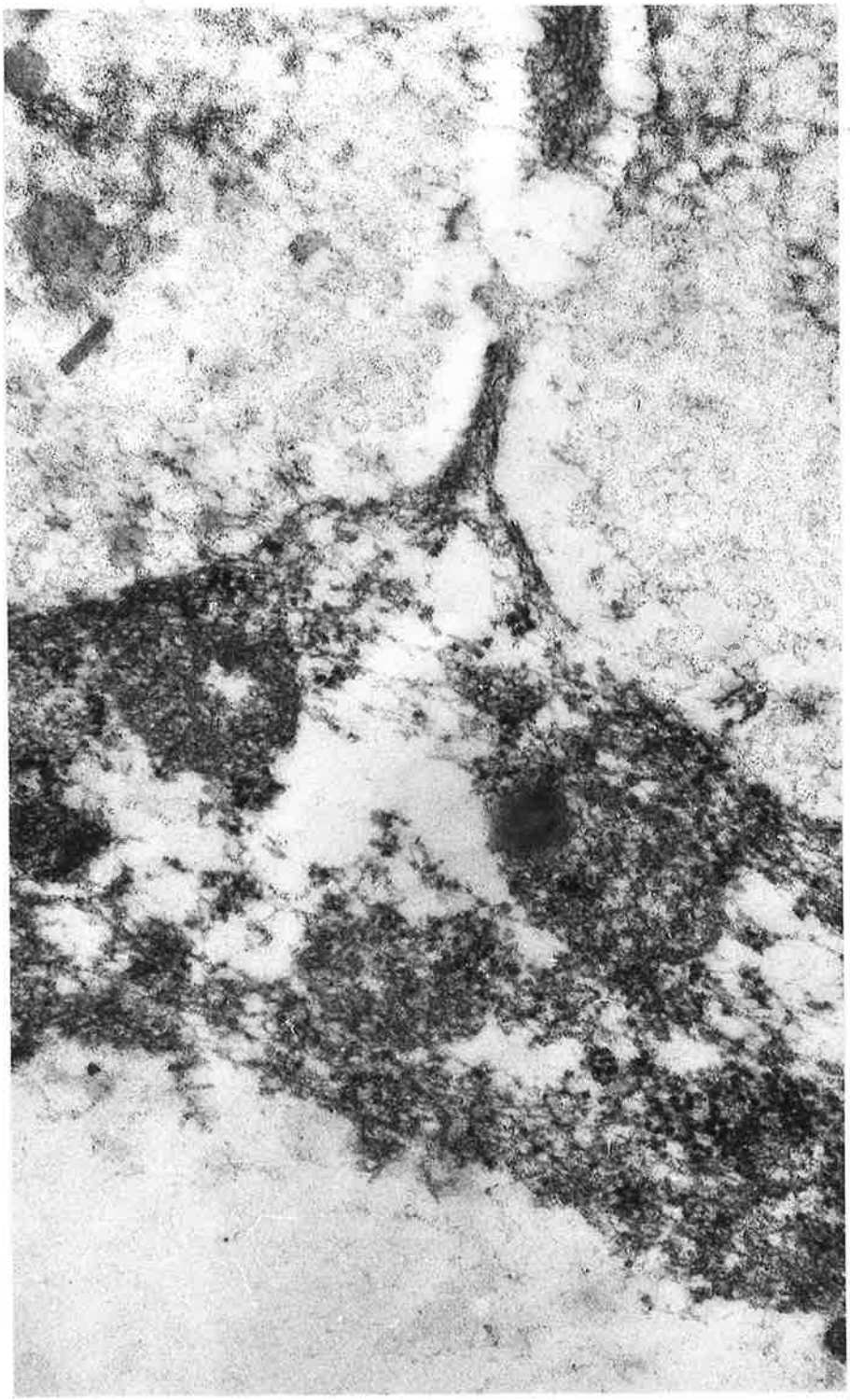


Fig. 28C. Detail of Figure 28B (Arrowed):

Ultramicrograph.	Osmium Tetroxide.
Uranyl Acetate.	Lead Citrate.
Uranyl Nitrate.	Decalcified.
	(10,000X).

