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ACETYLCHOLINESTERASE AND NORADRENALINE IN THE NERVES OF MAMMALIAN DENTAL PULPS

by

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INTRODUCTION

The primary physiological function of acetylcholinesterase in limiting the transmitter action of acetylcholine at synaptic and neuroeffector sites is well established. In addition, the presence of acetylcholinesterase in varying quantities in non-cholinergic neurones and in some non-nervous tissues is known (*Koelle*, 1963).

Avery and *Rapp* (1959) demonstrated acetylcholinesterase activity in human teeth. They found acetylcholinesterase in the nerve fibers underlying the predentine and throughout the odontoblasts, including their secondary processes. Avery and Rapp suggested that an acetylcholine-mediated synapse between the odontoblasts and the adjacent free nerve endings was the mechanism of pain conduction. *Ten Cate* and *Shelton* (1966) were unable to confirm these histochemical findings and denied the possibility of a cholinergic synapse. Later, *Rapp*, *Avery* and *Strachan* (1967) found no acetylcholinesterase activity in the odontoblastic processes of human primary teeth with complete roots, but they noticed intense activity in the coronal parietal nerve plexus.

In the dental germs of rats and bovines acetylcholinesterase activity is linked with the presence of nervous elements, although a fully formed bovine incisor also displays an acetylcholinesterase-negative nervous component (*Quintarelli*, 1961; *Cavallazzi*, *Veggetti & Callegari*, 1966).

The presence of noradrenaline in the human pulpal nerves has been demonstrated simultaneously by *Anneroth* and *Norberg* (1968) and *Pohto* and *Antila* (1968). The visualization of the transmitter of adrenergic nerve endings rendered it possible to study the distribution of the sympathetic nerves in the pulp. Both teams found a close connection of sympathetic fibers with blood vessels and attributed a vasoregulatory function to these fibers.

There is also physiological evidence for the presence of vasoconstrictor fibers in the dental pulp, although the results of experiments on stimulation of sympathetic nerves are somewhat contradictory (*Taylor*, 1950; *Neidle & Liebman*, 1964a, 1964b; *Ogilvie, Gillilan & Knapp*, 1966). Even with combined methods it is difficult to distinguish between the nerve terminals on the basis of anatomical features. *Arwill* (1958) found two types of unmyelinated nerve fibers in the human tooth pulp, but only one of them was supposed to be of autonomic origin and located in the vascular wall. As *Gerebtzoff* (1959) has pointed out, the fact that nerves run in association with blood vessels does not mean that they terminate in these vessels.

Histochemical demonstration of noradrenaline and acetylcholinesterase affords more specific information on the nature of nervous elements. The amine histochemistry of neurones provides methods for the monoamine-ergic nervous systems (*Eränkö*, 1967a). High concentrations of acetylcholinesterase are localized in cholinergic, e.g. parasympathetic, neurones, and lower, variable concentrations are present in sensory and in some adrenergic neurones (*Koelle*, 1955, 1963). However, it is still a matter of debate whether acetylcholinesterase and noradrenaline can be present in the same axon, or are actually in closely associated axons which cannot be resolved by light microscopy (*Eränkö*, 1967b).

MATERIAL AND METHODS

Material for the histochemical demonstration of acetylcholinesterase and noradrenaline was obtained from rat incisors, rabbit incisors and molars, and cat cuspids and molars. Five to eight animals of each species were included. The human material consisted of permanent incisors, cuspids, premolars and molars. Altogether about 1500 sections were examined.

Specimens for acetylcholinesterase were taken from freshly extracted teeth, which were split open. The pulps were fixed in 4 % formaldehyde solution with 1 % $CaCl_2$. The fixation time ranged from 2 hours to 2 days. Sections of washed pulps were cut with a freezing microtome at 15—25 μ. The free-floating sections were preincubated for 30 minutes in the substrate-free

medium with the inhibitor of nonspecific cholinesterase or acetylcholinesterase or both together. The inhibitors were 10^{-5}M tetra-isopropylpyrophosphoramide (iso-OMPA; Koch-Light Laboratories LTD., Colnbrook, England) and 10^{-5}M 1,5-bis (4-allyldimethyl-ammoniumphenyl) pentan-3-one dibromide (284C51; The Wellcome Research Laboratories, London). Incubation proper was carried out at 37°C for 2 to 18 hours with the above-mentioned inhibitor combinations. The substrate, pH 6.0, was made up according to a »direct-colouring» thiocholine method (*Karnovsky & Roots*, 1964). The incubated and rinsed sections were mounted in polyvinylpyrrolidone.

Ground sections were prepared from fixed or unfixed human and rabbit teeth at about 200 μ. The treatment was as above. Frozen sections were also made of rat and rabbit parotid glands and striated muscle and treated identically for the evaluation of results.

The formaldehyde-induced fluorescence of catecholamines after the freeze-drying of specimens was used for the demonstration of noradrenaline (*Falck, Hillarp, Thieme & Torp*, 1962). The procedure followed has been described in detail in a previous paper (*Pohto & Antila*, 1968).

