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Contribution to the Microscopic Appearance of Ultracapillaries — Reticular Fibers.

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It has long been known (KUPPFER, VOLTERRA, CLARA, NAGEL, HUZELLA, and others) that the types of connective tissue fibers known as Gitterfasern, argyrophil fibers, precollagen fibers, and now generally termed reticular fibers, are anatomically related with the circulatory system. These fibers have their source above all in the adventitia of the capillaries.

In earlier papers I advocated that reticular fibers are pertinent to the circulatory system, not only anatomically but also physiologically, that they conduct the blood plasma to the paracapillary tissue, and that they are thus ultracapillaries. I based my conclusion on the results of experiments by which I showed that it is possible to fill the reticular fibers with colloidal solutions, injected into one of the main arteries.

As is known, the reticular fibers are hardly visible in ordinary stained sections and become manifest only if impregnated successfully with silver, when they are seen as closely packed black or gray formations. In the hard dental tissues the large ultracapillary trunks, enamel tufts, and Neumann's sheaths, exhibit a distinct lumen when stained with hematoxylin, especially with Mallory's fibrin staining.

The purpose of the present study was to find out whether the electronmicroscopic picture of reticular fibers might reveal evidence to confirm or disprove the existence of lumina in reticular fibers, no attempt being made to investigate the finer

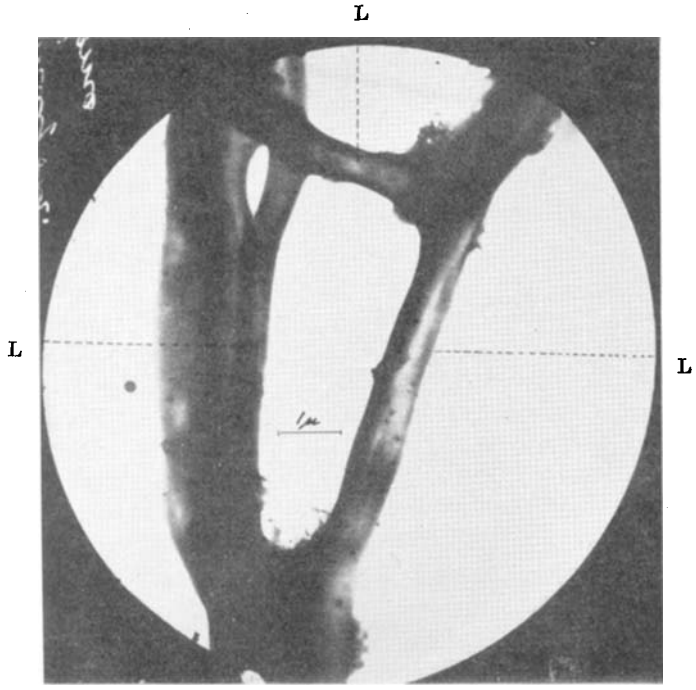

Fig. 1. Enamel tuft in the electronmicroscope, Homo. The structure shows bifurcations like those of blood vessels. Most of the branches have been broken off in the course of preparation. This applies to all of the preparations shown here. Anastomosis is also manifest (*AL*), cf. Fig. 10. Concerning the light central areas *L* and *AL*, see text, cf. Figs. 8, 9, and 10.

morphological structure of the ultracapillaries but solely to find answers to the following fundamental questions:

1) Do ultracapillaries — reticular fibres — arborize in the same manner as blood vessels?

2) Do they anastomose as do blood vessels?

3) Do they bud like blood vessels?

4) Is it possible, in spite of the weak penetration capacity of electron rays, to see the lumina in those cases in which for some natural reason, or on account of fixation etc., lengths of the capillaries are practically void of blood plasma?

The material consisted mainly of dental enamel, a tissue in which the reticular fibers are practically pure — apart from possible nervous structures. Preparations from young healing tissue, in which the reticular fibers play an essential role, were also studied.