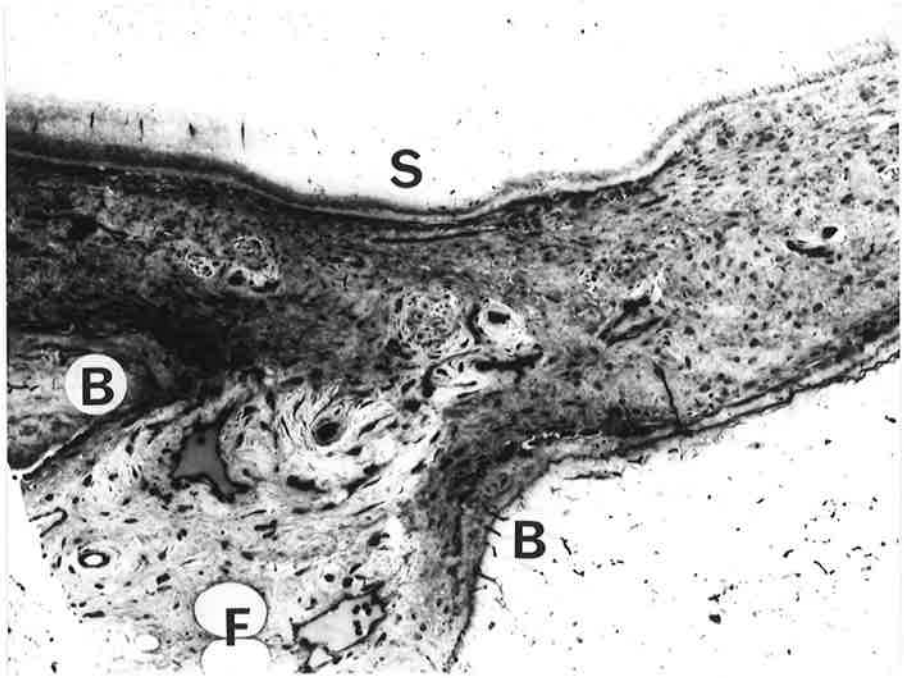


Fig. 29A. Foramen in Alveolar Wall of Tooth Socket:

Fat spaces (F), cemental pearl (S), foramen between alveolar bone (B,B).

Light micrograph. Toluidine Blue.
Decalcified. (150X).

