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INNERVATION OF THE DENTAL PULP

I. INTRA- AND SUPRAVITAL FLUOROCHROMATION OF NERVE FIBERS IN THE DENTAL PULP

by

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INTRODUCTION

The demonstration of nerve fibers has generally been carried out in thin tissue sections, thus limiting the follow-up of the continuity of the neural components. The present paper, actually initiated as a study to determine the existence of lymphatics in the dental pulp, will describe a method which enables vital and supravital staining of the neural elements in whole or sectioned tooth pulp.

MATERIAL AND METHOD

The dental pulps were obtained from the lower and upper incisors of white Sprague-Dawley rats, and also from intact human premolars extracted for orthodontic reasons. A total of 64 rats and 6 human teeth were used in the experimental series.

The experimental procedure in the rat material is outlined in Fig. 1. Thus an anodic fluorochrome solution, Thioflavine S[1] (abbreviation THFLS), 1:5000 in Ringer's solution was either injected intravitally in the pulp tissue,

[1] Chroma-Gesellschaft, Schmid & Co. distributed by ROBOZ Surgical Instrument Co., Inc., 810—18th St. N.W., Washington, D.C. 20006, U.S.A.

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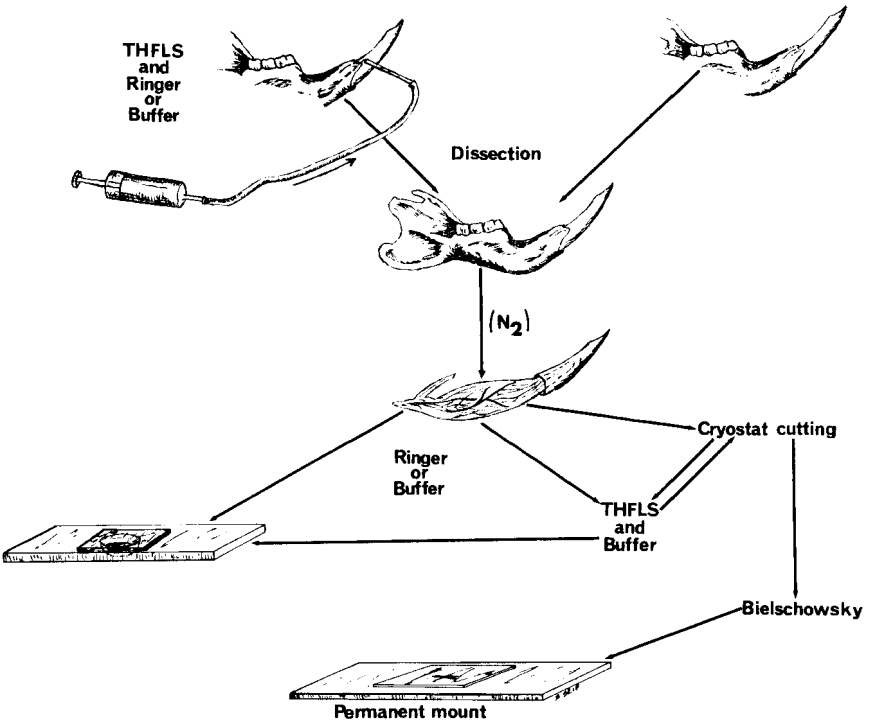


Fig. 1. Diagram outlining the experimental procedures.

or the entire dental pulp organ was immediately after dissection stained supravitally with THFLS in Ringer's or Sörensen's buffer solution.

Intravital injection. The injection was carried out in animals anaesthetized with Nembutal[1], immediately after securing a tight-fitting cannula, diameter 0.022″, in a cylinder-shaped cavity reaching the pulp. The preparation of the cavity was carried out by means of a special selfcentering spiral drill (Unitek)[2], diameter 0.021″, originally intended for the friction lock retention pin technique. The amount injected varied from 0.01 to 0.1 cc.