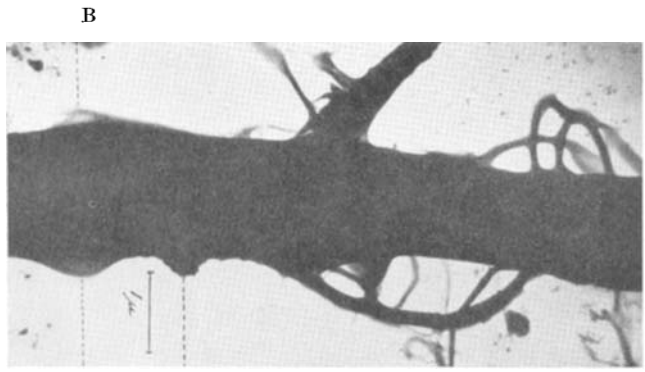

Fig. 2. Reticular fibers from the enamel of a dog's tooth. Some of the branches have been twisted out of position in the course of preparation. If allowance is made for this, the structure exhibits bifurcations in the same manner as blood vessels. *B* vesicular dilatations see text. *Br* broken branch (?).

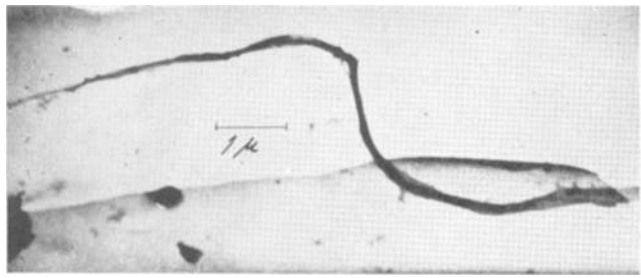

Fig. 3. Reticular fiber from a 48-hour-old wound left by an extraction of a tooth. Transverse bifurcations are beginning to bud.

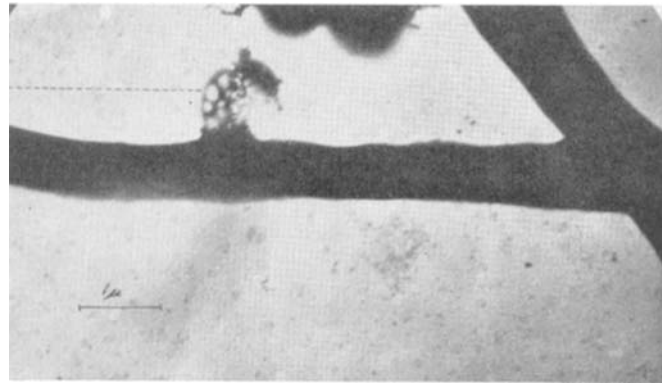

Fig. 4. Reticular fiber from human enamel. *E*, see text.

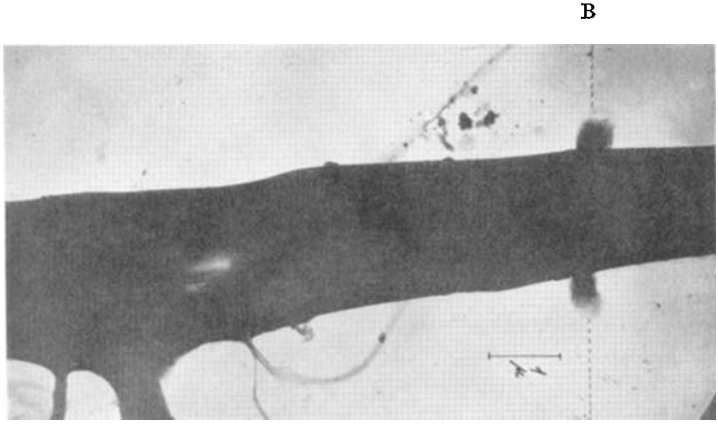

Fig. 5. Reticular fiber trunk from the enamel of a dog's tooth, most of the branches broken off in the course of preparation. *B* vascular buds. Note the thin terminating vesicles, cf. Fig. 2.

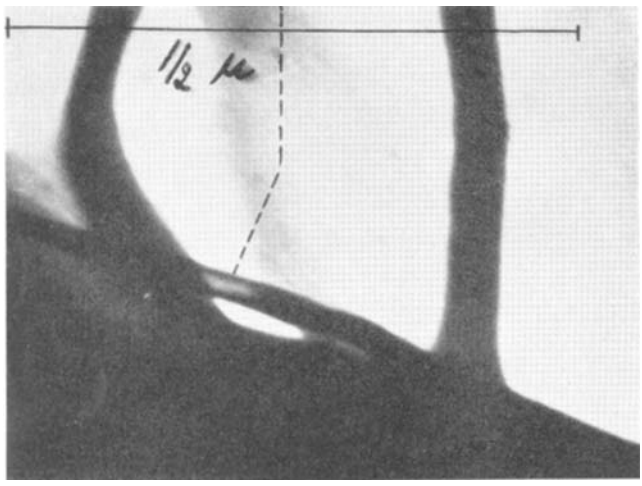

Fig. 6. Reticular structures from dog enamel. *L* lighter central area, see text.

