

## Lymphatic Vessels in Human Alveolar Bone

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### Summary

There are large vessels within the trabecular spaces of human alveolar bone buccal to the maxillary first premolars. In form and fine structure the vessels are collecting lymphatics which originate as initial lymphatics within the periodontal membrane and extend through the buccal plate of bone. By contrast, present evidence suggests that such vessels do not exist in long bones.

### Introduction

It is said that lymphatic vessels are not present in bone (8, 13, 15). Supporting this, *Hudson* and *Yoffey* (7) studied the fine structure of long bones but were unable to find evidence of lymph vessels. *Brahim* and *Osmond* (1, 2) showed in guinea pigs that selective tibial bone marrow labelling with  $^3\text{H}$ -thymidine was followed by transport to the popliteal lymph node of DNA-labelled lymphocytes; however, the method of transport was unable to be determined. Similarly, "intramedullary injections of radio-opaque material in the long bones of dogs will produce rapid imaging of the local lymph nodes . . . nevertheless the true route is still a mystery" (*Deysine* 1980, personal communication, supported by *Olszewski* and *Sawicki* (11)).

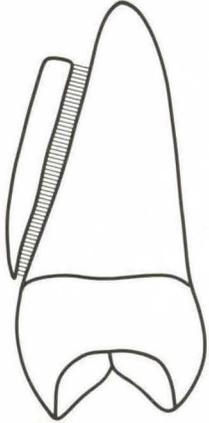
Texts of dental histology claim that lymphatic vessels are present in alveolar bone, the structure "containing the sockets of functional teeth, and the crypts of developing teeth" (14). Satisfactory evidence for the presence of these vessels is never given prompting *Mjör* and *Pindborg* (10) to state, "the lymph drainage, although inadequately known, presumably follows the path of the blood vessels". However, to the present author's knowledge there is one

publication which to a certain degree supports the contention. Accordingly, *Ruben* et al. (12) back-perfused the large regional lymphatic collecting vessels for the mandibles of dogs to demonstrate that the infused carbon occupied large-vessel lumens in the trabecular spaces of the alveolar bone. These vessels were examined under the light microscope. Without stating identifying criteria, the vessels were thought by the authors to have the histological appearance of lymphatics communicating with the overlying buccal mucosa by means of bony Volkmann's canals and with the periodontal membrane by means of foramina in the walls of the tooth sockets.

To the present author, identification of lymph vessels is possible only by means of the transmission electron microscope. However, when it is considered in conjunction with the present report which describes the fine structure of similarly distributed human alveolar vessels, the canine experiment of *Ruben* et al. (12) is highly significant. Thus, in contrast to available published evidence on long bones, there are lymph vessels in human alveolar bone which it will be shown can be readily identified by transmission electron microscopy.

### Materials and Methods

A maxillary first premolar together with attached buccal bone and periodontal membrane was biopsied under local anaesthesia (Xylocaine, Astra Chemicals Ltd., North Ryde, NSW) from each of 6 children. The subjects were males and females ranging between 11 and 15 years in age. The teeth which were removed for orthodontic treatment purposes were healthy, in functional occlusion and had