Dissection. The animals were sacrifized within 5 to 60 min following the injection and the jaws were dissected free of the soft tissues. The jaws were then quickly frozed in liquid nitrogen, the incisors removed, and the pulps carefully removed. In later experiments the dental pulps were handled without freezing, by means of careful dissection in a stereo-microscope (Fig. 2).

[1]) Abbot Laboratories, 14th & Ceridan Road, North Chicago, U.S.A.

[2]) Unitek Corporation, 950 Royal Oaks Drive, Monrovia, Calif., U.S.A.

The intravitally stained pulps were then placed in Ringer's or Sörensen's buffer solution, the pH of the latter varying between pH 5.5 to 9.0.

Supravital staining. The unstained dental pulps were supravitally stained for 5 min with THFLS 1:1000 to 1:5000 in Ringer's or Sörensen's buffer solution, the pH of the latter varying as stated above. The pulps were then rinsed in the corresponding buffer solution for additional 5 minutes.

Mounting. The intra- or supravitally stained whole pulps were then immersed in the aforementioned buffers on a cavity slide and covered by a coverglass.

Sectioning. Part of the stained or unstained pulps were cut in a cryostat (International Cryostat I.E.C., Model C T I)[1]. These sections, cut to 20 μ in thickness, were handled as described above.

Handling of human tooth material. Intact mandibular premolars to be extracted for orthodontic reasons, were anaesthetized by applying a mandibular block. Immediately after extraction a cavity reaching the pulp was prepared as described, and 0.02 cc of THFLS 1:5000 in Ringer's solution injected intrapulpally within one minute after the extraction. The injected teeth were then frozen in liquid nitrogen, split and the pulps then handled as already described in the animal material.

Control staining. Six rat incisor pulps were fixed in a 10 % neutral formalin solution, cut to 20 μ sections and stained according to Bielschowsky as modified by Gros (*McManus & Mowry*, 1961).

Microscopical examination. The examination of the fluorochromated mounts was carried out in a fluorescence microscope (Carl Zeiss-Photomicroscope)[2]. The light source was a HBO 200 W/4 super pressure mercury lamp. The sections were examined either in transmitted or incident UV-light. The latter observations were carried out by using Epiplan-objectives in a UV-incident light attachement, type II/Fl vertical illuminator, all by the same manufacturer.

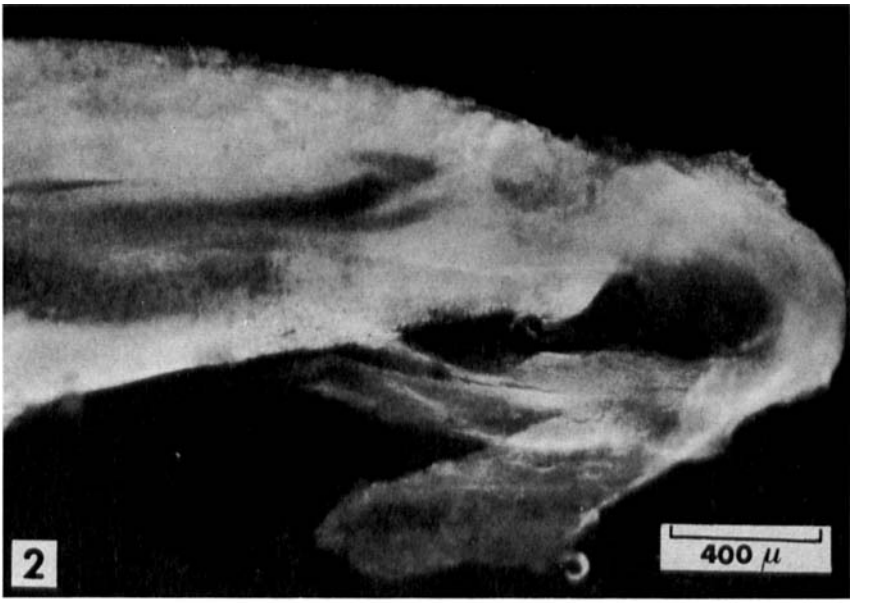
The microphotographs were made on 35 mm Kodak Panatomic and Kodachrome II Daylight emulsion film.

