J. Anat. (1973), **115**, 2, pp. 277–288
With 10 *figures*
Printed in Great Britain

The free penicillate nerve endings of the human hairy skin

NIKOLAJS CAUNA

Department of Anatomy and Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

(*Accepted* 13 *April* 1973)

INTRODUCTION

The skin possesses two morphologically distinct kinds of sensory receptor organs: (1) the 'corpuscular' receptors and (2) the so-called 'free' nerve endings (Cauna, 1966). The corpuscular receptors can easily be recognized histologically in unstained preparations, and even some time after degeneration of their nerves, because of the non-nervous components within their structure. The free nerve endings, on the other hand, can only be visualized by the use of neurohistological or specific histochemical procedures that reveal nerve fibres. The free nerve endings have a widespread distribution in the skin, mucous membranes, joints, periosteum, bone and viscera. In some situations they constitute the only kind of sensory receptor organ (Bradlow, 1939; Lele & Weddell, 1956; Rapp, Avery & Rector, 1957; Whitear, 1960; Cauna, Hinderer & Wentges, 1969; Schulman & Dorfman, 1970). Yet so far these receptor organs have attracted relatively little attention from morphologists.

In addition to the tactile hairs and Merkel corpuscles, the superficial corium of the human hairy skin and the overlying epidermis contain at least two morphologically distinct kinds of free nerve endings. The dermal papillae surrounding the hair orifices reveal simple papillary endings that exhibit a positive butyrylcholinesterase activity. They are derived from nerve fibres that ascend towards the surface alongside the hair sheaths. The rest of the superficial corium contains free endings of 'horizontal' distribution, frequently referred to as the subepidermal nerve net. The present paper reports a study of the endings that compose this network. It is proposed to call them 'penicillate endings' because of the typical tuft-like manner of their arrangement.

MATERIAL AND METHODS

The material was obtained from 22 individuals of both sexes ranging in age from 11 months to 54 years. Skin samples were obtained from the deltoid region with a high-speed dermal punch without anaesthesia. For routine electron microsopy the tissue was immediately cut into strips oriented perpendicularly to the external surface of the epidermis with the use of two stainless-steel razor blades mounted parallel and 0·3 to 0·5 mm apart. The slices were fixed in 1 % osmic acid solution buffered at pH 7·4 with phosphate buffer for 2 h at 4 °C. During dehydration through the alcohol series, the strips were divided into blocks of desired orientation. They were embedded in an Epon–Araldite mixture (Mollenhauer, 1964), cut on a Cambridge–Huxley microtome with a diamond knife in serial ribbons and stained on grids with