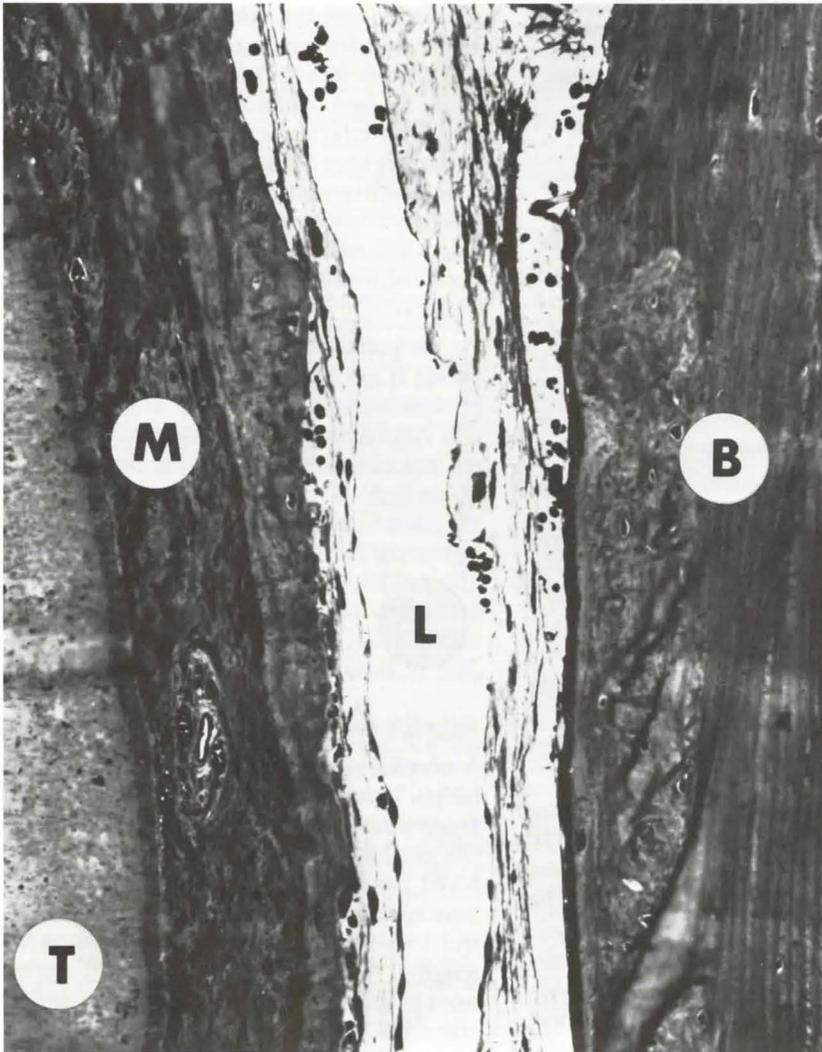


**Fig. 1** Sagittal aspect of biopsy. Cross-hatching represents the periodontal membrane with tooth on one side and attached buccal bone on the other

not been subjected to any previous orthodontic treatment procedure.

The cervical two-thirds of the root of each premolar was surgically removed together with a 1 mm to 2 mm wide sliver of intact buccally attached periodontal membrane cum buccal bone (Fig. 1).

Upon excision the intact specimens were washed briefly in physiological saline at room temperature, fixed in 4% glutaraldehyde at 4 °C and prepared for embedding in either Epon or Spurr's resins according to standard techniques (Ladd Research Laboratories, Burlington, Vermont). The intact composite spe-



**Fig. 2** Vessel (L) in alveolar trabecular space. Tooth (T), periodontal membrane (M), alveolar bone (B) LM 250 x

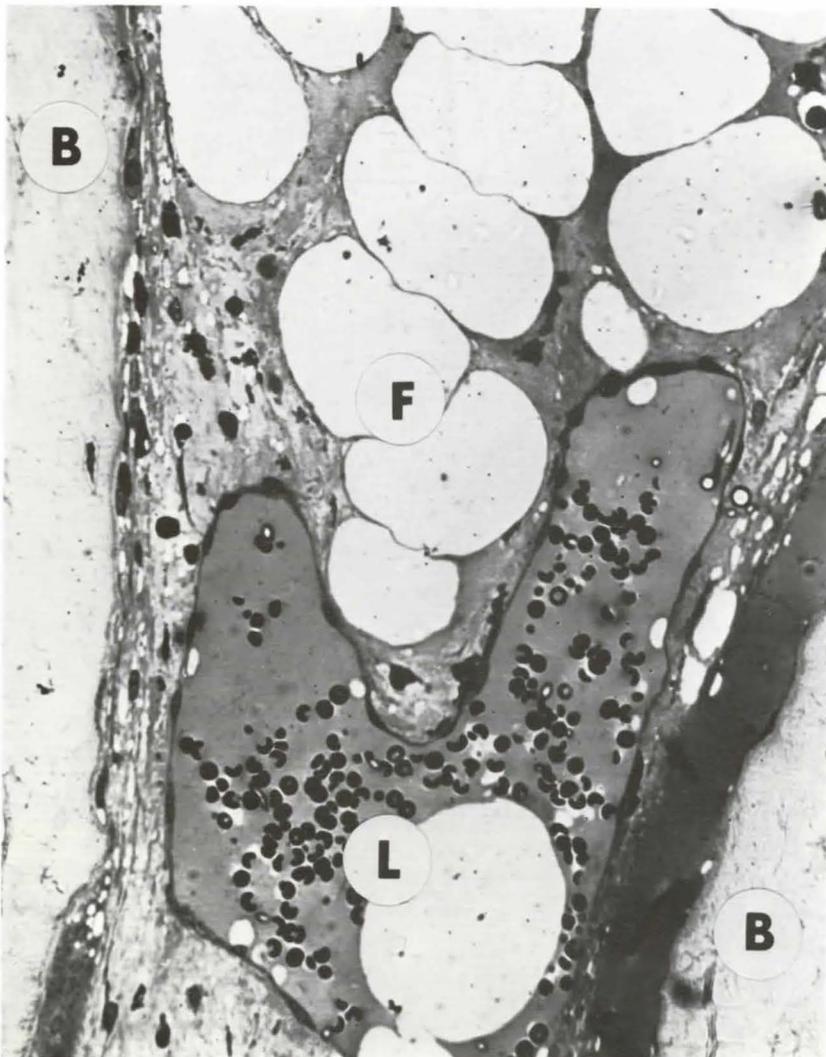
cimens of tooth, bone and periodontal membrane were decalcified in 0.1M buffered ethyldiaminetetraacetic-acid in 2.5% glutaraldehyde pH6 at 4 °C. Specimens were post-fixed in 2% osmium tetroxide at 4 °C for 1 hr, dehydrated in acetone and embedded in resin.

Each of the 6 blocks was serially sectioned horizontally through tooth, bone and periodontal membrane. Specimens 1  $\mu$ m thick were taken with a glass knife and stained with 1% toluidine blue for viewing under the light microscope. Vessels thus found in the block face were ultramicrotomed with a

diamond knife, the resulting silver sections being stained with 5% uranyl acetate followed by lead-staining with a modified *Reynolds'* solution (*Casley-Smith* 1980). These 60 nm-thick sections were examined in a Siemens Elmiscop I fitted with a Faraday cage. Sections viewed by light microscopy (Axiomat, Zeiss, West Germany) were photographed on high contrast film (Recordak, Kodak).