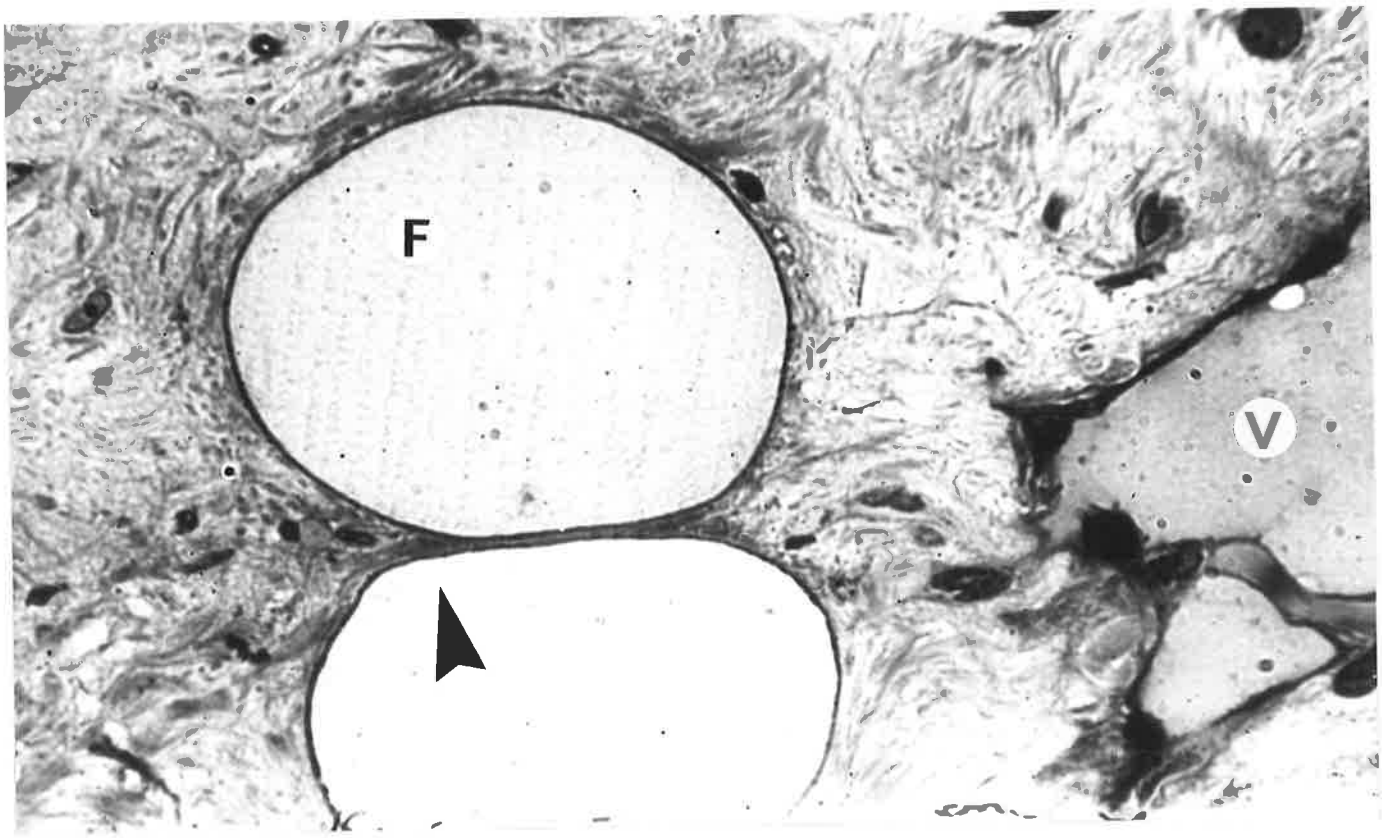


Fig. 29B. Detail of Fat Spaces in Figure 29A.

Large vessel (V), fat space (F).

Light micrograph. Toluidine Blue.
Decalcified. (1,000X).

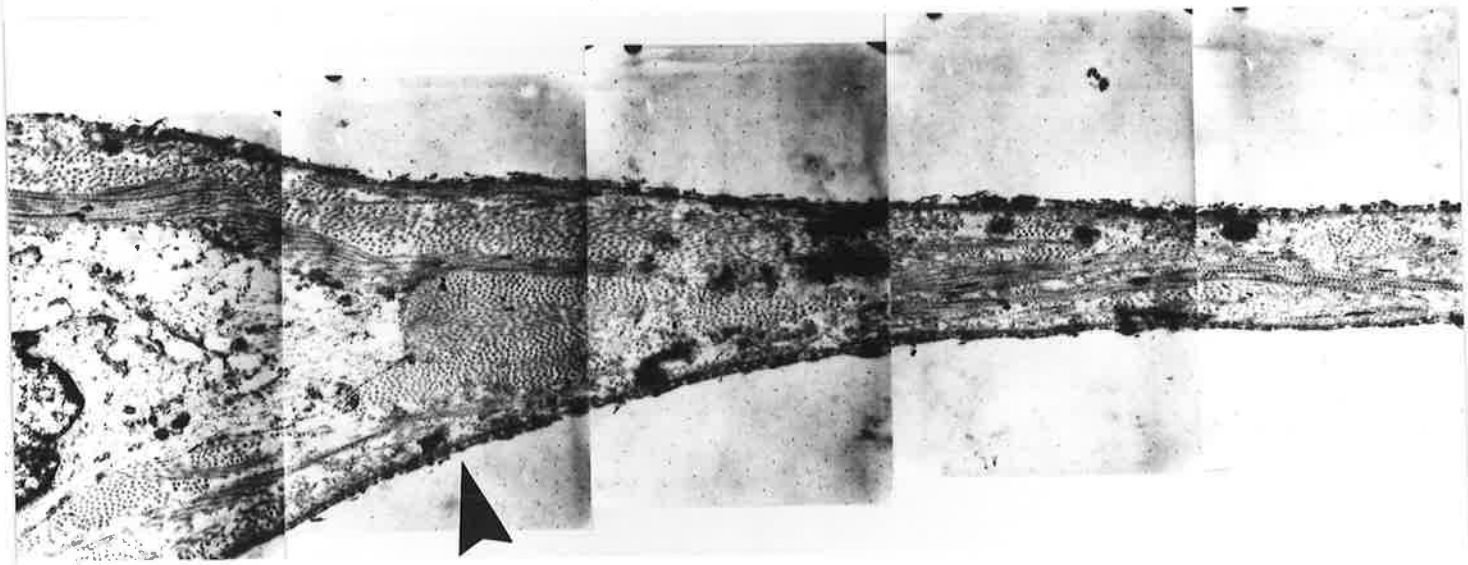


Fig. 29C. Detail of Arrowed Wall Between Fat Spaces in Figure 29B.

Ultramicrograph Montage. Osmium Tetroxide.
Uranyl Acetate. Lead Citrate.
Uranyl Nitrate. Decalcified.
(5,000X).