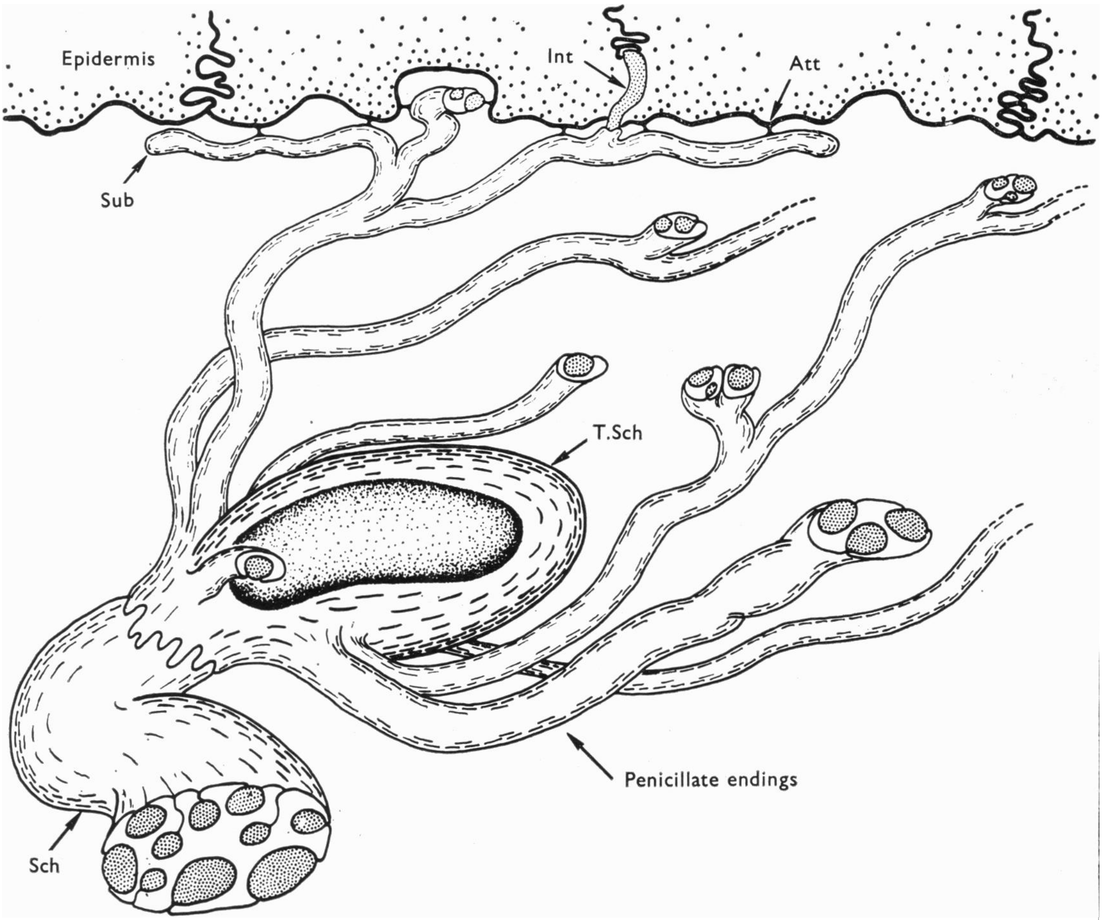


Fig. 1. Diagram showing the manner of origin of the free penicillate nerve endings. Sch, Schwann sheath of a preterminal nerve fibre carrying non-myelinated sensory axons. T.Sch, terminal Schwann cell which gives origin to penicillate endings and provides them with cytoplasmic sheaths for the length of their corial course. The endings terminate either in the subepidermal zone of the corium (Sub) or intraepidermally (Int). Att, attachment between the basal laminae (basement membranes) of the epidermis and the ending.

1 % uranyl acetate solution for 10 minutes (Watson, 1958) and lead citrate solution for 1 minute (Reynolds, 1963). Electron micrographs were made on a Philips 300 microscope. Particular effort was made to trace structures in serial and semiserial sections. Cholinesterase activity was demonstrated in frozen sections using a modified Koelle technique (Naik, 1963). Ethopropazine hydrochloride was used in a concentration of 10 μM as a selective inhibitor of butyryl cholinesterase (Bayliss & Todrick, 1956). Eserine controls were employed with both substrates, and they gave negative reactions.

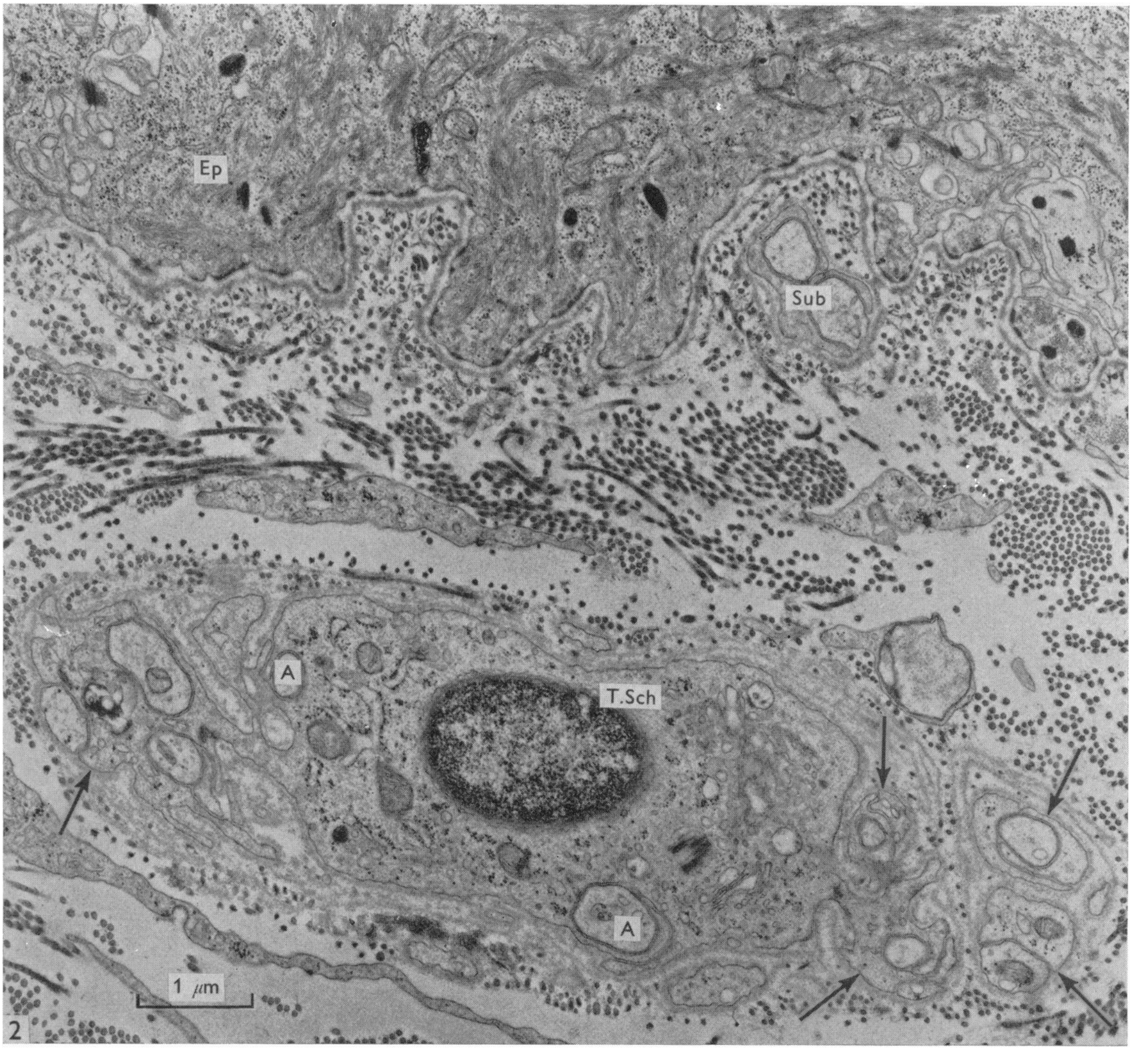


Fig. 2. Terminal Schwann cell (T.Sch) cut transversely through its proximal part (cf. with Fig. 1) showing the origin of penicillate endings (arrows). Two axons (A) are still enfolded within the Schwann cell body. The latter is characterized by well-developed Golgi systems (seen to the right of the cell nucleus) and a somewhat distended cisternae of the granular endoplasmic reticulum (to the left of the nucleus). The basal lamina of the terminal Schwann cell and the endings consists of several layers interleaved with collagen fibres. Ep, epidermis of the hairy skin. Sub, subepidermal nerve terminal, containing two axons. The basal lamina of the ending is connected to that of the epidermis. Male, 11 years old. ×15000.