### Results

Within each trabecular space were large vessels. These were irregular in outline, often hundreds of microns long and tens of microns wide (Fig.2).



**Fig. 3** Large alveolar trabecular vessel. Lumen (L), fat cells (F), trabecular bone (B). LM 450 x

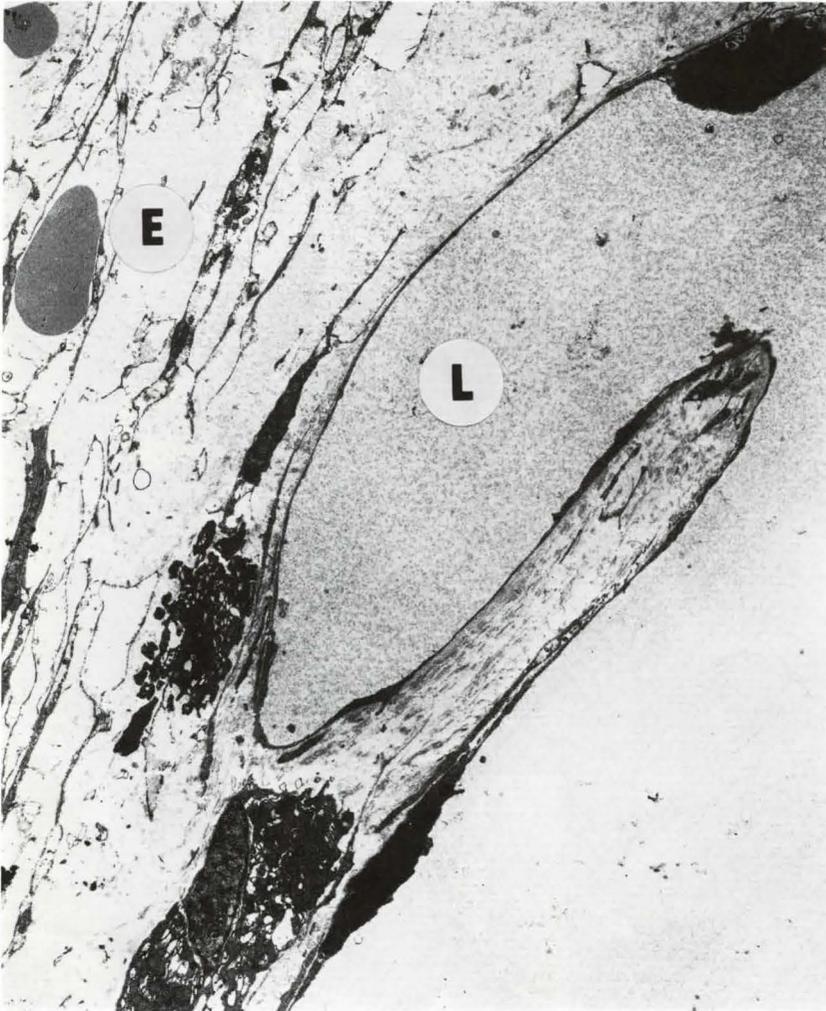
These vessels together with large fat cells occupied the greatest part of each trabecular space (Fig. 3).

The medullary connective tissue was flimsy in appearance, poorly fibrous and contained scattered erythrocytes (Fig. 4).

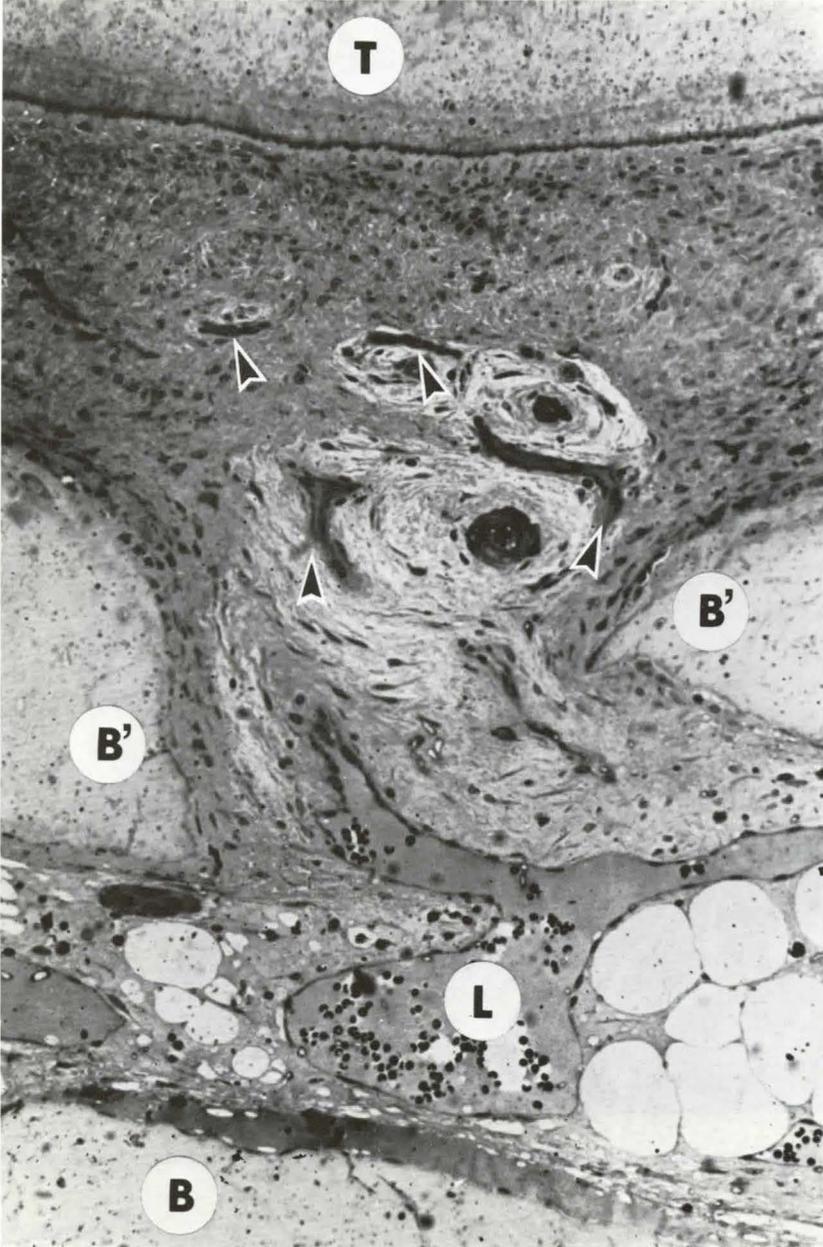
The large vessels ramified through foramina in the tooth socket walls (Fig. 5) to terminate in blind-ended vessels (Fig. 5, Fig. 6) in the periodontal membrane. Furthermore, the trabecular vessels communicated with the overlying buccal tissues through Volkmann's canals.