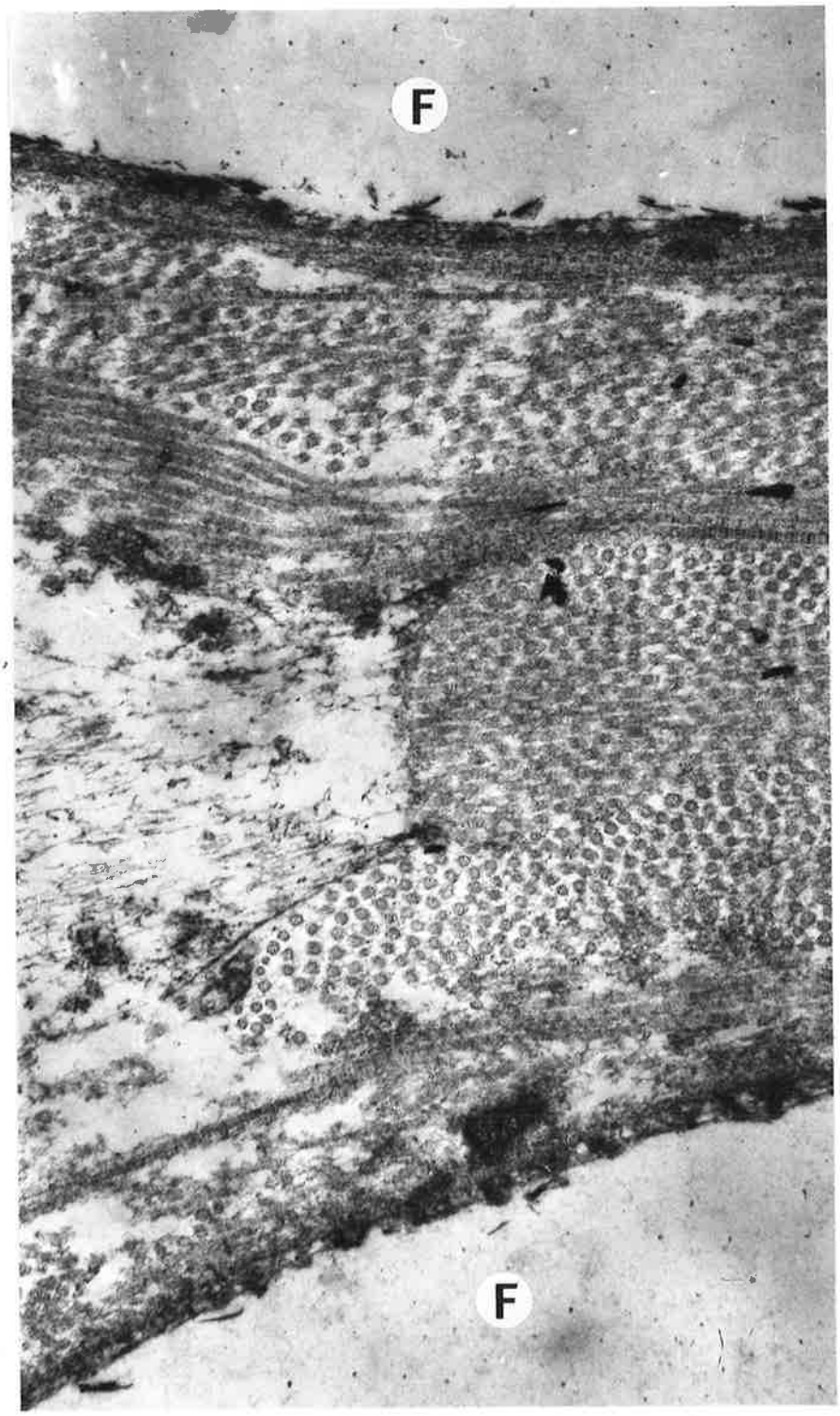


Fig. 29D. Detail of Arrowed Wall Between Fat Spaces in Figure 29C.

Fat (F).

Ultramicrograph. Osmium Tetroxide.

Uranyl Acetate. Lead Citrate.

Uranyl Nitrate. Decalcified.

(10,000X).

SECTION 6.

DISCUSSION.

(i) IDENTITY OF PERIODONTAL LARGE VESSELS.

1. Veins:

If Kindlova and Matena (1962) were correct, periodontal large vessels are veins which drain from the periodontal ligament to a "central septal venous plexus". Cliff (1976) stated that veins "have a low ratio of wall thickness to lumen diameter"; thus, like periodontal large vessels, veins have very thin walls. Elastic tissue, although meagre, exists in venous walls, "the surprising strength (of which) is due to their well developed collagenous components" (Cliff 1976).

Hence, unless the structure of periodontal veins has been greatly modified to accommodate to function within a special infrabony environment, the periodontal large vessels would appear not to be veins.