RESULTS

The endings in the subepidermal network were found to originate from non-myelinated preterminal nerve fibres in a typical pattern (Fig. 1). The point of origin of the endings was marked by the perikaryon of a modified Schwann cell which constituted the terminal cell of the Schwann sheath. This cell was usually found

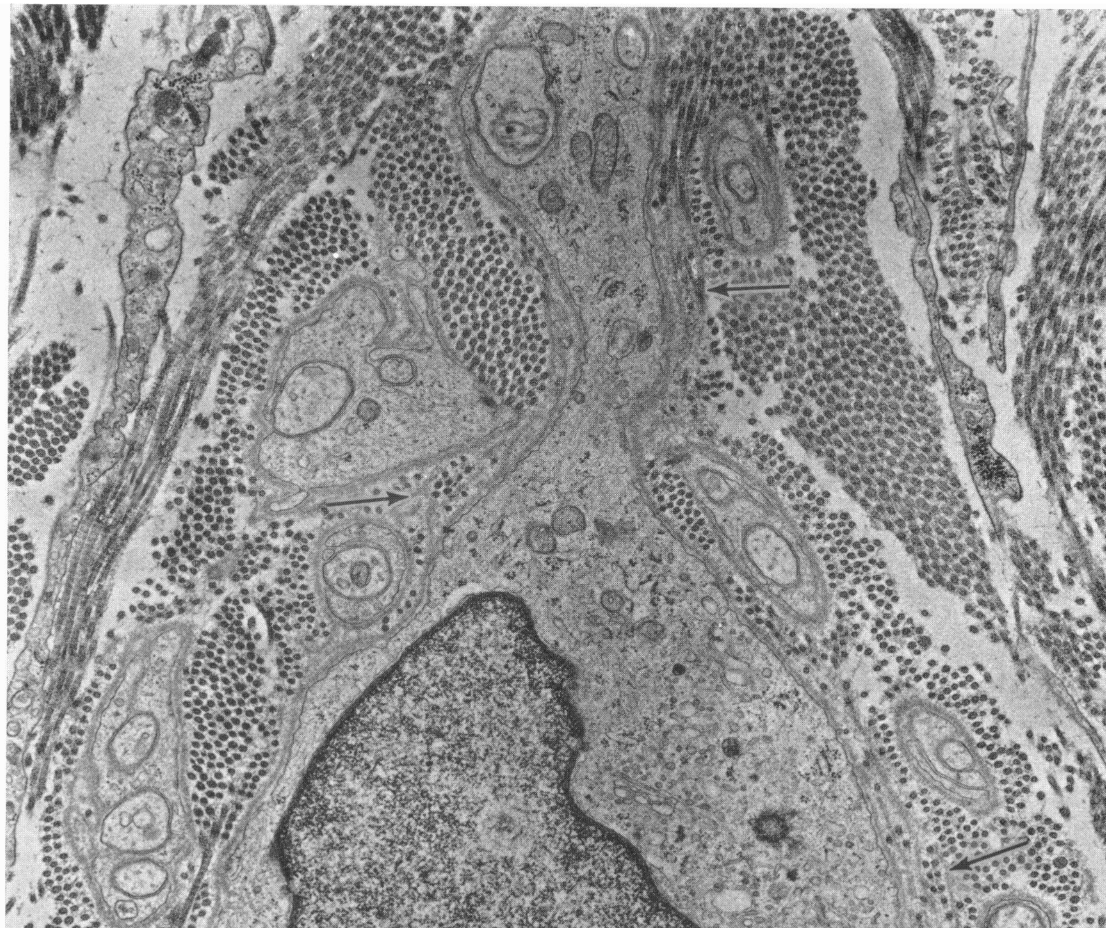


Fig. 3. Terminal Schwann cell cut transversely through its middle part, and the penicillate endings derived from the cell. The penicillus is surrounded by a zone of collagen fibres oriented the same way as the nerves. In some areas the fibres are tied to the cell body and to the endings by layers of the basal lamina (arrows). Male, 24 years old. × 15000.