RESULTS

The microscopic examination of the unsectioned dental pulp organ revealed the existence of myelinated nerve fiber bundles. This type of fibers was observed both in the human (Fig. 3) and in the rat material, particularly in

[1]) International Equipment Company, Needham Heights, Mass., U.S.A.

[2]) Carl Zeiss, Oberkochen, Württ., Federal Republic of Germany.



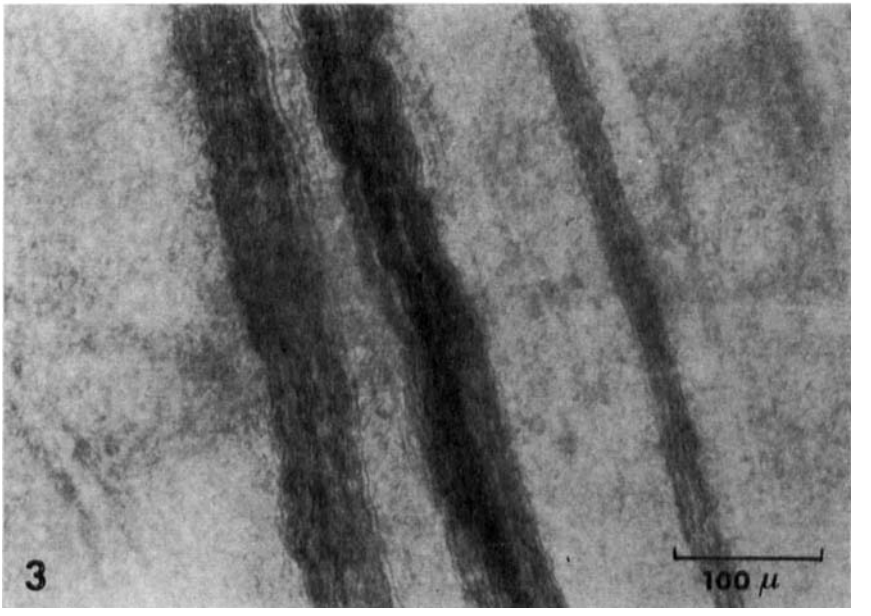


Fig. 2. Low-power photomicrograph of a mandibular incisor pulp of a rat. Note the sharp bend in the immediate periapical region.

Fig. 3. Myelinated nerve fiber bundles in the pulp of a human mandibular premolar tooth. Microphotograph in ordinary transmitted light.

the apical part of the pulp in the latter species. Single myelinated nerve fibers had a diameter of 5 μ, and could also be detected in ordinary transmitted light even in an unstained condition (Figs. 3, 4a).

Intravital or supravital fluorochromation and subsequent examintion in transmitted or incident ultraviolet light resulted, however, in a strong secondary fluorescence of the nerve fibers in the dental pulp (Figs. 4b, 5, 6, 7b). Fluorochromation with Thioflavine S stained the myelin sheaths yellow, the lipid center of these fibers and the Schwann cells exhibiting a reddish-grey fluorescence, which showed up as dark areas in the black and white microphotographs (Figs. 4b, 5).

In addition to the myelinated nerves, another type of thin fibers was found, particularly in the apical part of the rat incisor pulp (Figs. 6, 7a, 7b). These thin nerve fibers, their diameter ranging from 2 μ to about 0.5 μ, were frequently connected to small bead-like structures (ø 2—3 μ) (Figs. 6, 7b).

The formalin-fixed Bielschowsky-stained sections showed a coiled appearance of the myelinated nerve fibers (Figs. 8, 9). Also in these sections, fibers with a diameter of 1—2 μ were recognized (Fig. 8). The continuity of the neural components was more difficult to ascertain in the silver-impregnated sections than in whole fluorochromated pulps.