RESULTS

Acetylcholinesterase

Acetylcholinesterase-positive nerve fibers were observed in the pulps of every species included in the investigation. The amount of these fibers varied not only according to species and tooth examined, but also according to the age of individual. Incubation times exceeding 10 hours were necessary to visualize acetylcholinesterase in the pulps, whereas 30 minutes was enough in the tests with the motor end-plates of striated muscle and 2 hours sufficed with the parasympathetic nerves of the rat parotid gland.

In sections of human root pulps incubated for 18 hours, the acetylcholinesterase activity was confined to the nerve bundles. Often these trunks followed blood vessels (Fig. 1). Higher magnification of the bundles revealed the rectilinear course of single fibers (Fig. 2). In the coronal portion of the pulp divergence of fibers from branching bundles was frequent. In addition to straight-running fibers, there was a delicate network of acetylcholinesterase-positive fibers on the surface of some of the larger blood vessels (Fig. 2). Below the coronal odontoblasts, it was not possible to identify the nerve plexus of Raschkow, with its characteristic loops. The peripheral fibers traversed the cell-poor zone of Weil, often spreading out fanwise (Fig. 3).

The area of odontoblastic bodies and predentine contained acetylcholinesterase, but no activity was found in the dentine or in the odontoblastic proc-

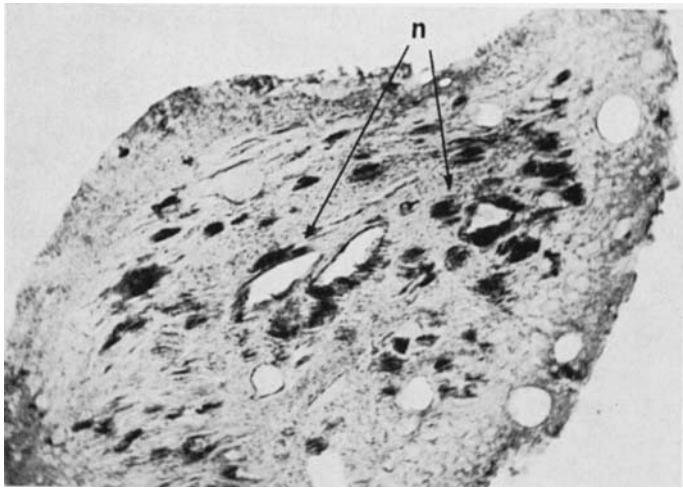

Fig. 1. Slightly oblique section of a human upper incisor pulp. Acetylcholinesterase-positive nerve bundles (n) of the cervical area are running close to blood vessels. Incubation 18 hours. × 180.

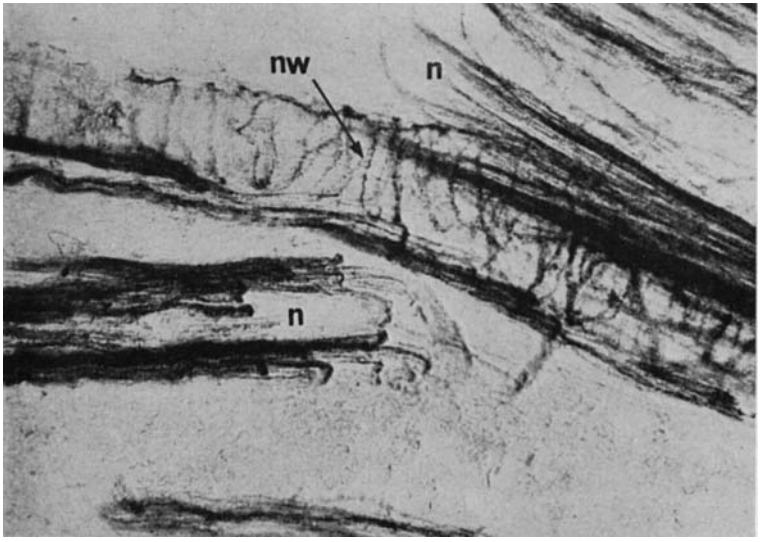

Fig. 2. Longitudinal section of a human upper incisor pulp. Nerve bundles (n) composed of straight-running single fibers. A delicate network (nw) of fibers on the surface of blood vessel. Acetylcholinesterase activity, incubation 18 hours. × 150.

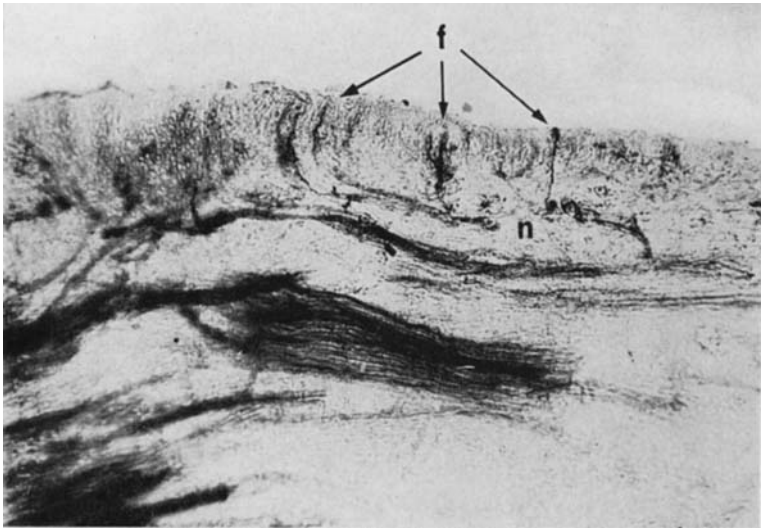

Fig. 3. Human upper incisor pulp. Longitudinal section of a coronal portion. Nerve fibers (f) of branching bundles (n) traversing the zone of Weil. Acetylcholinesterase activity, incubation 18 hours. × 125.