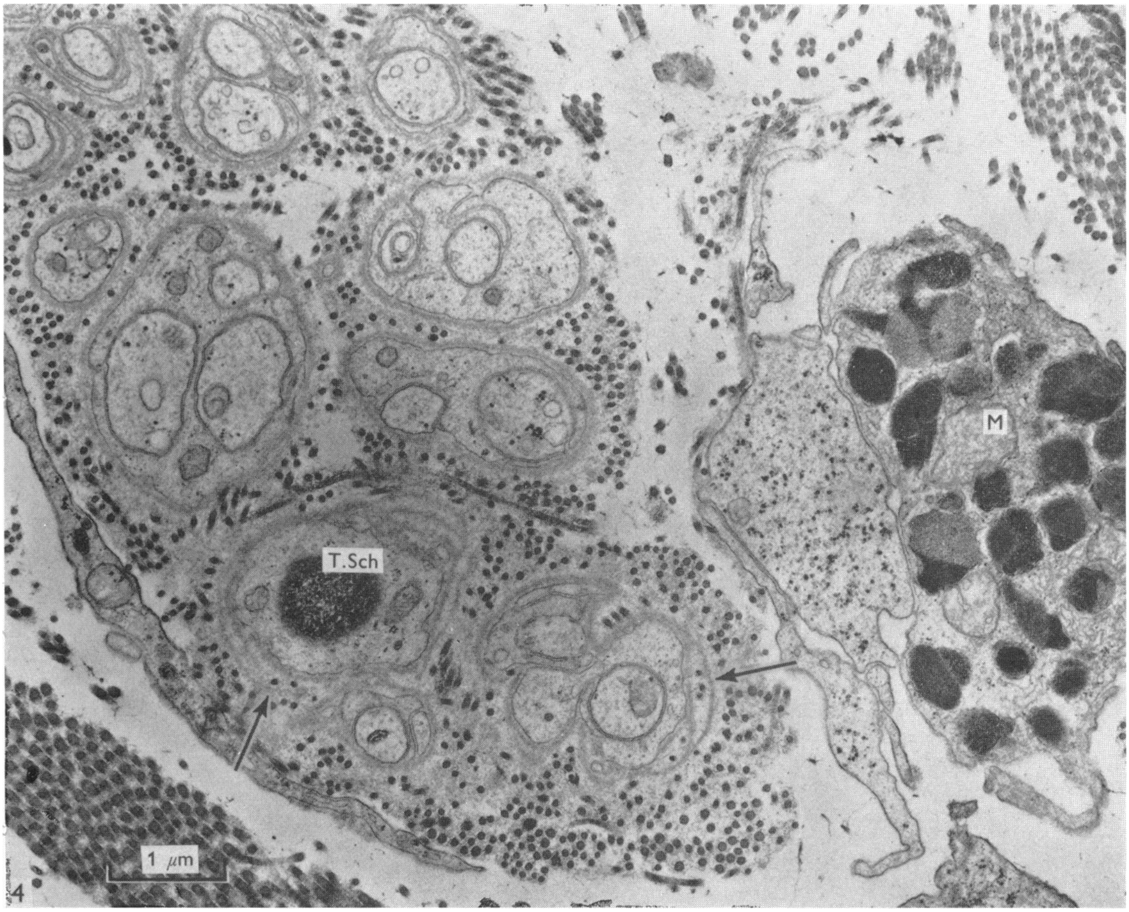
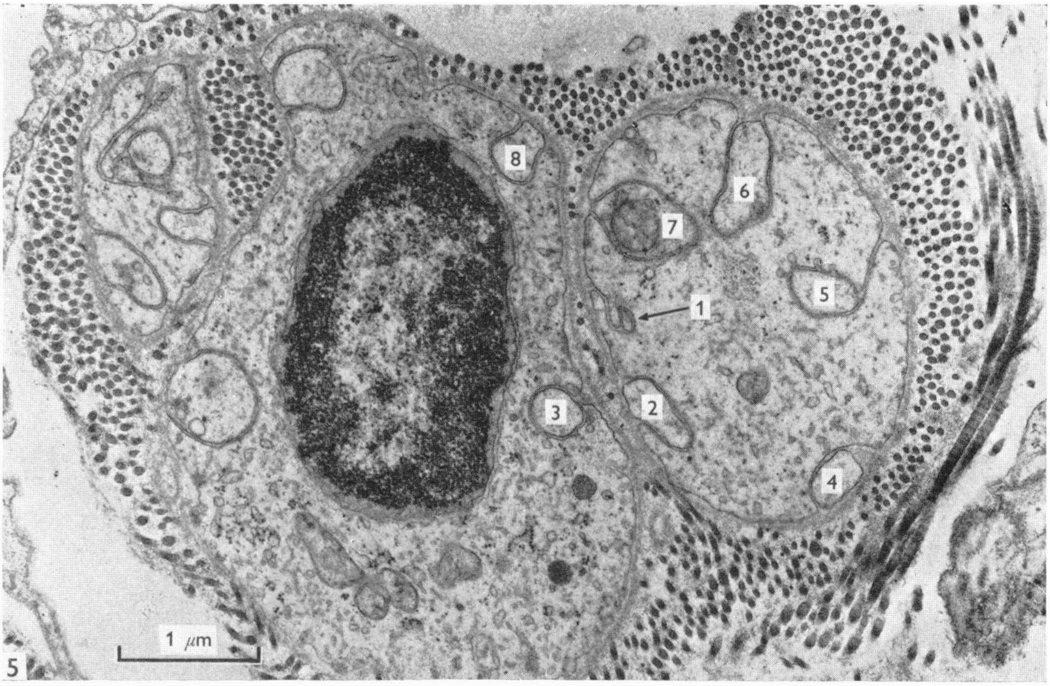