Only two types of vessel were present in the alveolar marrow spaces, large and very small. The latter, identified without exception as pericytic venules, were situated often to within a few microns of the walls of the large vessels (Fig. 7).

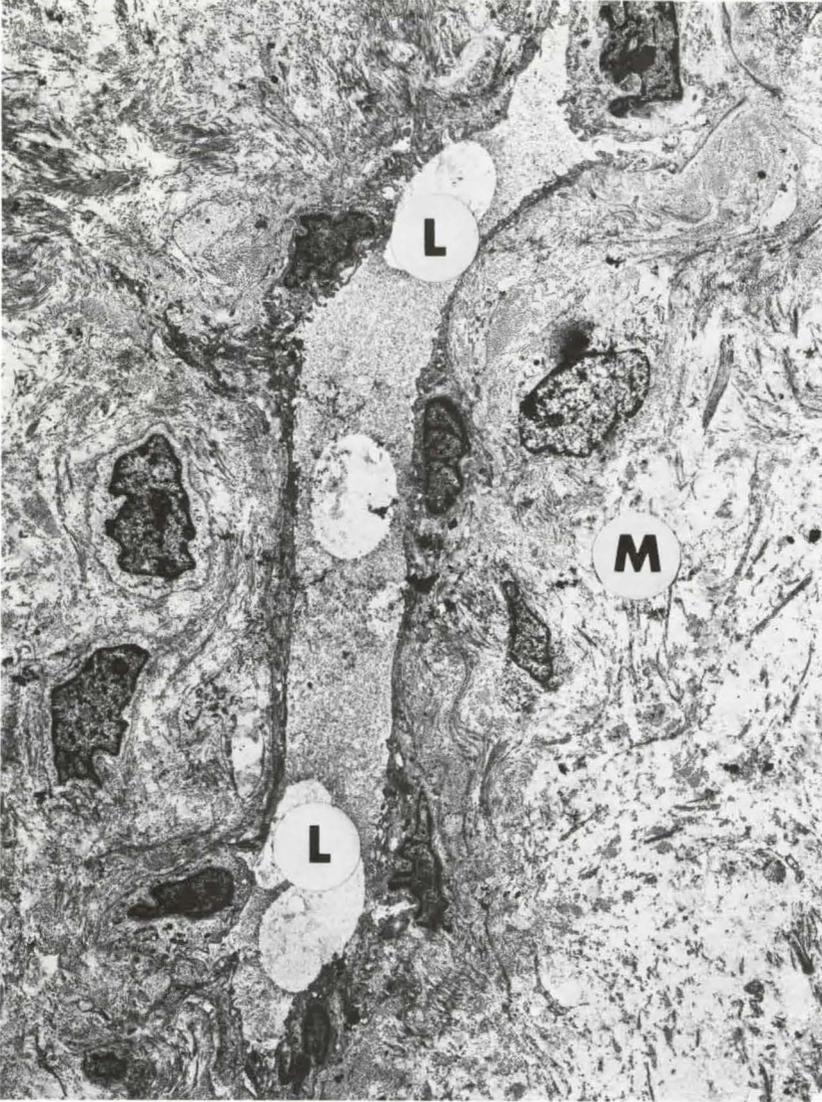
Based upon their ultrastructural appearance all trabecular large vessels and their extensions were classified as lymphatic according to the published criteria of *Casley-Smith* (3, 4) and *Cliff* (6). Thus, the walls of the large vessels consisted of a simple layer of endothelium often so thin that few vesicles or other inclusions were present (Fig. 8, Fig. 9, Fig. 10, Fig. 11, Fig. 12).