2. Initial Lymphatics:

The reported alveolar large vessels are of the same appearance as the aortic lymphatics

pictured on page 131 of Cliff's (1976) textbook, "Blood Vessels". In this illustration, a large fat globule is situated extravascularly to within a few microns of the lymphatic, which has a "wide lumen" and "tenuous endothelium". Cliff, in his book, reaffirms the statement of Yoffey and Courtice (1970) that "lymphatics of blood vessel walls" ... hence, lymphatics of the type illustrated on page 131 ... "conform in appearance to lymphatic vessels elsewhere in the body". In the same tract, Cliff advises that the ultramicrographs of Jellinek, Varess, Balint and Nagy (1970) exemplify fine structural criteria used to identify lymphatics. These ultramicrographs are similar, in all respects, to the reported fine structure of periodontal large vessels; very thin endothelial cytoplasm is shown, having no apparent basement membrane, the illustrated junctions are similar, lipid filled endothelial vesicles are identified, collagenous fibrils insert into the endothelial lining, the lumens contain particulate matter and intravascular lipid droplets, and macrophages loaded with lipid droplets approximate to the vessels' walls.

Casley-Smith (1979) has adopted the same ultrastructural criteria for identifying initial

lymphatics, adding that they tend to form "two dimensional plexuses" and are usually about 0.5mm long and 75μm wide. He has postulated a functional role for the inserting fibrils which are characteristic of initial lymphatics, the fibrils themselves being from 5nm to 10nm in diameter. The wider fibrils connect particular parts of lymphatic junctions to the general collagen constituent of the interstitial tissue (Casley-Smith and Florey 1961, Leak and Burke 1966), and the ultrastructure of lymphatic junctions may be so poorly defined as to make their identification impossible (Dobbins and Rollins 1970). Casley-Smith has claimed that initial lymphatics contain fewer erythrocytes than blood vessels and that, when fixed in certain stages of the lymphatic cycle, these vessels may contain a higher concentration of protein than do pericytic venules. The endothelial walls are irregular in outline, and are very thin in relation to the vessels' diameters.

The ultrastructural evidence published by Jellinek et al (1970) satisfied both the authors of the paper, and Cliff (1976), who quoted the article, that the illustrated aortal vessels were, indeed, lymphatics.

However, although previous workers had identified the presence of lymphatics in the walls of rat abdominal aortae by means of India ink, no such confirmation was made in the quoted article. Thus, Jellinek et al and Cliff accepted the lymphatic identity of the micrographed vessels on the evidence of fine structure alone.

Here, there is conflict. On the one hand, Cliff (1980) has verbally stated to the present author that tracer studies, together with ultrastructural evidence, are essential for confirming the identity of a lymphatic; this seems to be reasonable, practical advice. However, on the other hand, by quoting the Jellinek, article he has accepted fine structure as being evidence enough for such an identification.

There is further conflict. If, ultrastructurally, periodontal large vessels appear to be lymphatic, there is no published precedent for the existence of initial lymphatic vessels in the bones of any animal (Yoffey and Courtice 1970, Vaughan 1975, Deysine 1980 personal communication). This, despite the use of radio-opaque dyes, tracers and ultrastructural

studies of long bones. No experiments of this type appear to have yet been carried out on alveolar bone, however.

3. Conclusions:

The periodontal large vessels cannot be unconditionally identified. Ultrastructurally, based on published criteria of Jellinek et al (1970), Cliff (1976) and Casley-Smith (1979), the large vessels are initial lymphatics. Indeed, by directly viewing the specimens in the TEM, Casley-Smith has 'confirmed' this identification. However, ultimate identification must await experimental proof using tracers which are selectively taken up by lymphatics. It is possible that the vessels might be veins, modified from the basic venous structure to accommodate to their encasement within alveolar bone.

If, by tracer studies, periodontal large vessels are eventually identified as initial lymphatics, their presence will be the first reported evidence that lymphatic vessels exist in bones.

(ii) CLASSIFICATION OF TRABECULAR INTERSTITIAL TISSUE.

1. Sinusoids:

Interstitial tissue supporting alveolar large vessels and fat spaces was reported to be 'flimsy' in character, and to contain occasional erythrocytes. It appears that this tissue is sinusoidal in nature.

In man and mammals, adipose tissue is the main component of yellow bone marrow where haematopoiesis is almost non-existent (Vague and Fenasse 1965). Certainly, within the trabecular spaces of alveolar bone studied in this report, there was no evidence of haematopoiesis. All erythrocytes were mature, and there was no sign of leucopoiesis.