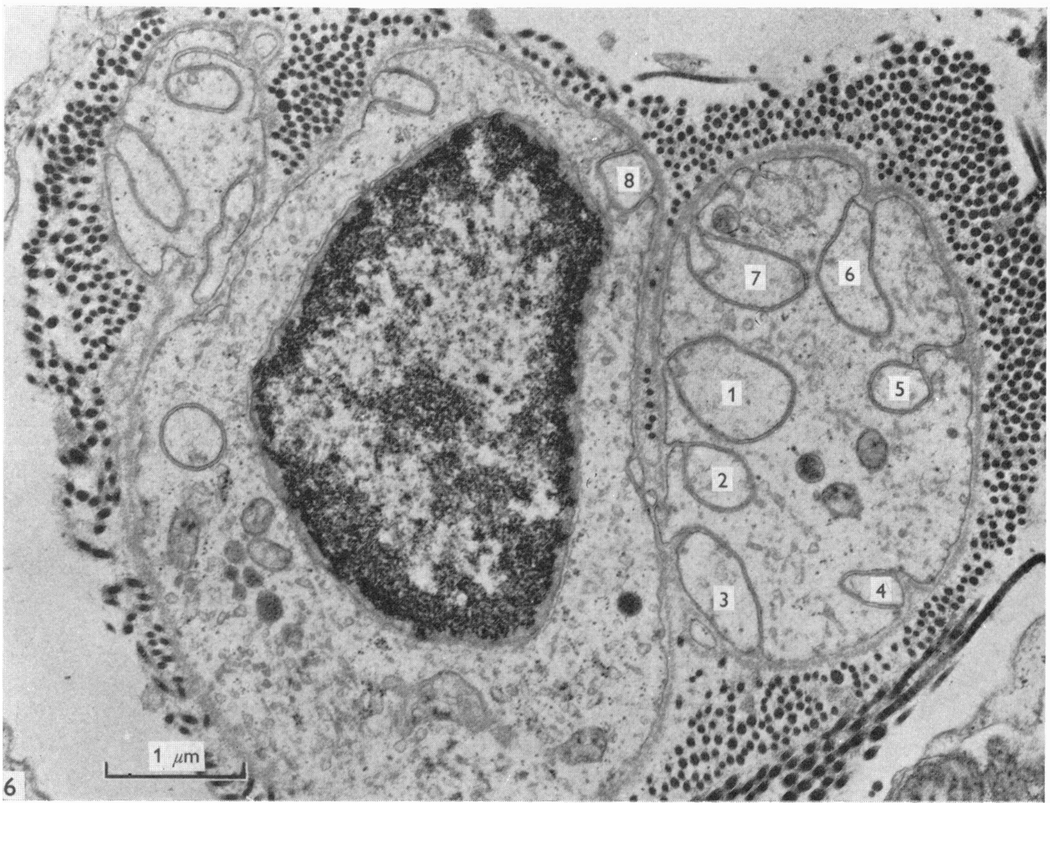
Fig. 4. A set of penicillate nerve endings and the associated terminal Schwann cell (T.Sch) cut transversely. The endings are accompanied by collagen fibres which follow the same course as the nerves. In some areas the fibres are tied to the endings by layers of basal lamina (arrows). M, mast cell. Male, 20 years old. × 15000.

2–6 μM deep to the epidermis (Fig. 2) and its proximal extremity joined the regular Schwann sheath to receive the sensory axons. The penicillate endings usually emerged from the proximal or middle part of the terminal Schwann cell so that most of its perikaryon projected distally beyond the origin of the nerves (compare Fig. 1 with Figs. 2–6). The terminal Schwann cell was the only source of the cytoplasmic sheaths for all its endings and their branches within the corium.

The cytoplasm of the terminal Schwann cell body was characterized by well-developed Golgi zones (Figs. 2, 3). Some slightly distended cisternae of the granular endoplasmic reticulum and some polyribosomes were usually present.

During their initial course the penicillate endings proceeded in a compact fascicle closely related to the terminal Schwann cell, frequently connected to one another and to the Schwann cell body by common layers of the basal lamina (Figs. 2, 3, 5, 6).


8
7
6
5
1
2
3
4
1 μm
5


8
7
6
1
5
2
3
4
1 μm
6

During this initial course axons sometimes departed from one sheath and joined another (e.g. axon no. 3 in Figs. 5 and 6). Such an exchange of non-myelinated axons was also observed in larger nerve fascicles of the corium.

The basal lamina of the terminal Schwann cell and that of the emerging penicillate endings was surrounded by a zone of collagen fibres oriented in the same direction as the nerves. In areas where the basal lamina formed multiple layers, the fibres were frequently interleaved with the laminae (arrows in Figs. 2–4). When the endings diverged, each received its share of collagen fibres, which accompanied the ending and its branches throughout their winding course almost to their termination (Figs. 7, 8). The collagen zone around the nerve endings could clearly be distinguished from the rest of the collagen fibres of the superficial corium in which the fibres formed a complex three dimensional meshwork (Fig. 7). Those belonging to the nerve endings were embedded in an amorphous material that appeared to be continuous with the basal lamina of the nerve ending and sometimes formed distinct compartments for small groups of fibres (arrows in Fig. 8). On reaching the immediate subepidermal zone, the endings usually proceeded within grooves on the deep aspect of the epidermis, avoiding the dermal papillae in the vicinity of the hair orifices. The accompanying collagen fibres gradually departed and connexions were established between the basal lamina of the nerve and that of the epidermis (compare Fig. 1, Att, with Figs 2, 9).

Most of the penicillate endings seemed to terminate in the subepidermal corium. Intraepidermal axon terminals (compare Fig. 10, arrow, with Fig. 1, Int) were exceedingly rare, especially in the adult skin. The subepidermal endings could seldom be traced successfully to their termination. In the few instances where tracing was possible the Schwann sheath element extended beyond the longest axon terminal for some distance.

The axons of the penicillate endings varied in diameter throughout their course. After divisions, the diameters of the branches did not decrease, but continued to vary within the same ranges, so that an axon of a proximal stem nerve could not be distinguished from that of a terminal branch on the basis of their diameters. Usually, the change in axon diameter was gradual and continuous. Sudden transitions between extremely narrow segments and bulbous enlargements were relatively infrequent but could be dramatic (Figs. 5, 6).