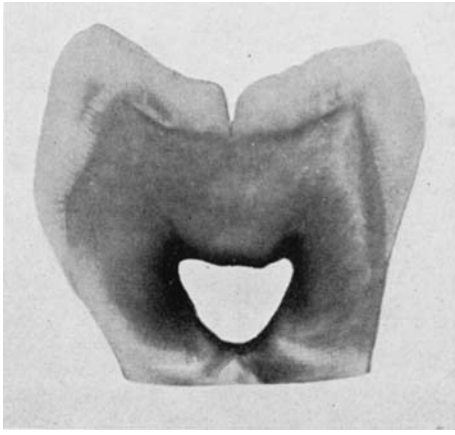

Fig. 4. Bucco-lingual ground section of the unerupted lower third molar of man. Acetylcholinesterase activity of the area of odontoblastic bodies and predentine is observed around the pulpal cavity. Incubation 13 hours. × 7.

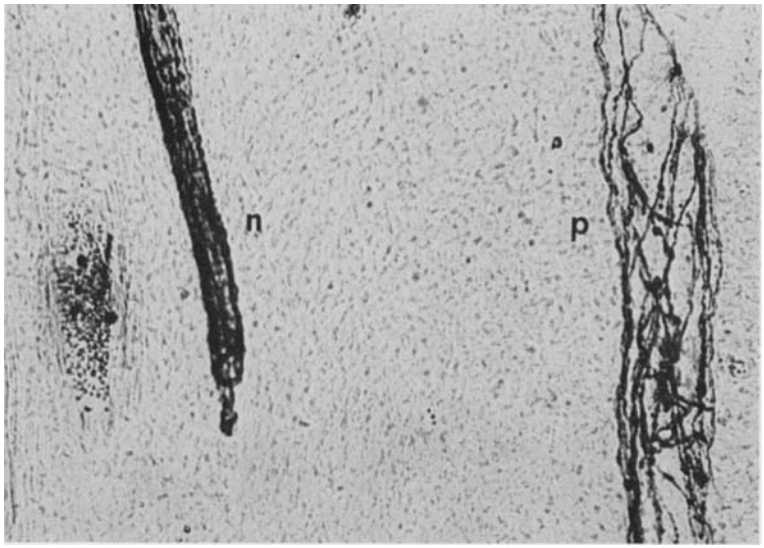

Fig. 5. Paravascular nerve plexus (p) and a nerve bundle (n) in a cat lower molar. Acetylcholinesterase activity, incubation 10 hours. × 200.

esses (Fig. 4). Some sample tests with butyrylthiocholine as substrate suggested that the predentinal area also contained nonspecific cholinesterase. In the human pulps nonspecific cholinesterase showed the same general distribution as acetylcholinesterase.

The incisors of rats of varying ages (80—300 g) showed sparse acetylcholinesterase-positive structures and sections totally devoid of stained neurones were found. The pulps of cat cuspids and molars contained fewer acetylcholinesterase-stained fibers than the human and rabbit material.

In the cat molars a paravascular fiber plexus was observed alongside some of the larger blood vessels (Fig. 5). The plexus was clearly visible after 10 hours' incubation. The finest nerve fibers detected in the cat pulps were 1—2 μ in diameter and were sharply outlined against an essentially cholinesterase-negative background (Fig. 6).

In the rabbit pulps the mature connective tissue matrix included numerous acetylcholinesterase-containing mesenchymal cells (Fig. 7). The neural staining of the rabbit pulps resembled that of the human pulps. Also an acetylcholinesterase-positive band coinciding with the area of the odontoblastic bodies was found in the rabbit.

In control tests with the cholinesterase inhibitors the specific staining was abolished or reduced significantly. In the substrate-free medium no staining was observed.

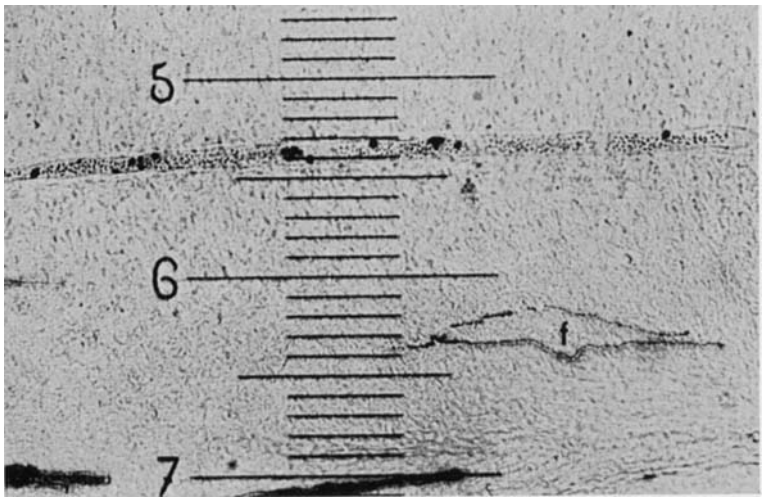

Fig. 6. Single nerve fibers (f) in a cat upper molar. A vessel with acetylcholinesterase-positive blood cells is seen. Incubation 10 hours. One scale division 17 μ.