**Fig. 4** Loose trabecular connective tissue with contained erythrocytes (E). Large vessel lumen (L). EM 2 K.



**Fig. 5** Tooth socket foramen (B'B') in alveolar bone (B). Tooth (T). Trabecular vessel (L) with arrowed extensions to the periodontal membrane. LM 125x



**Fig. 6** Membranous extension of large alveolar trabecular vessel. Collagen fibres of peridontal membrane (M), vessel lumen (L). EM 1 K

Occasional fat vesicles were seen in the walls (Fig. 9). It was common to see fine fibrils or "anchoring filaments" (5, 9) inserting into the endothelium (Fig. 8, Fig. 10, Fig. 11, Fig. 12). Junctions were close, tight or open. There were no fenestrae and no definable adventitia. Erythrocytes were scattered through the lumens of the lymphatic vessels. Based

on size, shape and ultrastructure the small membranous vessels were classified as initial lymphatics and the large tortuous foraminal and trabecular vessels were identified as collecting lymphatics. Thus, pericytic venules closely related to the walls of trabecular collecting lymphatics might be designated as 'vasa lymphorum' (Fig. 7).

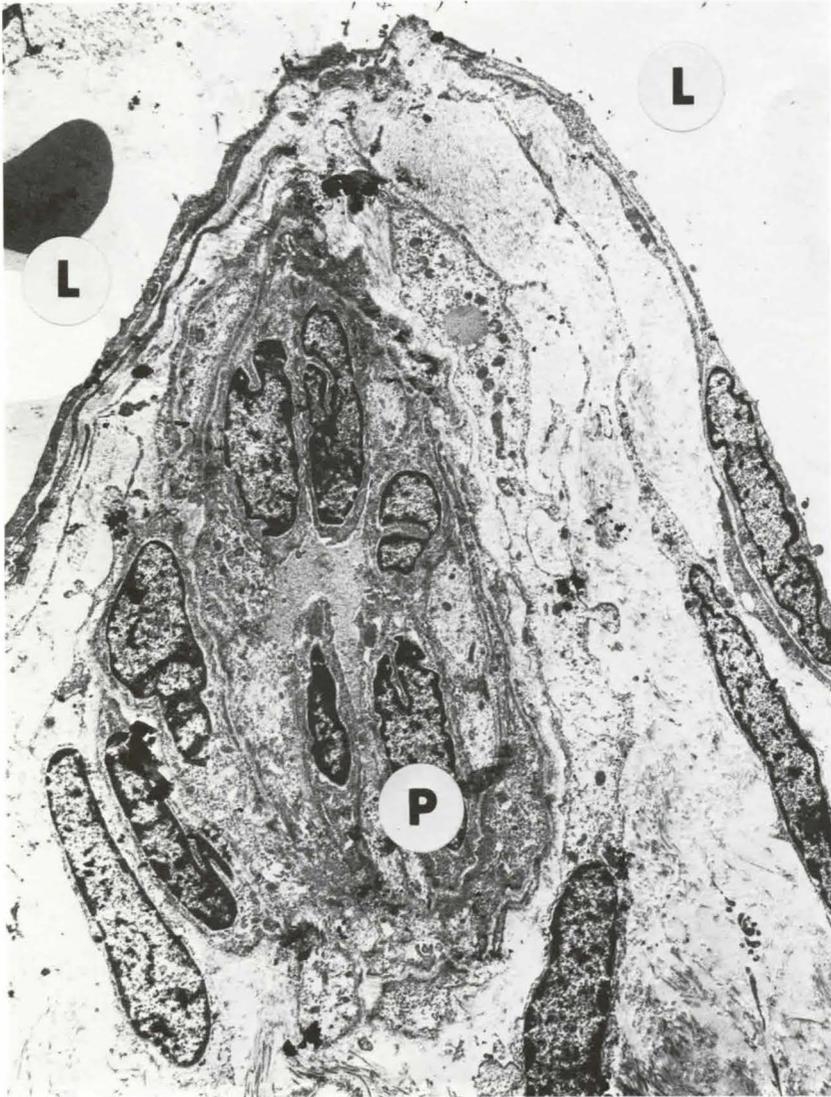


Fig. 7 Pericytic venule (P), lumen of trabecular vessel (L). EM 1 K

### Discussion

The canine alveolar and membranous vessels which were filled with carbon by retrograde perfusion through a major collecting lymphatic (12) have the same distribution and histological appearance as the large human alveolar vessels ultrastructurally defined as lymphatic, presently described. The combined informa-

tion is the basis for conclusively identifying as lymphatic the canine and human large trabecular vessels and their extensions.

It was often not possible to identify such vessels from single sections, however, fine structural identification was readily made when many sections of the same vessel were viewed (3, 4).



**Fig. 8** Fine structure of wall of trabecular large vessel. Lumen (L), endothelial cytoplasm (E), connecting filaments (C). EM 10 K

If, indeed, long bones contain no evidence of lymphatic vessels, why should alveolar bone be different? It may be a result of the presence of teeth which are embedded by means of a gomphosis joint within its substance. By contrast with long bones this situation is unique. The teeth act as levers to transmit masticatory forces to the heart of the alveo-

lus via the periodontal membrane. Thus, alveolar bone would appear to be a vehicle for lymphatic drainage of teeth and periodontal membranes. Furthermore, if each trabecular space throughout alveolar bone (Fig. 13) contains lymphatic vessels, the volume and surface area of the alveolar lymphatics must be very great.