The following technique was adopted for the preparation of the enamel material. Teeth of humans, dogs and pigs, fixed in alcohol and thoroughly cleaned, were decalcified at atmospheric pressure. As is known, the dental enamel dissolves and "disappears" in the course of decalcification. The acid solution was then centrifugated, the sediment washed several times in distilled water to eliminate the dissolved lime salts, and then placed

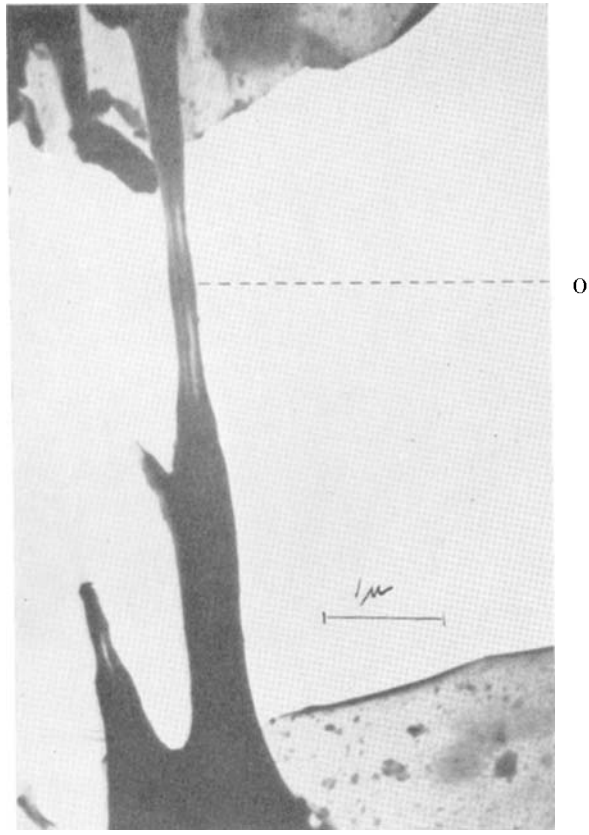

Fig. 7. Reticular fiber that was stretched when the aluminium film of the object holder cracked under the electron beam. When stretched, a vessel only partially filled ought to have this very appearance. *O* centre of the vessel-like stretched length.

on the object holder of the electronmicroscope. Optical control preparations were made in the same manner. No use was made of metal shadowing technique.

The investigation produced the following results:

1. Reticular fibers seem to arborize in the same manner as blood vessels (Figs. 1, 2, 3, 4, and 5).

2. Judging by Fig. 1 they seem to anastomose like blood vessels (Cf. Fig. 10).

3. Occasionally, short even lateral processes, often symmetric in occurrence and bordered peripherally by a thin uniform vesicle were seen (Figs. 2 and 5).

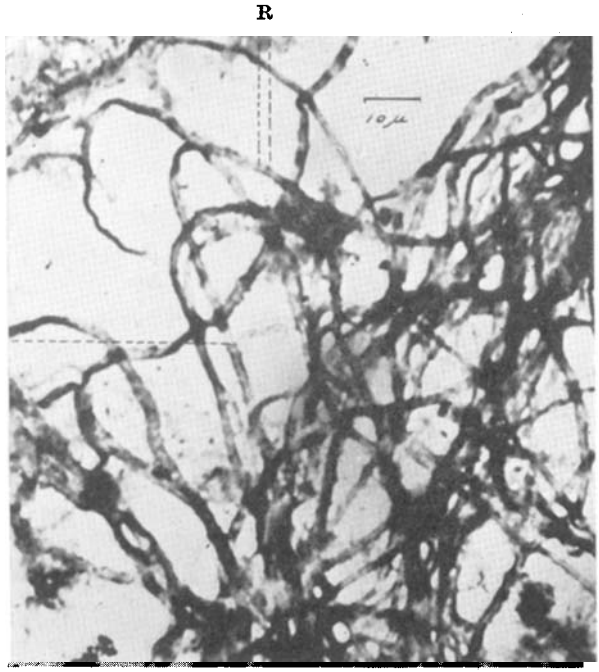

Fig. 8. Optical control preparations from human enamel, prepared in the same manner as those for the electronmicroscope. Staining: Mallory's fibrin staining. At *R* denser transverse structures and lumina of reticular fibers.