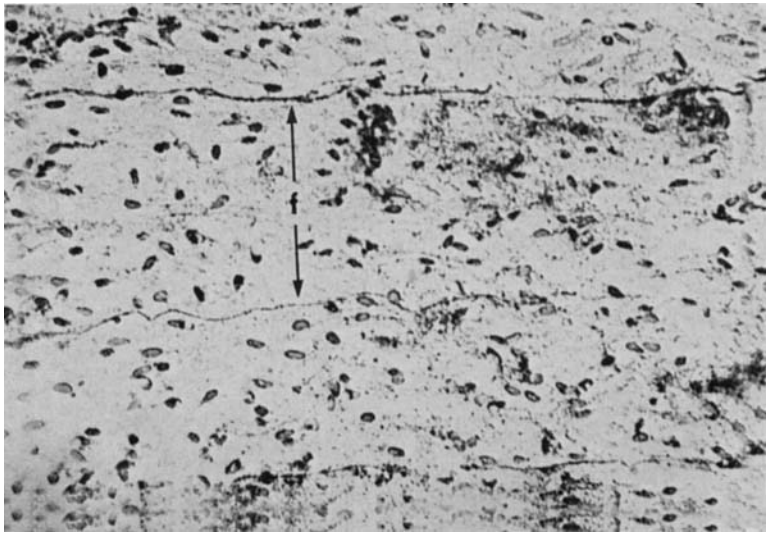

Fig. 7. Rabbit lower incisor with single nerve fibers (f). Acetylcholinesterase containing mesenchymal cells are evenly distributed throughout the pulp. × 200.

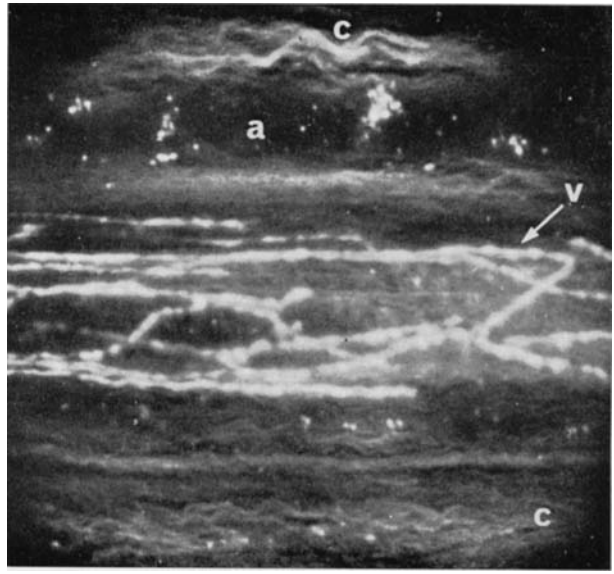

Fig. 8. Longitudinal section of a cat lower cuspid pulp showing a sympathetic adrenergic ground plexus on an arteriole. Individual green-fluorescent fibers with bead-like varicosities (v). Paraxial arteriole (a) without sympathetic innervation. The specific fluorescence of 5-hydroxytryptamine of platelets is seen within the arteriole. Yellowish autofluorescence of collagenous fibers (c). Dark-field condenser. × 325.

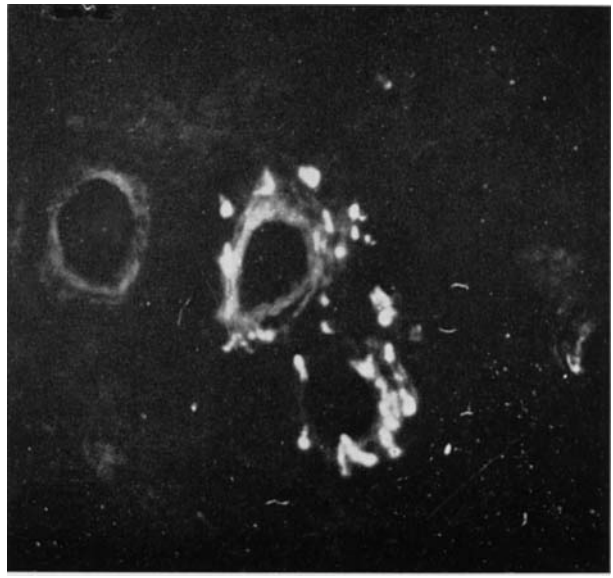

Fig. 9. Transverse section of the same pulp as in Fig. 8. Dark-field condenser. × 340.