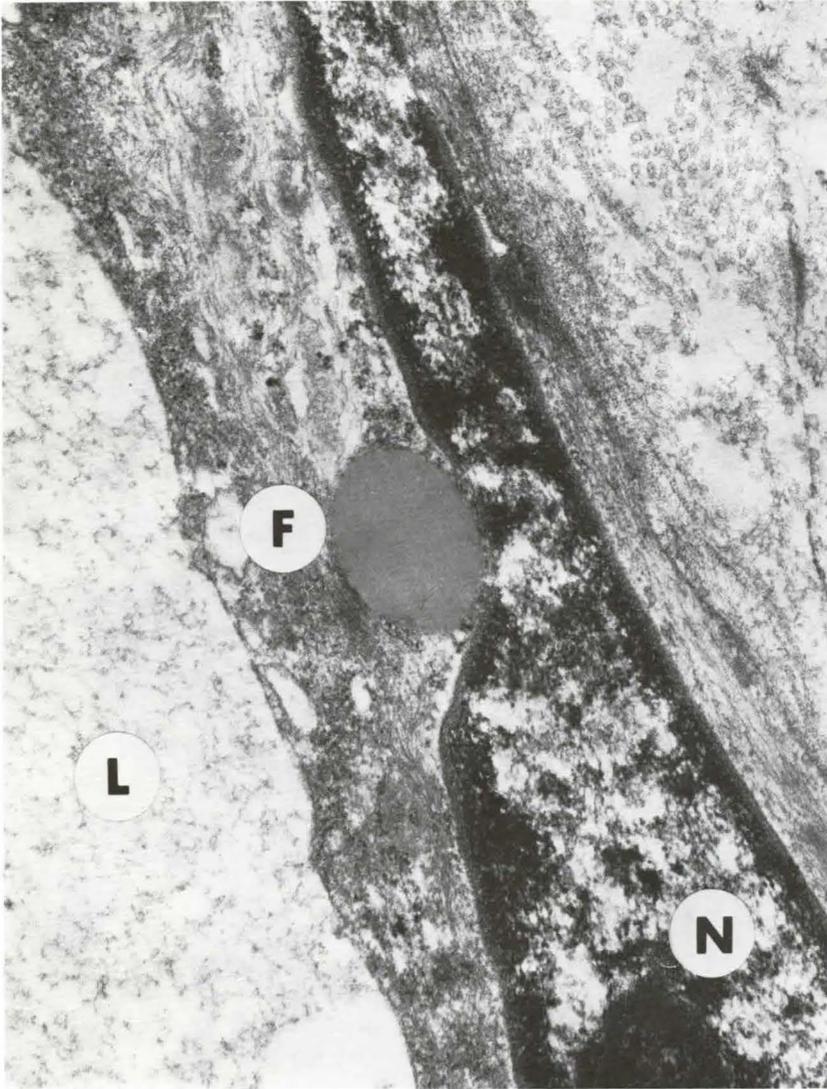
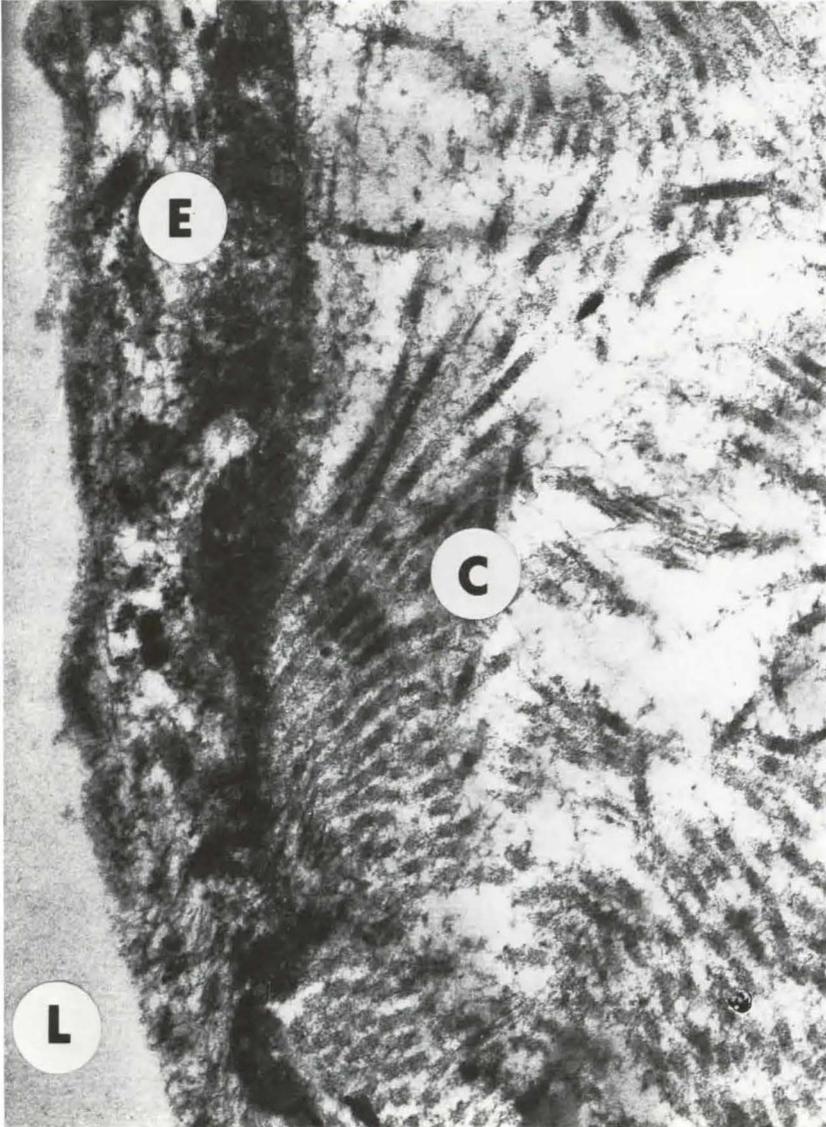