Experiments with increased concentrations of the THFLS-solution up to 1:1000 revealed no appreciable concentration effect when compared to the fluorochromation produced by the 1:5000 solution. The use of Sörensen's buffer solution at pH 5.5 anf 6.0 revealed a somewhat stronger fluorochromation than acheived at higher pH-values.

In addition to the nerves, other tissue components also accepted the stain. The staining was, however, limited to easily recognizable structures, the odontoblast nuclei, and the endothelium of some blood vessels accidentally injected. Blood vessels and their during vital fluorochromatory cellular contents showed normally a very weak dark brown or no fluorescence.

DISCUSSION

An abundance of nerve fibers in the rat incisor and in the human dental pulp could be demonstrated by the fluorescence microscopy technique described. *Hals* (1953) has previously in a comprehensive way studied the primary and secondary fluorescence of developing and adult dental structures, with the exception of the pulp. *Bennett* (1953) has previously discussed the appearance of fluorochromated nerves in the dental pulp, the

secondary fluorescence being attributed to the fibrous connective tissue along the nerve trunks. The present findings show, however that the myelin sheaths accepted an anodic fluorochrome, and that the thin fibers also accepted the stain. The present method offers thus a possibility to study the entire innervation of the dental pulp, particularly as the continuity of the nerve fibers and their connections could be followed by examining the entire dental pulp organ.

Contrary to the observations of *Hattyasy* (1959) the present study clearly revealed the existence of myelinated nerve fibers in the rat incisor pulp. The occurrence of these nerve bundles was limited to the apical part of the pulp.

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SUMMARY

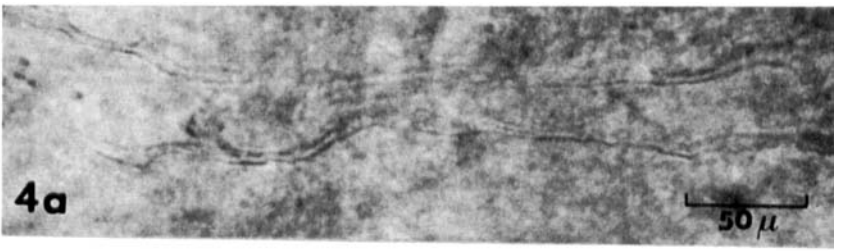
Intravital or supravital fluorochromation of the dental pulp by using an anodic fluorochrome (Thioflavine S) resulted in a strong secondary fluorescence of the nerve fibers in the dental pulp. The observations on the continuity and interconnections of these nerves were facilitated by the possibility of examining the dental pulp in its entire thickness.

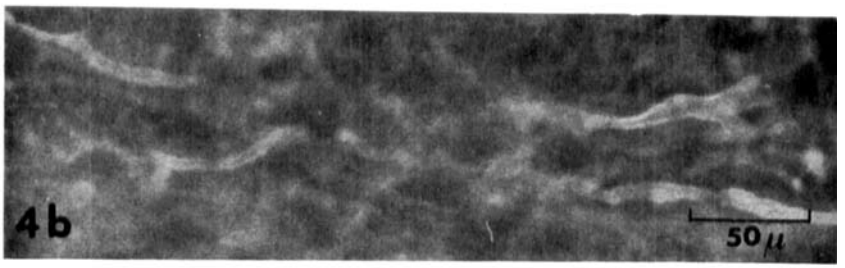
Fluorescence microscopy revealed the existence of myelinated nerve fibers in the rat incisor pulp, as well as thin fibers ranging from 0.5 to 2 microns in diameter. The latter elements also contained bead-like structures.

Fig. 4. Myelinated nerve fibers in a human premolar pulp.
a. Ordinary transmitted light.
b. Incident UV-light supravital fluorochromation with Thioflavine S, 1:5000 in Ringer's solution.