The axoplasm of the penicillate nerve endings was characterized by the paucity of organelles. Some single thin-walled microvesicles ranging from 40 to 80 nm in diameter were occasionally present in the stem axons as well as in the axon terminals. An accumulation of mitochondria was almost never observed, except in some intraepidermal terminals (Fig. 10).

Figs. 5 and 6. Terminal Schwann cell cut transversely through its proximal (Fig. 5) and middle regions (Fig. 6). The two figures represent the most proximal and the most distal sections of a series of five. The axons in the larger penicillate trunk and in the adjacent aspect of the Schwann cell body are numbered from 1–8 to facilitate comparison. Note in particular that axon no. 1 has increased in diameter from approximately 0·15 μm to 1 μm, and that axon no. 3 has moved from the perikaryon of the terminal Schwann cell to the trunk of the penicillate endings. Male, 24 years old. × 18000.

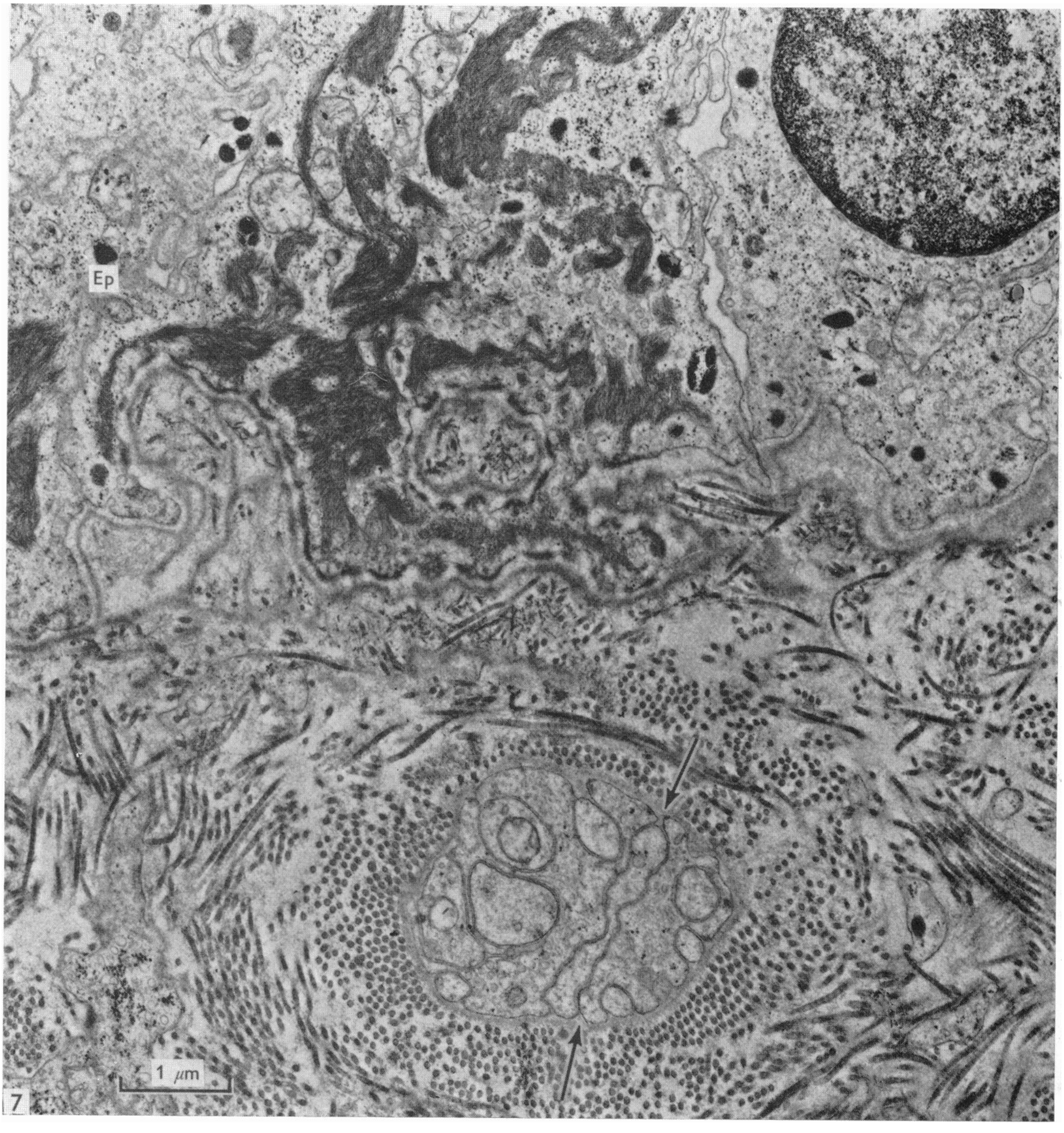


Fig. 7. Penicillate ending cut transversely a short distance from its origin. The ending is about to divide along the cleavage lines indicated by the arrows. It is surrounded by a zone of collagen fibres that faithfully follow the same course as the winding nerve. In contrast, the collagen fibres of the superficial corium form a complex three dimensional meshwork. Ep, epidermis. Male, 24 years old. × 15000.