Beard and Beard (1927), Majno (1965) and De Bruyn, Breen and Thomas (1970) described sinusoids in mammalian long bone marrow to have the reticulo-endothelial capacity of avidly taking up large particles from the blood stream. Williams (1951) described splenic sinusoids as "multilocular labyrinthine channels" which were extremely labile structures continuously forming and disappearing.

There is doubt as to the exact nature of the wall of sinusoids; some, which have intact identifiable walls, communicate with those which have no identifiable walls. Williams considered that, where walls were intact, they probably consisted of endothelium. Bennett, Luft and Hampton (1959) and Majno (1965) described the walls as "endothelial linings characterised by endothelial gaps". McCuskey (1968) demonstrated in foetal liver that erythrocytes could penetrate sinusoidal spaces from haematopoietic tissue, and this has been confirmed by Weiss (1960) in ultrastructural studies on the marrow of rabbit long bones. Weiss (1960) has also shown the existence in myeloid tissue of large fat spaces associated with the sinusoids.

There appear to be no published studies of loose trabecular tissue in alveolar bone.

Consequently, it appears that the extravascular fat-filled spaces and the flimsy, loose stroma of alveolar trabeculae might represent vestigial haematopoietic tissue through the sinusoids of which erythrocytes and other vascular components can, apparently, circulate.

The reported 'flimsy' nature of this type of tissue was described by Majno (1965) to consist of "vessels of such labile construction (that they) could not exist elsewhere but inside the rigid casing of a bone". Zamboni and Pease (1961) described them thus: "the marrow sinusoids stand almost alone in the simplicity of their organizational pattern".

2. Conclusions:

Alveolar medullary interstitial tissue consists of a poorly fibrous loose network of spaces, some of which contain large globules of fat, others containing both mature erythrocytes and lipid containing macrophages. It appears that, by virtue of the protective casing of alveolar bone, this tissue gives adequate support to alveolar large vessels. The tissue might represent vestigial sinusoids of inactive haematopoietic tissue.

(iii) SIGNIFICANCE OF VASCULAR DISTRIBUTION.

Periodontal specimens, reviewed in this report, were taken from a restricted area of each subject. Consequently, it is probable that the described histology and ultrastructure of the reviewed

periodontal tissues is not representative for the alveolar bone and ligaments of all teeth.

The following discussion is qualified by this major consideration.

Furthermore, future LM and TEM studies of human periodontium should be made on large blocks of alveolar bone with teeth in situ. However, block removal of such large pieces of tissue from living humans is a rarely performed oral surgical procedure.

It is possible that the radiographically obvious trabecular spaces throughout alveolar bone might be filled with large vessels. If this is so, the volume and surface area of these vessels must be very great.

1. Periodontal Microcirculation:

All small vessels of ligament and alveolar bone were pericytic venules. The large vessels were either veins or initial lymphatics (or they served both functions, if that is at all possible). No vessels, either small or large, were identified as being on the arterial side of the circulation. It is therefore probable that arterial vessels were

not included in the biopsy sites.

Pericytic venules are exchange vessels on the venous limb of the circulation (Casley-Smith 1979). Within the ligament, pericytic venules proximal to foramina were reported to be 'encircled' by the ligamentous limbs of large vessels. It seems reasonable to conjecture that net extravasation of fluid, protein and particles from the venules may be taken up by the ligamentous large vessel extensions and transported to the trabecular spaces. It is further speculated that, whereas flow within ligamentous vessels might be severely interrupted by masticatory forces, interstitial tissue pressure variations acting upon the walls of alveolar vessels might be slight. Thus, the term 'spongiosa' might be appropriate to describe the function of the trabecular spaces, the sinusoids and large vessels acting as a 'sponge' to continually absorb fluid, protein and cells from the ligament, eventually to return them to the systemic circulation. Such a role is played by lymphatics (Casley-Smith 1979) and, whatever their identity, large vessels might similarly function to conserve protein and fluid, thus preventing periodontal oedema.

The 'vasa vasorum' might not only nourish large vessels and the surrounding tissues but they might also resorb some of the extravasated constituents from these vessels.

Whether fluid in pericytic venules flows from ligament to alveolar bone, or vice versa, cannot be conjectured upon. However, in either case, a countercurrent mechanism might act, similar to that which operates in the kidney.

It is possible that, when masticatory forces are applied to the teeth, flow within the pericytic venules might be reversed. It is also possible that material extravasated into large vessels during occlusion might be resorbed from large vessel extensions within the ligament during release of masticatory pressure.