4. Concerning the lumina a lighter central zone was seen in some of the ultracapillaries-reticular fibers. This light central portion may possibly be due to the plasma having left the capillary for some natural reason or on account of the fixation, but as these light centres may also be ascribed to other causes, the electronmicroscopic pictures made in this investigation are insufficient by themselves to prove the existence of the lumina of the reticular fibers. It has been claimed that electron rays are capable of disintegrating or actually melting certain structures. The light central areas seen in the reticular fibers cannot, however, be attributed to such melting or dissolution because they were observed immediately the preparation was placed in the path of the electron beam. In no case did prolonged radiation produce a lighter central zone in initially uniformly dark sections of reticular fibers. The belief that reticular fibers are not round but flat with relatively thick borders is likewise unable to explain the light central areas of the fibers, because the light

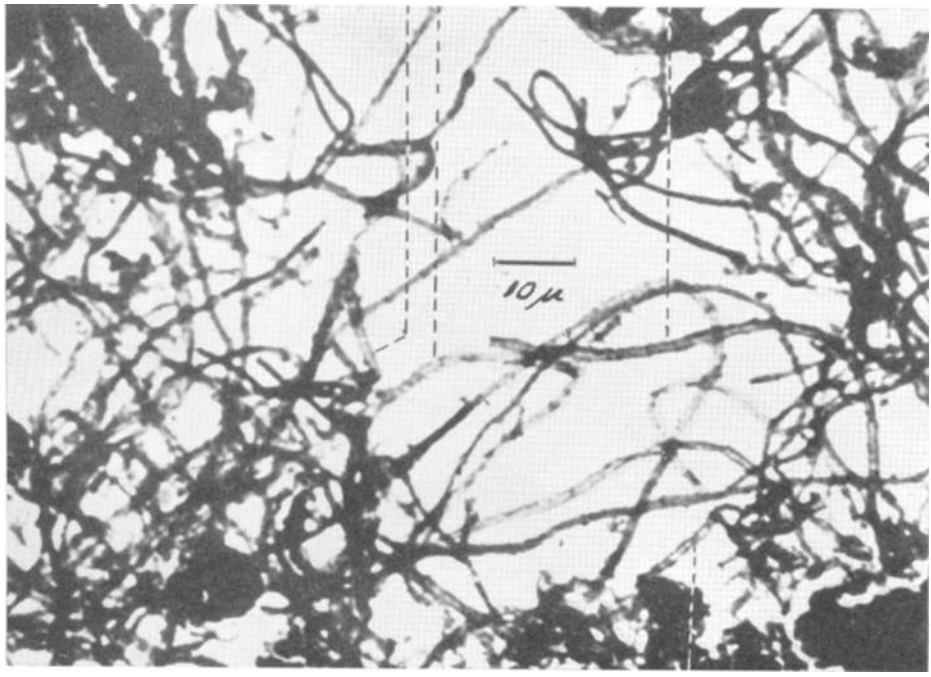

Fig. 9. Optical control preparations from dog-enamel, prepared in the same manner as those for the electronmicroscope. Staining: Mallory's fibrin staining. At *L* lumina of reticular fibers are to be seen.

centres were seen but occasionally and then only along a limited length of the fibers.

As will be apparent from Figs. 8, 9 and 10, which are reproductions of optical microphotographs of preparations made in the same manner as those used for electronmicroscopic inspection, the reticular fibers exhibit distinct lumina, visible along short distances, for which reason the lighter centres cannot be electronic artefacts.