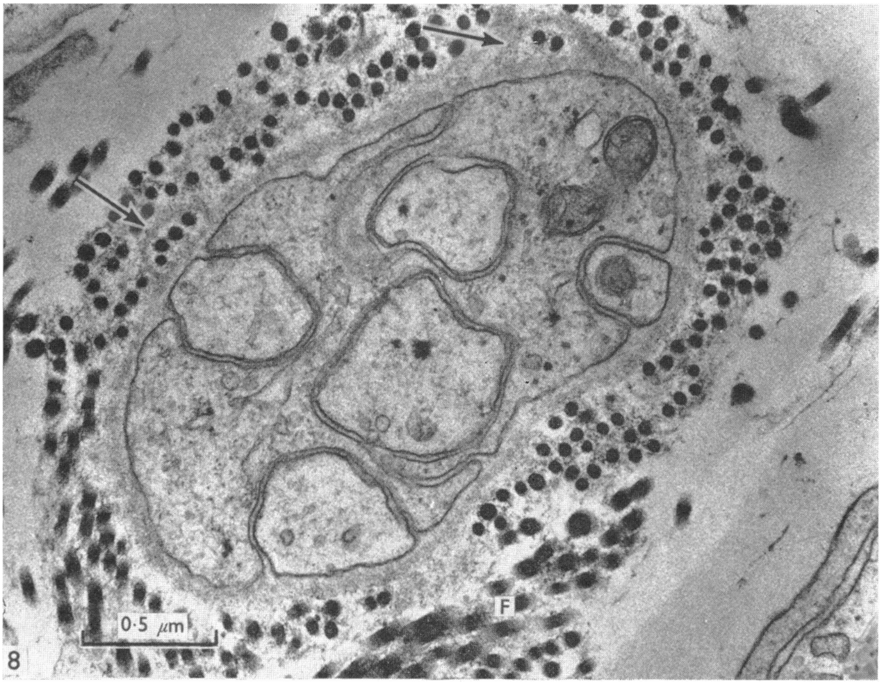


Fig. 8. Penicillate ending containing five axon terminals, accompanied by a zone of collagen fibres. These are partly embedded in an amorphous material that appears to be continuous with the basal lamina of the nerve, sometimes forming distinct compartments (arrows). F, fascicle of collagen fibres not associated with the nerve ending. Male, 11 years old. ×35000.

Histochemically, the penicillate endings gave negative acetyl- and butyrylcholinesterase reactions even after prolonged periods of incubation. This was in contrast to the papillary endings which were encountered in the immediate vicinity of the hair orifices and which exhibited butyrylcholinesterase activity.

DISCUSSION

The typical tuft-like origin of the nerve endings from the terminal Schwann cell should justify the proposed term 'penicillate'. Each penicillus seems to constitute a unit dependent upon the integrity of the respective terminal Schwann cell, since the latter is the only source of the cytoplasmic sheaths for all endings. It can be presumed that irreparable damage to this cell would result in loss of the whole penicillus and its eventual regeneration from the proximal nerve segment.

The endings appear to be derived from non-myelinated nerve fibres. This is a speculation since the preterminal non-myelinated fibres of the penicillate endings were never traced to larger nerve fascicles of the corium. However, there is no other ending of a widespread distribution in the hairy skin that could account for the presence of the sensory non-myelinated axons in the cutaneous nerves. Moreover, the number of myelinated axons encountered in the cutaneous nerve fascicles seems

Fig. 9. Subepidermal penicillate ending cut transversely, containing four axon terminals. The ending is accommodated within a groove on the deep aspect of the epidermis. The basal lamina of the ending is connected to that of the epidermis in two areas (arrows). Male, 11 years old. ×25000.

to be insufficient to supply the tactile hairs and the associated receptors, and to give origin to the penicillate endings as well.

While the non-myelinated origin of the penicillate endings seems to be reasonably established, the homogeneity of their axons may be disputed. Adjacent non-myelinated nerve bundles regularly exchange axons along their course, so that those enclosed within a common Schwann sheath near the sensory ganglion are not necessarily the same as those encountered in the periphery. Consequently, the penicillate endings may be composed of different types of non-myelinated axons having different destinations and may be associated with one or several sensory modalities.

The functional role of the penicillate endings is subject to speculation. The paucity of organelles in the axoplasm of the endings suggests that they are rapidly adapting receptors. They seem to be protected against acute deformation because of the