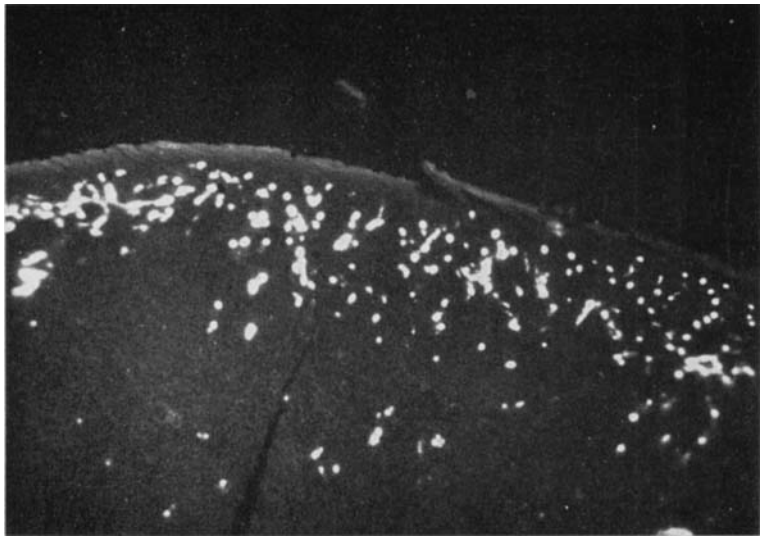

Fig. 10. Rabbit lower incisor pulp. Transverse section through the radicular portion. Intensely fluorescent fibers running in the peripheral pulp. Bright-field condenser. × 120.

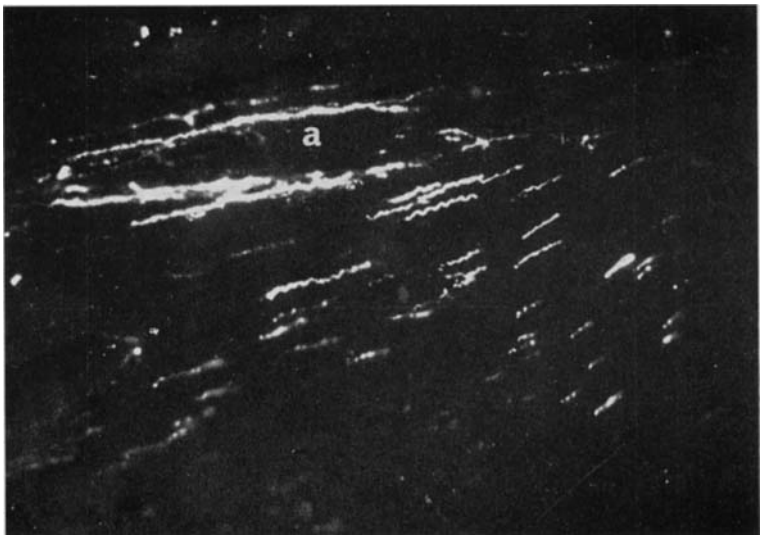

Fig. 11. Longitudinal section of the same area as in Fig. 10. The section is made through a sympathetically innervated arteriole (a). Nonvaricose coiled fibers of unknown nature are not associated with blood vessels. For details see text. Bright-field condenser. × 120.

Noradrenaline

The induced fluorochrome from the noradrenaline of the sympathetic postganglionic terminals was seen in the human, cat and rabbit pulps studied. The incisor pulps of rats were devoid of adrenergic fibers.

Within the teeth the main arteries ascended the coronal pulp and arborized into arterioles. Some, but clearly not all, of these vessels were surrounded by an adrenergic ground plexus (Fig. 8 and 9). Often it was an adjacent paraxial arteriole which lacked the adrenergic fibers. In the human, rabbit and cat pulps the thin walls of some veins also had a sympathetic innervation. In the serial sections the terminal axons containing intensely fluorescent varicosities could often be followed on blood vessels up to about 15 μ in diameter. Neither the subdentinal capillaries of cat or rabbit pulps nor the anastomotic peripheral capillary network of human pulps were supplied with sympathetic fibers.

The upper and lower incisors of the rabbit contained fluorescent fiber of a type which was not seen in the other species. These green-fluorescent fibers were located peripherally only in the radicular portion of the pulps (Figs. 10 and 11). The fibers were not associated with blood vessels and disappeared in a borohydride reduction test. These fibers differed from autofluorescent

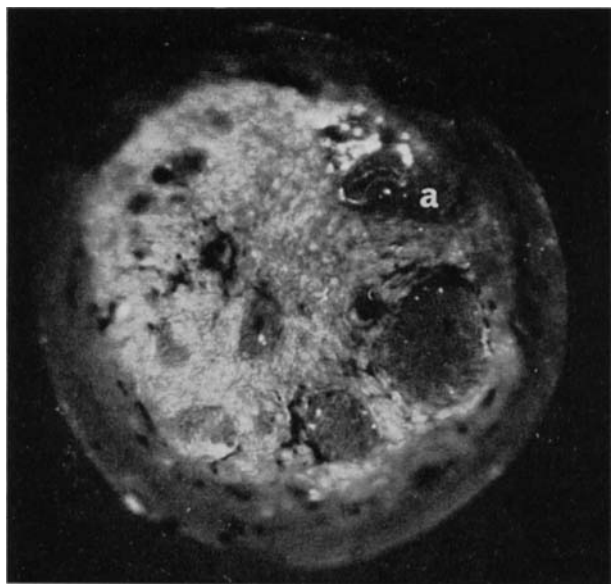

Fig. 12. Root pulp of aged human lower molar displaying a strong nonspecific background fluorescence. One sympathetically innervated arteriole (a) is observed. Bright-field condenser. × 150.

collagenous fibers in sharpness, intensity, colour and distribution (Figs. 8 and 12). On the other hand, the varicosities typical of the sympathetic terminal axons were lacking and regeneration of the fluorescence by repeated exposure to formaldehyde gas was unsuccessful.