Fig. 9 Fine structure of wall of foraminal trabecular extension. Lumen (L), fast inclusion (F) in endothelial cytoplasm. Endothelial nucleus (N). EM 10 K

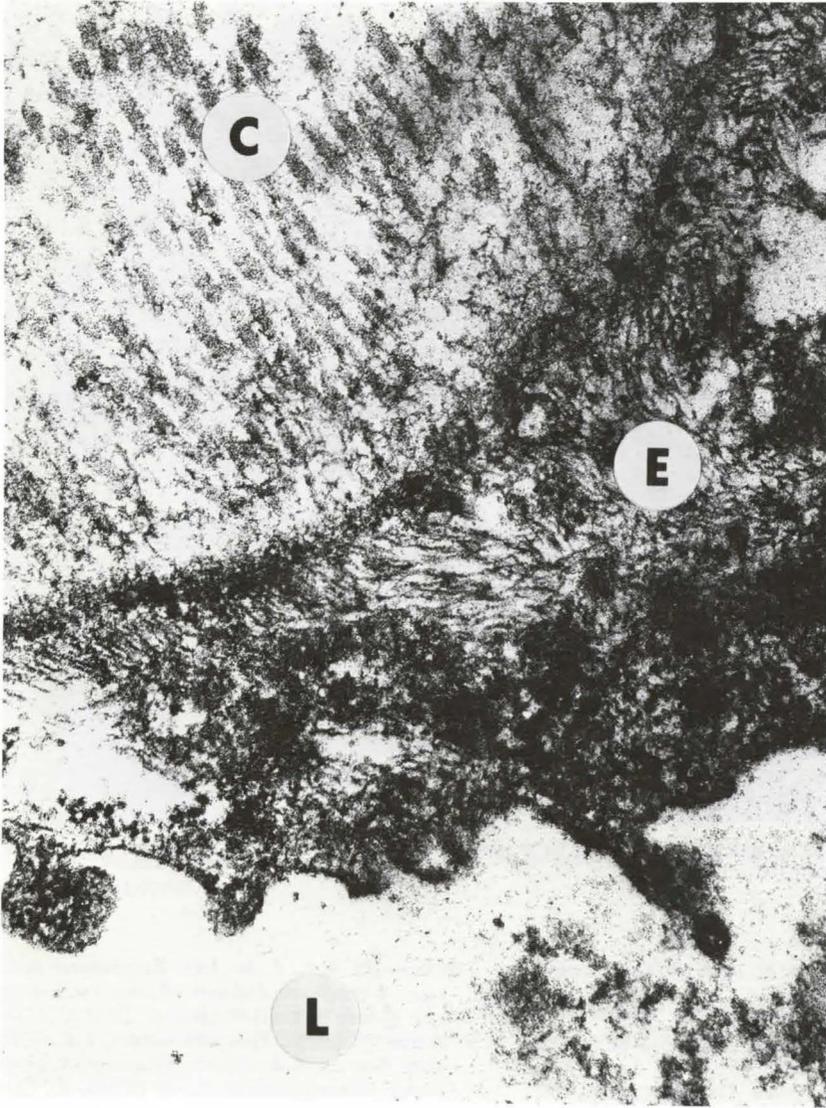
Studies are under way to determine the direction and nature of lymphatic flow through the trabecular spaces and Volkmann's canals in response to variations in the degree and direction of masticatory forces applied to the teeth.

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**Fig. 10** Fine structure of wall of trabecular large vessel. Lumen (L), endothelial cytoplasm (E), connecting fibrils (C), EM 10 K



**Fig. 11** Fine structure of wall of trabecular large vessel. Lumen (L), endothelial cytoplasm (E), connecting filaments (C). EM 19 K