Fig. 5. Myelinated nerve fiber in a rat incisor pulp. Incident UV-light, supravital fluorochromation with Thioflavine S, 1:5000 in Sörensen's buffer, pH 5.5. Arrow indicates location of a Schwann cell.

Fig. 6. Fine-caliber fibers connected to bead-like structures (arrows) in apical part of a rat incisor pulp. Supravital fluorochromation with Thioflavine , 1:1000 in Sörensen's buffer, pH 6.0.


4a
50 μ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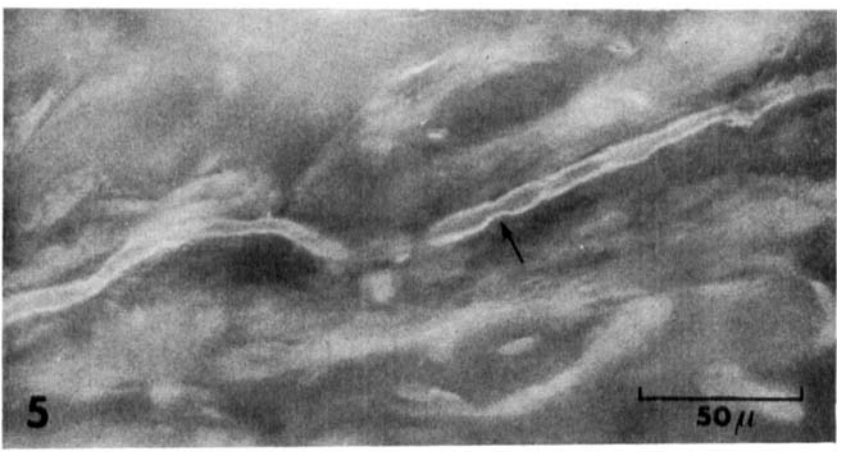
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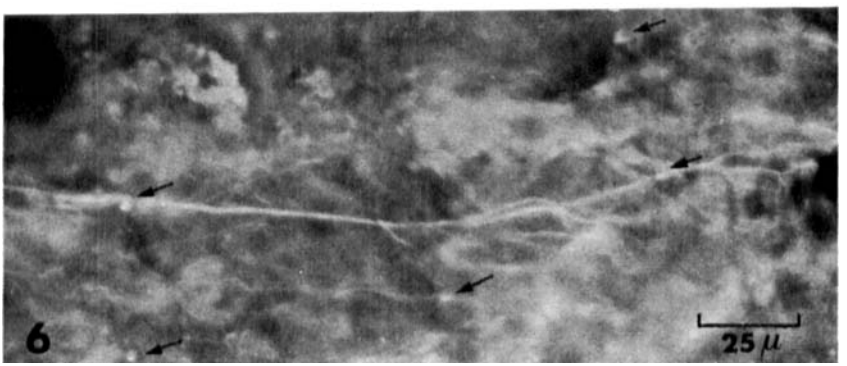
50 μ