In this report, it has been shown that ligamentous pericytic venules run in columns of loose connective tissue, and that the foramina are filled with similarly loose tissue which is contiguous with the sinusoids of the trabecular spaces. The ligamentous extensions of the large vessels appear to

'cross link' the vertically running columns. Thus, from this evidence, it is conjectured that extravasated material from parallel, longitudinally running pericytic venules might be 'funnelled' into foraminal large vessels by their ligamentous extensions.

A complicated study of tissue fluid pressure variations and flow patterns, during mastication, would be necessary to clarify these suggestions.

2. Medullary Canalicular Relationships:

The reported close association of alveolar large vessels, endosteal cells and canalicular processes mirrors the microanatomy of long bones, illustrated in Figure 1. Thus, it is probable, though undemonstrated, that macromolecules, ions and fluid contained within alveolar large vessels and sinusoids might influence the composition of bone tissue fluid and, hence, the activity of osteoblasts, osteoclasts and lacunal osteocytes.

Variation in masticatory or orthodontic forces acting upon a tooth would, conceivably,

alter the composition of fluid in the alveolar large vessels which, via foramina in the alveolar socket wall, drain the affected periodontal ligament. Similarly, such forces might change the interstitial tissue fluid pressure, hence, the vascular pressure of the trabecular spaces.

Consequently, it is conjectured that this model offers, in addition to Bassett's proposed piezo electric effects (1965), structural evidence for masticatory and orthodontic forces to effect a direct influence on the remodelling elements of alveolar bone through changes in microvascular physiology.

3. Periodontal Infection:

In this dissertation, direct communication was reported to exist between ligament and overlying soft tissues, by way of large periodontal vessels. Such a low resistance path for the spread of pulpal and periodontal infections would explain the high incidence of parulis from these sources.

To the layman, the classical sign of a dento-alveolar infection is a swollen cheek.

Indeed, dental infections, non infectious traumatic occlusions and alveolar surgical interventions often cause not only 'cellulitis' of the overlying cheek but also pain in adjacent muscles as well as enlargement of local lymph nodes. The former signs and symptoms may be localised neurogenic manifestations of the inflammatory triple response. However, to this might now be added the inflammatory effects of direct transalveolar transport, to soft tissues, of inflammatory hormones from the infrabony site of injury.

Furthermore, if periodontal large vessels are indeed lymphatics, the possibility exists that their functional disruption might result in localised lymphadenopathy, upon which might be superimposed the signs and symptoms of inflammation.

4. Intra-osseous Local Anaesthesia:

The technique of intra-osseous anaesthesia involves gaining access to trabecular spaces via a hole in the buccal or labial plate of the alveolar process. Local anaesthetic solution is then deposited as near as possible to the apex of the tooth to be anaesthetised.

Although the amount of solution necessary to achieve dental anaesthesia is less than that usually required by the infiltration technique (Gilchrist 1980, personal communication), it is probable that the intraosseously deposited solutions are injected intravascularly.

(iv) VALUE OF A SYSTEMATIC APPROACH TO THE STUDY OF PERIODONTAL STRUCTURE.

Given their great size, it is difficult to understand the reasons that periodontal large vessels have not previously been studied. Perhaps dental histologists have not been very interested in periodontal vessels.

More significantly, periodontal large vessels may not have been clearly seen. TEM studies of periodontium are rare. However, Gilchrist (1979) failed to describe the structure and distribution of large vessels in his analysis of tissue biopsied from the same area of human subjects. In the present author's opinion, three factors have contributed to his description of large vessels:

1. The use of TEM resin embedding procedures preserved the fine structure of endothelial cells and sinusoids, enabling thin (1µm) sections to be taken for LM, and ultrathin

(50nm) sections for TEM. It appears that this technique has enabled description of large vessel histology and cytology.

By contrast with this, most previous workers have used harsh paraffin embedding techniques known to result in tissue deterioration obvious at the fine structural level. Moreover, paraffin embedded sections are usually about 8μm thick. Often, in such sections, the present author has found that periodontal vessels are obscured, only scattered erythrocytes being visible within trabecular spaces.

Hence, the use of thin, resin embedded sections has revealed the presence of all periodontal vessels.

2. In the present study, the biopsied specimens were serially sectioned at intervals of 1μm. Each section was studied by LM. Accordingly, where a structure of special interest was observed at a certain level of the block face, it was ultramicrotomed, and its fine structure determined. Consequently, by using this approach, no detail was lost to the observer.

This necessary procedure was very time-consuming. For this reason, previous workers

might have avoided the technique.

3. Periodontal large vessels might not be present in animals other than humans. Most histological studies are of animal periodontal tissue.

   For this reason, large vessels might not have been seen in the past.

(v) SUMMARY.