When examining human material there occurred a bi-finding which is worthy of special study and which will be briefly mentioned here in the hope that it might stimulate investigation. In Fig. 4, we see another structure hitherto observed exclusively but often in human material. As a rule teeth crack when being extracted. If during extraction and cracking an ultracapillary is ruptured, blood plasma will probably escape from the broken branch. Immediately after extraction when the tooth

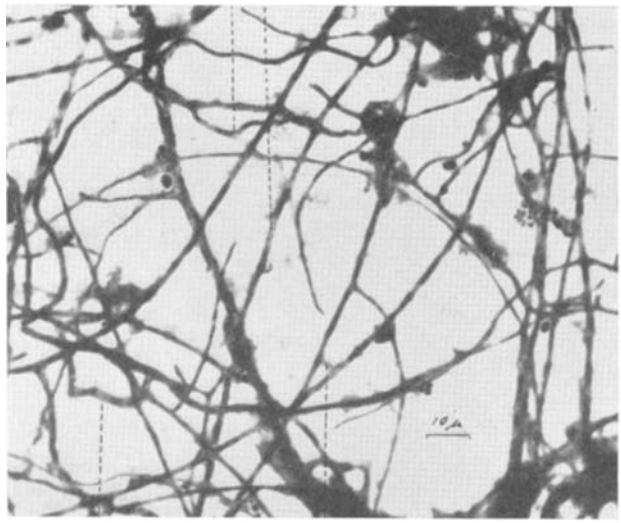

Fig. 10. Optical control preparations from pig-enamel, prepared in the same manner as those for the electronmicroscope. Staining: Mallory's fibrin staining. At *L* distinct lumina where the lengths of the vessels for some reason are void of their contents. *A* anastomosis.

is placed in alcohol, those portions of the tooth around the cracks will surely be fixed rapidly and the escaping plasma coagulate instantly. Does the tooth after treatment with acid then exhibit the picture shown in Fig. 4?

The author wishes to express his deepfelt appreciation to Professor Dr. MANNE SIEGBAHN, director of the Nobelinstitute of Physics, for the privilege of carrying on these investigations also in his institution. He is also greatly indepted to the Dean, Professor Dr. GÖSTA WESTIN, and the Council of the State Dental College for financial support received from the funds for medical research at the college.

All the preparations and the photographs in this study had been done by Mrs. ULLA BRITTA BJÖRKMAN, to which the author extends his deepfelt appreciation.

Summary.

In earlier papers it was shown that the reticular fibers, whose anatomical pertinence to the vascular system has long since been known, also belong physiologically to the blood system in so

far as they transport blood plasma to the paracapillary tissue. I therefore termed them ultracapillaries. In the present study it is shown that they branch in the same manner as blood vessels; they also seem to anastomose like blood vessels; they bud like blood vessels, and they have a lumen. The existence of a lumen was determined after a comparative study of electronmicroscopic pictures and optical control photographs.

Zusammenfassung.

In einigen früheren Publikationen wurde gezeigt, dass die Retikulinfasern, deren anatomischen Zugehörigkeit zu dem Gefässystem schon längst bekannt ist, auch physiologisch dem Blutsystem angehören in so fern, dass sie Blutplasma zu dem parakapillaren Gewebe transportieren. Deswegen nannte ich sie Ultrakapillaren. In der vorliegenden Arbeit wird gezeigt, dass sie sich in gleicher Weise wie Blutgefässe verzweigen; sie scheinen auch wie Blutgefässe zu anastomosieren; sie knospen wie Blutgefässe und sie haben einen Hohlraum. Das Vorhandenseins eines Hohlraumes wurde durch vergleichenden Studien von elektronenmikroskopischen Bildern und optischen Kontrollaufnahmen festgestellt.

Résumé.

Dans des publications précédentes il a été montré que les fibres réticulaires, dont la communication anatomique avec l'appareil circulatoire est connue depuis longtemps, aussi physiologiquement appartiennent à l'appareil sanguin en tant qu'elles transportent du plasma sanguin au tissu paracapillaire. C'est pourquoi nous les appellons des ultracapillaires. Par l'étude présente il a été montré qu'elles se ramifient de la même manière que les vaisseaux sanguins; aussi elles semblent anastomoser comme des vaisseaux sanguins; elles bourgeonnent comme des vaisseaux et elles ont un lumen. L'existence d'un lumen a été décelée par une étude comparative de photos électronmicroscopiques et de photos optiques de contrôle.

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