The most disturbing collagen autofluorescence in the specimens studied was encountered in the aged human pulps (Fig. 12), and generally, the human pulps displayed the most intense nonspecific background fluorescence.

DISCUSSION

Acetylcholinesterase

The four accepted types of cholinergic nerve fibers are somatic motor, some fibers of the central nervous system, preganglionic autonomic and postganglionic parasympathetic, including some sympathetic fibers. Primary sensory neurones with their terminations are generally assumed to be noncholinergic, in spite of their acetylcholinesterase content (*Koelle*, 1963). Thus, it is reasonable to presume that the acetylcholinesterase-positive neurones of pulps are of postganglionic autonomic and sensory origin.

The long incubation period required for the accumulation of a sufficient amount of reaction product suggests that the acetylcholinesterase-positive nerve bundles and fibers in the pulps studied are sensory. Formaldehyde fixation improves the preservation of structural detail, but a simultaneous loss of cholinesterase activity occurs (*Taxi*, 1952; *Couteaux*, 1958). However, the intense staining of fixed motor end-plates and parotid parasympathetic neurones with incubation times shorter than those necessary for similarly fixed pulpal neurones indicates that the acetylcholinesterase content of nerve fibers in the pulps is better correlated with sensory than with parasympathetic neurones.

The observed coronal penetration of afferent sensory fibers to an odontoblastic layer seems understandable, since sensory terminals are found lying between the process of an odontoblast and the wall of a dentinal tubule (*Fearnhead*, 1967).

It is more difficult to interpret the acetylcholinesterase-positive fiber network on some blood vessels. With the exception of the digital arteriovenous anastomoses of human skin (*Hurley & Mescon*, 1956), there are few cholinergic terminals of parasympathetic origin along the veins and arteries of various species and organs. *Spriggs*, *Lever* and *Graham* (1968) gathered evidence which militates against the presence of acetylcholinesterase in the periarteriolar sympathetic plexus. *Koelle* (1963) has drawn attention to the difficulty of distinguishing between stained nerve terminals and vascular

smooth muscle fibers. The localization of the faint reticular staining on some of the larger blood vessels of human pulps remains unclear for the present.

The more prominent acetylcholinesterase-positive network of fibers associated with some of the blood vessels of cat molars resembles the autonomic ground plexus described by *Hillarp* (1959). A group of cells in the cat superior cervical ganglion has a high concentration of acetylcholinesterase and in the corresponding axons the enzyme remains proportional to the perikaria (*Koelle*, 1955; *Giacobini*, 1957). Sympathetic neurones of this kind have been postulated to be cholinergic and to have vasodilatory function (*Folkow, Frost, Haeger & Uvnäs*, 1948; *Bolme & Fuxe*, 1967). However, we think that the nervous network observed in the cat molars is not of autonomic origin, but represents paravascular sensory nerves which may respond to algesic stimuli (*Lim*, 1968). Besides the long incubation time required, the sensory nature is substantiated by the greater distance of the fibers from the vascular wall than was observed with the perivascular sympathetic ground plexus.

The acetylcholinesterase-positive staining of the band lining the human pulp cavity does not imply that the odontoblastic bodies contain acetylcholinesterase. Many small capillary loops are found in the odontoblastic layer (*Saunders & Röckert*, 1967). The acetylcholinesterase content of human erythrocytes is 100-fold that of the cat, although the plasma contains only traces of acetylcholinesterase in most species (*Koelle*, 1963). In the ground sections, diffusion of acetylcholinesterase from the sensory terminals or from the haemolyzed erythrocytes may be responsible for the staining. Thus, no confirmatory evidence was obtained for the proposed cholinergic synapse between the odontoblasts and the free nerve endings in the teeth.

In the study of *Ten Cate* and *Shelton* (1966), the cholinesterase staining of the pulps was marred by a nonspecific precipitate and continuous prolonged incubation was impossible. Obviously, the phosphate buffer used instead of a maleate buffer and the lack of an inhibitor for the nonspecific cholinesterase were the causes.

The parasympathetic innervation of ox and horse pulps was described in morphological terms by *Armenio* and *Laforgia* (1955). Although the present method allows only careful quantitative conclusions about acetylcholinesterase, in the light of the foregoing findings we suggest that the pulps studied were not supplied with cholinergic, e.g. parasympathetic, neurones.

Noradrenaline

The pattern of sympathetic innervation associated with the blood vessels of cat and rabbit pulps agreed with earlier findings in man (*Anneroth &*

Norberg, 1968; *Pohto & Antila*, 1968). The possible existence of nonterminal axons is difficult to demonstrate because of their low amine concentration (*Dahlström & Fuxe*, 1964). No nonterminal axons were found in the cat or rabbit material, but in the human molars a faint fluorescence was observed in the structures suggested to be trunks, as reported earlier (*Pohto & Antila*, 1968). This fluorescence was abolished by borohydride reduction in the specificity test, but the fact that it could not be regenerated may be due to the low noradrenaline content.