6
25 μ

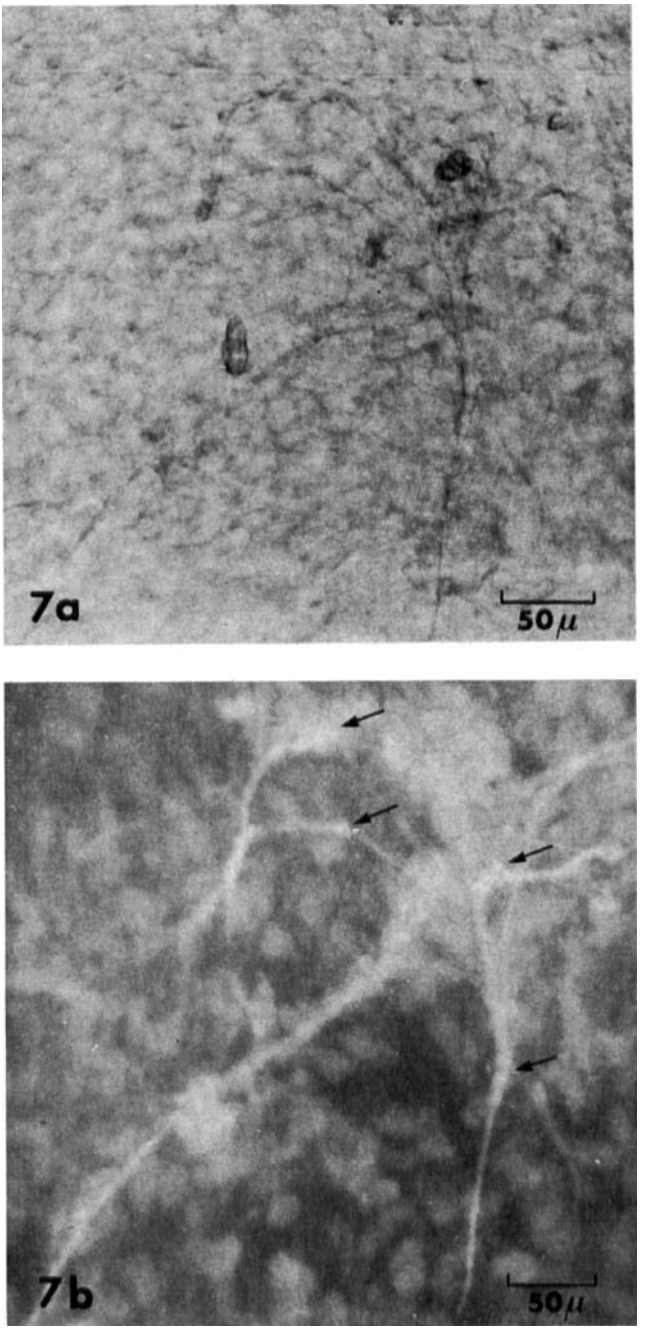
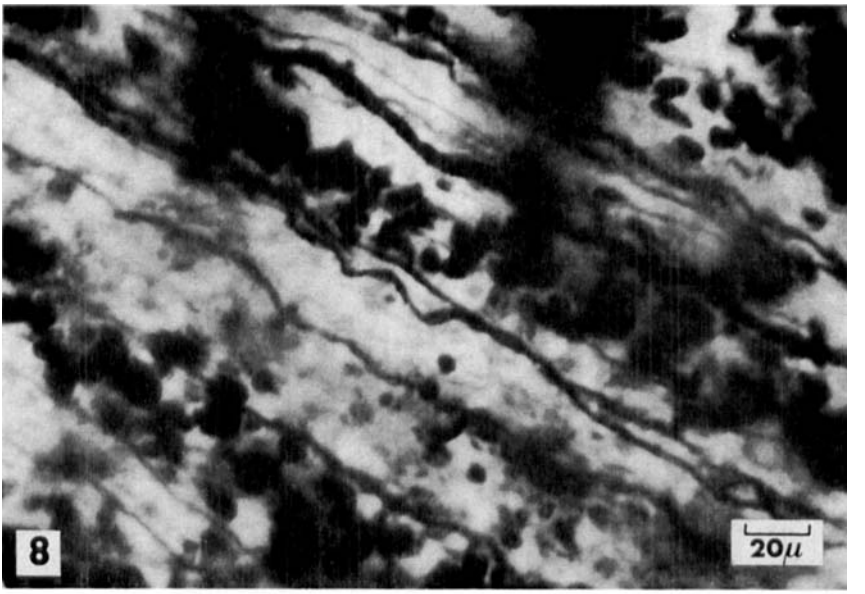


Fig. 7. Fine-caliber fibers and bead-like structures (arrows) in apical part of rat incisor pulp. Supravital fluorochromation with Thioflavine S, 1:5000 in Sörensen's buffer, pH 7.5.
a. Ordinary transmitted light.
b. Transmitted UV-light.