Based upon a systematic LM and TEM study of blood vessels, in a limited segment of human periodontal tissue -

1. the basis for a functional microcirculation within human periodontium has been proposed; and

2. some clinical applications of the reported findings have been suggested.

SECTION 7.

CONCLUSIONS.

These conclusions are based on the reported structure and distribution of tissues within periodontium biopsied from a restricted area. However, the children were in their early teens, their extracted teeth and associated periodontal tissue being healthy, and in functional occlusion.

1. The periodontal ligament is metabolically highly active (Ten Cate 1976). Based upon the present report, if pericytic venules alone can nourish the buccal ligaments of functional premolars, these same vessels, though fewer in number, appear to be adequate (together with venous or lymphatic large vessels) to satisfy the nutrient requirements of functional alveolar bone.

2. Anatomically, the periodontal ligament, alveolar bone and overlying gum tissues are closely interconnected by vessels and connective tissue, which communicate via foramina. Hence, the periodontal circulation cannot be considered separately from that of the alveolar bone. Consequently, it is concluded that ligamentous function is inseparable from that of alveolar bone. Furthermore, the

overlying buccal soft tissues might influence periodontal behaviour and, conversely, might be affected by it.

Orthodontic forces and changed occlusal patterns can modify the structure of tooth, bone and ligament (Langford 1980). It is considered that these responses should be analysed in the light of the present findings.

3. Because of doubt as to their nature, the clumsy term 'periodontal large vessels' has been used throughout this dissertation. Further study is needed to determine their identity. However, the cited ultrastructural evidence points to the vessels being 'initial lymphatics', the most peripheral vessels of the lymphatic system.

4. In addition to large vessels, trabecular spaces contain tissue of a basically sinusoidal structure which contains numerous large fat deposits. This tissue is characteristic of vestigial haematopoietic tissue.

5. As is described in this dissertation, a systematic approach using LM and TEM is essential for determining periodontal structure.

6. No hypothesis of periodontal function should be proposed without a further carefully conducted qualitative and quantitative analysis of the tissue.

SECTION 8.

FURTHER SUGGESTED RESEARCH.

1. A stereologic and morphometric analysis (Barker 1979) should be made of healthy, functional periodontal samples biopsied at random from different sites in human beings. This will enable a sound functional analysis to be made of human periodontium. Moreover, the baseline data so obtained can eventually be used to assess, by similar techniques, the periodontal effects of tooth succession, ageing, disease and orthodontic tooth movement.

2. Tracers, selectively taken up by lymphatics, should be injected into the periodontium of a primate. This will be necessary to determine whether periodontal large vessels are, indeed, initial lymphatics.

3. A meal (for example, milk or cream) high in fat content should be given to a subject prior to biopsy of periodontal tissue. Using this 'auto-injection' technique, quantitative analysis of trabecular fat might assist determination of regulating mechanisms controlling its deposition. From this information, a functional role might be ascribed to the fatty deposits.

4. Fat globules should be dissected from unfixed, non-decalcified, unstained frozen alveolar bone, sectioned with a sharp razor blade. The composition of the fat should then be analysed to determine its function.

5. Each of the previous proposals requires biopsy of a basic 'sandwich' of tooth, periodontal ligament and bone. For more information, a 'club' sandwich is suggested; in addition to the original periodontal biopsy, the buccal periosteum and attached soft tissue should be retained. Such a small sliver of gingival tissue would compromise neither flap replacement nor the well-being of the patient.

SECTION 9.

APPENDICES.

APPENDIX I - CLARK'S BUFFER.

Stock Solution A:

NaCl	14.00gm
KCl	0.75gm
$MgSO_4$ anh.	0.55gm
$Ca(NO_3)_2 4H_2O$	1.50gm

Mix with double-distilled water and make up 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 anh.	0.525gm
Phenol Red	0.01gm

Mix with double-distilled water and make up to 100ml.

50ml of Solution A was added to 50ml of Solution B, and the mixture was made up to 2,000ml with double-distilled water. The pH was adjusted to 7.4 at $4^{o}C$.

APPENDIX II - MODIFIED REYNOLDS' SOLUTION.

1.33gm Lead Nitrate $Pb(NO_3)_2$

1.76gm Sodium Citrate $Na_3(C_6H_5O_7)\ 2H_2O$

30ml Double-distilled H_2O

Shake vigorously for 1min, then intermittently for 30min. To this solution add 8ml of IN Sodium Hydroxide, and dilute with double-distilled water to 50ml.

Filter this solution on to paraffin paper through Whatman 40 filter paper. The solution should be used within one week of its preparation.

SECTION 10.

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