The sparse varicose fibers reached blood vessels of suitable calibre to be called metarterioles. Whether the pulpal precapillary sphincters described by *Provenza* (1958) receive sympathetic innervation or not remains to be revealed by more refined techniques.

The nonvaricose fibers in the root pulps of the continuously growing rabbit incisors resembled the sympathetic terminals in a penile retractor muscle of the bull, even though the pulpal fibers were not so strongly coiled (*Klinge, Pohto & Solatunturi*, 1968). These single incisal fibers were not related to the blood vessels, but no extravascular smooth muscle is known to exist in a pulp. The nature of the fibers remains obscure.

There was a pronounced difference between the incisors of the two rodents examined. In contrast to those of the rabbit, the pulps of young or adult rats were not supplied with sympathetic axons. *Taylor* (1950) observed that stimulation of the cervical sympathetic nerves retarded or stopped the flow of blood in the rat incisor, but there was no change in an arteriole calibre within the pulp. The present finding also indicates a more proximal sympathet ic control of the blood flow. On the other hand, the pulpal vessels of a rat incisor may contain sympathetic α-receptors, since adrenaline in local analgesic solutions affected the circulation in the dental pulp (*Pohto & Scheinin*, (1962). The species variation in the background fluorescence of the pulps was obviously dependent on qualitative differences in the autofluorescent collagen fibers (*Prenna & Sacci*, 1964; *Bailey*, 1968).

Acknowledgements

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SUMMARY

Histochemical methods were employed to visualize acetylcholinesterase and noradrenaline in human, rabbit, cat and rat pulps. All the pulps examined contained acetylcholinesterase-positive nerve fibers; these were fewest in

the rat incisors. In the light of the present findings, there appears to be no convincing evidence for the cholinergic nature of these fibers, which are suggested to be afferent sensory fibers. Thus, no parasympathetic terminals were identified.

Of the pulps examined, those of man, rabbit and cat were supplied with adrenergic sympathetic nerves associated with blood vessels; the incisal pulps of rats were devoid of vasomotor innervation.

RÉSUMÉ

ACÉTHYLCHOLINESTÉRASE ET NORADRÉNALINE DANS LES NERFS DES PULPES DENTAIRES DES MAMMIFÈRES

Les auteurs ont utilisé des méthodes histochimiques pour mettre en évidence l'acétylcholinestérase et la noradrénaline dans les pulpes de l'Homme, du Lapin, du Chat et du Rat. Toutes les pulpes examinées contenaient des fibres nerveuses acétylcholinestérase-positives; c'était dans les incisives du rat que ces fibres étaient le moins nombreuses. A la lumière des résultats de la présente étude, il ne semble pas y avoir de preuve évidente de la nature cholinergique de ces fibres, qui seraient des fibres sensitives afférentes. On n'a ainsi pas identifié de terminaisons parasympathiques.

Parmi les pulpes examinées, celle de l'Homme, du Lapin et du Chat recevaient des nerfs sympathiques adrénergiques associés à des vaisseaux sanguins; les pulpes des incisives des rats étaient dépourvues d'innervation vaso-motrice.

ZUSAMMENFASSUNG

ACETYLCHOLINESTERASE UND NORADRENALIN IN DEN NERVEN DER ZAHNPULPA EINIGER SÄUGETIERE

Zum Nachweis von Acetylcholinesterase und Noradrenalin in der Pulpa von Menschen, Kaninchen, Katzen und Ratten wurden histochemische Methoden angewandt. Alle untersuchten Pulpas enthielten acetylcholinesterase-haltige Nervenfasern, am wenigsten die der Rattenschneidezahne. Angesichts dieser Ergebnisse sprechen keine überzeugenden Anhaltspunkte für die cholinergische Natur dieser Fasern, die man ausschliesslich für afferente sensorische Fasern hält. Dementsprechend wurden keine parasympathischen Nervenenden gefunden. In den untersuchten Pulpas von Menschen, Kaninchen und Katzen waren die adrenergischen Fasern mit Blutgefässen verbunden; in der Pulpa der Schneidezähne von Ratten fanden sich keine vasomotorischen Nerven.

REFERENCES

Anneroth G. & *K. A. Norberg,* 1968: Adrenergic vasoconstrictor innervation in the human dental pulp. Acta odont. scand. *26:* 89.

Armenio G. & *P. D. Laforgia,* 1955: Sulla innervazione parasimpatica della polpa dentale. Clin. Odont. *10:* 191.

Arwill T., 1958: Innervation of the teeth. Transactions of the Royal Schools of Dentistry. Dissertation, Stockholm.

Avery J. K. & *R. Rapp,* 1959: An investigation of the mechanism of neural impulse transmission in human teeth. Oral Surg. Oral Med. Oral Path. *12:* 190.

Bailey A. I., 1968: The nature of collagen. In: Extracellular and supporting structures, ed. M. Florkin and E. H. Stotz. p. 297. Elsevier publishing comp., Amsterdam.

Bolme P. & *K. Fuxe,* 1967: Identification of sympathetic cholinergic nerve terminals in arteriole of skeletal muscle. Acta pharmacol. toxicol. *25:* 79.