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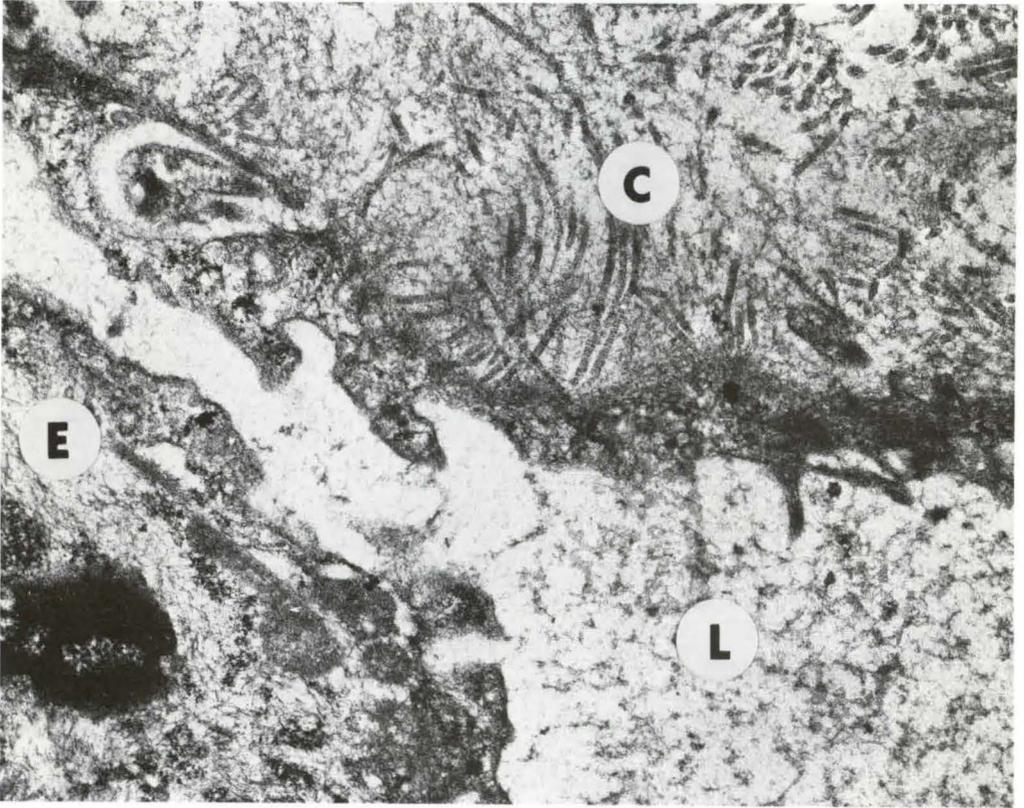
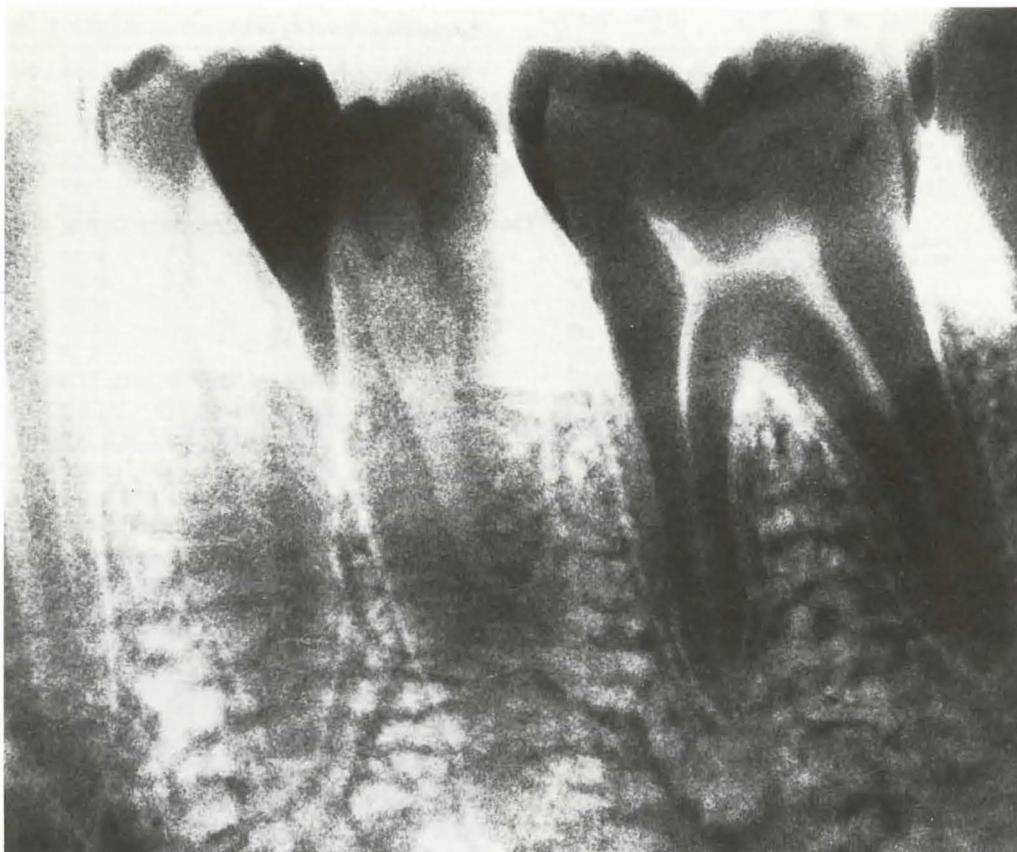


Fig. 12 Fine structure of wall of membranous large vessel. Lumen (L), endothelial cytoplasm (E), connecting filaments (C). EM 10 K

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**Fig. 13** Bucco-lingual radiograph of human mandibular teeth in alveolar bone indicating distribution of alveolar trabecular spaces

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