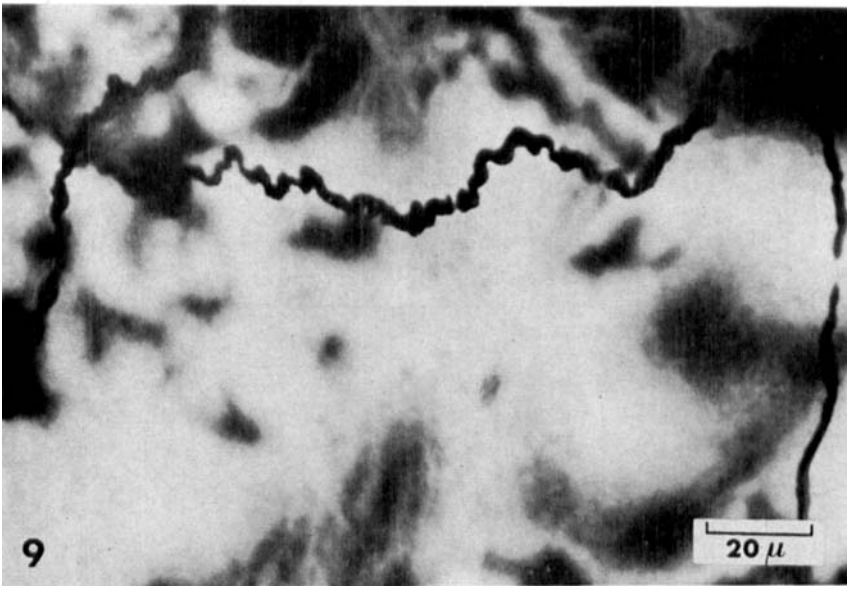


Fig. 8. Paravascular nerve fibers in apical part of rat incisor pulp, silver-impregnated according to Bielschowsky.

Fig. 9. Coiled myelinated nerve fibers in rat incisor pulp. Bielschowsky-stain.

RÉSUMÉ

INNERVATION DE LA PULPE DENTAIRE

I. IMPRÉGNATION VITALE (INTRA-VITALE ET SUPRA-VITALE) DES FIBRES NERVEUSES DE LA PULPE DENTAIRE PAR LES FLUOROCHROMES

L'imprégnation vitale (intra-vitale et supra-vitale) de la pulpe dentaire par un fluorochrome anodique (Thioflavine S) déterminait une fluorescence secondaire marquée des fibres nerveuses dans la pulpe dentaire. Les observations concernant la continuité de ces nerfs et les connexions entre eux étaient facilitées par la possibilité d'examiner la pulpe dentaire dans toute son épaisseur.

La microfluoroscopie a mis en évidence l'existence de fibres nerveuses à myéline dans la pulpe de l'incisive du rat, ainsi que l'existence de fibres fines d'un diamètre allant de 0,5 à 2 microns. Ces derniers éléments contenaient aussi des formations en forme de boutons.

ZUSAMMENFASSUNG

DIE INNERVATION DER ZAHNPULPA

I. INTRA- UND SUPRAVITALE FLUOROCHROMATION VON NERVENFASERN DER ZAHNPULPA

Die intra- oder supravitale Fluorochromation der Zahnpulpa unter Anwendung eines anodischen Fluorochroms (Thioflavin S) ergab eine kräftige Sekundärfluoreszenz der Nervenfasern in der Zahnpulpa. Die Beobachtung des Verlaufs dieser Nervenfasern und ihrer Verbindungen untereinander wurde erleichtert durch die Möglichkeit die Pulpa in ihrer ganzen Dicke zu untersuchen.

Fluoreszenzmikroskopisch entdeckte man in der Pulpa der Ratteninzisiven sowohl das Vorhandensein von myelinhaltigen Nervenfasern als auch von dünnen Fasern im Durchmesser von 0,5 μ bis 2 μ. Die letzteren enthielten auch perlähnliche Strukturen.

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