Cavallazzi C., A. Veggetti & *E. Callegari,* 1966: Acetylcholinesterase activity in the dental pulp of various age bovines. Riv. Biol. *59:*125.

Couteaux R., 1958: Morphological and cytochemical observations on the post-synaptic membrane at motor end-plates and ganglionic synapses. Exp. Cell Res. *5:* 294.

Dahlström A. & *K. Fuxe,* 1964: A method for the demonstration of adrenergic nerve fibres in peripherial nerves. Z. Zellforsch. Mikroskop. Anat. *62:* 602.

Eränkö O., 1967a: The practical histochemical demonstration of catecholamines by formaldehyde induced fluorescence. J. Roy. Micr. Soc. *87:* 259.

Eränkö O., 1967b: Histochemistry of nervous tissues: Catecholamines and cholinesterases. Ann. Rev. Pharmacol. *7:* 203.

Falck B., N-Å. Hillarp, G. Thieme & *A. Torp,* 1962: Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. *10:* 348.

Fearnhead R. W., 1967: Innervation of dental tissues. In: Structural and chemical organization of teeth vol. 1, ed. A.E.W. Miles. p. 247. Academic Press, N.Y.

Folkow B., J. Frost, K. Haeger & *B. Uvnäs,* 1948: Cholinergic fibres in the sympathetic outflow to the heart in the dog and cat. Acta physiol. scand. *15:* 421.

Gerebtzoff M., 1959: Cholinesterases: A histochemical contribution to the solution of some functional problems. Pergamon Press, N.Y.

Giacobini E., 1957: Quantitative determination of cholinesterase in individual sympathetic cells. J. Neurochem. *1:* 234.

Hillarp N.-Å., 1959: The construction and functional organization of the autonomic innervation apparatus. Acta physiol. scand. *46:* Suppl. 157.

Hurley H. J. & *H. Mescon,* 1956: Cholinergic innervation of the digital arterio-venous anastomoses of human skin. J. appl. Physiol. *9:* 82.

Karnovsky H. J. & *L. Roots,* 1964: A »direct-colouring» thiocholine method for cholinesterases. J. Histochem. Cytochem. *12:* 219.

Klinge E., P. Pohto & *E. Solatunturi,* 1968: To be published.

Koelle G. B., 1955: The histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. J. Pharmac. exp. Therap. *114:* 167.

Koelle G. B., 1963: Cytological distributions and physiological functions of cholinesterases. In: Cholinesterases and anticholinesterase agents. Handbuch der experimentellen Pharmakologie XV, ed. G. B. Koelle. p. 187. Springer-Verlag, Berlin.

Lim R. K. S., 1968: Neuropharmacology of pain and analgesia. In: Pharmacology of pain, ed. R.K.S. Lim. p. 169. Permagon Press, Oxford.

Neidle E. & F. Liebman, 1964a: Effects of vasoactive drugs and nerve stimulation on blood flow in the tooth pulp and allied structures of the cat. J. dent. Res. *43:* 412.

Neidle E. & F. Liebman, 1964b: The effect on blood flow to the mandibular area of stimulation of the peripheral mandibular nerve. J. dent. Res. *43:* 907.

Ogilvie R. W., L. A. Gillilan & D. E. Knapp, 1966: Physiologic evidence for the presence of vasoconstrictor fibers in the dental pulp. J. dent. Res. *45:* 980.

Pohto M. & A. Scheinin, 1962: Effect of local analgesic solutions on the circulation of the dental pulp. Int. dent. J. *12:* 227.

Pohto P. & R. Antila, 1968: Demonstration of adrenergic nerve fibres in human dental pulp by histochemical fluorescence method. Acta odont. scand. *26:* 137.

Prenna G. & S. Sacci, 1964: Primi resultati di microspettrofluorometria qualitativa del tessuto elastico e collagene. Boll. Soc. med.-clin. *78:*779.

Provenza D. V., 1958: The blood vascular supply of the dental pulp with emphasis on capillary circulation. Circulat. Res. *6:* 213.

Quintarelli G., 1961: Cited by Cavallazzi C. et al. 1966.

Rapp R., J. Avery & D. Strachan, 1967: Cholinesterase enzymes in human primary teeth. J. Dent. Child. *34:* 336.

Saunders R. L. de C. H. & H. Röckert, 1967: Vascular supply of dental tissue, including lymphatics. In: Structural and chemical organization of teeth vol. 1, ed. A.E.W. Miles. p. 199. Academic Press, N.Y.

Spriggs T. L. B., J. D. Lever & J. D. P. Graham, 1968: Evidence against the participation of acetylcholine in transmission of sympathetic adrenergic nerve terminals. Naunyn-Schmiedebergs Arch. Pharmak. u. exp. Path. *259:* 197.

Taxi J., 1952: Action du formol sur l'activité de diverses préparations de cholinestérases. J. Physiol. Path. gén. *44:* 595.

Taylor A. C., 1950: Microscopic observation of the living tooth pulp. Science. *111:* 40.

Ten Cate A. R. & L. Shelton, 1966: Cholinesterase activity in human teeth. Arch. oral Biol. *11:* 423.

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