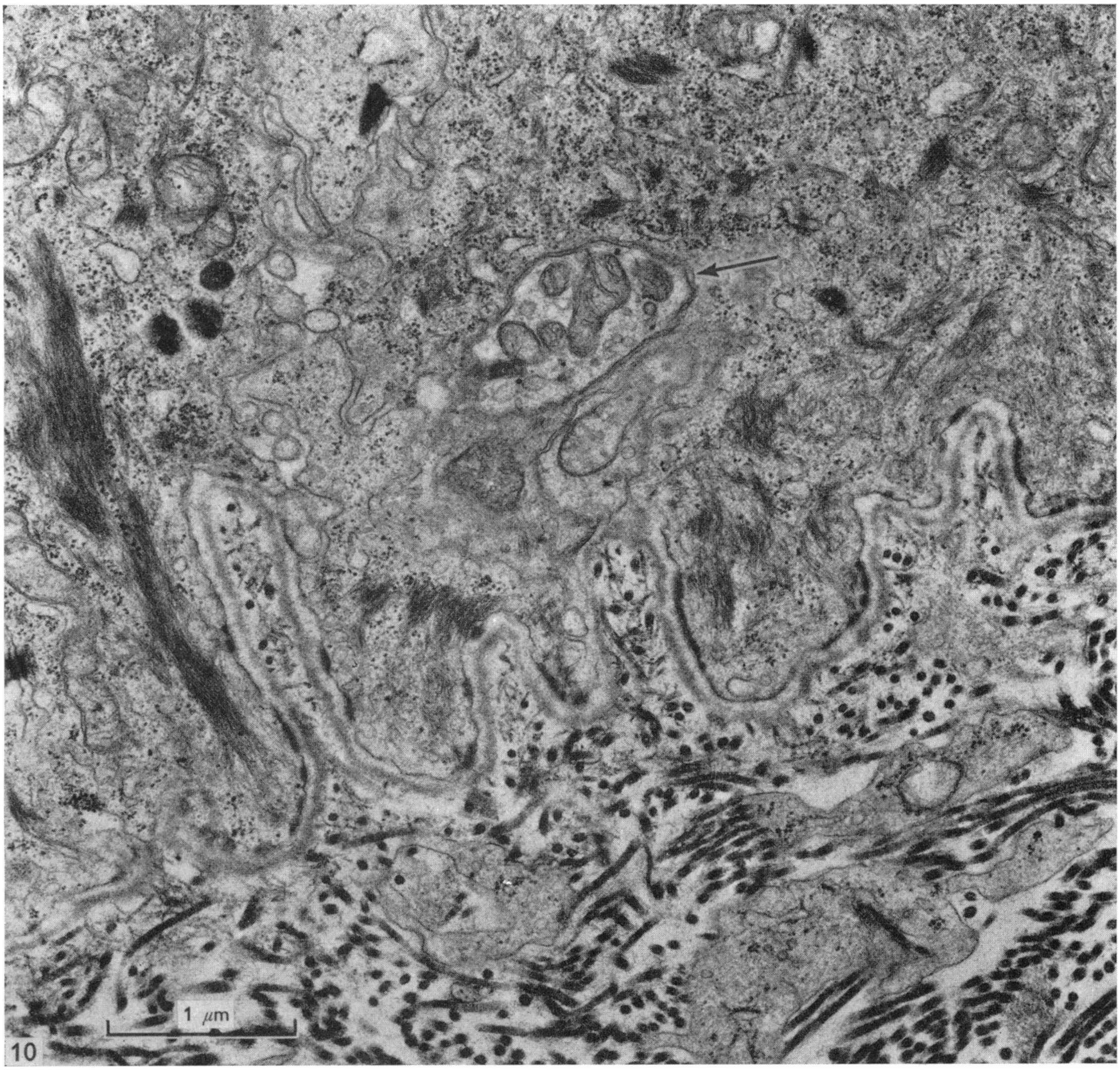


Fig. 10. Intraepidermal axon terminal (arrow) of a penicillate nerve ending enfolded by the cell membrane of a keratinocyte. Female, 21 years old. × 25000.

accompanying zone of collagen fibres and the discrete attachments between the endings and the epidermal basal lamina. Therefore they do not seem to be designed for effective mechanical stimulation. The main morphological characteristic of this receptor organ is the extensive neural surface area distributed in the corium next to and inside the epidermis. This arrangement secures the efficiency of its components in collecting the appropriate stimuli, but renders them inefficient in precise localization or two-point discrimination. The exact function or functions of the penicillate nerve endings remains an open question to be resolved by experimental investigation.

SUMMARY

The human hairy skin has been investigated electron microscopically and histochemically in 22 individuals aged 11 months to 54 years.

The free penicillate endings are derived from non-myelinated nerve fibres. Their origin in the superficial corium is marked by the perikaryon of a modified Schwann cell which constitutes the terminal cell of the Schwann sheath. This cell is the only source of the cytoplasmic sheaths to all endings and their branches.

The endings are poor in axoplasmic organelles. They give negative reactions to acetyl- and butyryl-cholinesterases. They do not seem to be designed for effective mechanical stimulation. Their main characteristic is the extensive neural surface area that is distributed in the corium next to and inside the epidermis. This arrangement secures their efficiency in collecting the appropriate stimuli, but renders them inefficient in precise localization or two point discrimination.

The author wishes to thank Miss Agnes Cralley for her technical assistance. This study was supported by U.S. Public Health Service Grant no. NB 04147.

REFERENCES

BAYLISS, B. J. & TODRICK, A. (1956). The use of selective acetylcholinesterase inhibitor in the estimation of pseudocholinesterase activity in rat brain. *Biochemical Journal* **62**, 62–67.

BRADLOW, R. (1939). The histology and histopathology of the dental innervation. *Proceedings of Royal Society of Medicine* **32**, 1040–1053.

CAUNA, N. (1966). Fine structure of the receptor organs and its probable functional significance. In *Ciba Foundation Symposium on Touch, Heat, and Pain* (Ed. A. V. S. de Reuck and Julie Knight), pp. 117–136. London: Churchill.

CAUNA, N., HINDERER, K. H. & WENTGES, R. T. (1969). Sensory receptor organs of the human nasal respiratory mucosa. *American Journal of Anatomy* **124**, 187–210.

LELE, P. P. & WEDDELL, G. (1956). The relationship between neurohistology and corneal sensibility. *Brain* **79**, 119–154.

MOLLENHAUER, H. H. (1964). Plastic embedding mixtures for use in electron microscopy. *Stain Technology* **39**, 111–114.

NAIK, N. T. (1963). Technical variations in Loelle's histochemical method for demonstrating cholinesterase activity. *Quarterly Journal of Microscopical Science* **104**, 89–100.

RAPP, R., AVERY, J. K. & RECTOR, R. A. (1957). A study of the distribution of nerves in human teeth. *Journal of the Canadian Dental Association* **23**, 447–453.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SCHULMAN, L. & DORFMAN, H. (1970). Nerve fibers in osteoid osteoma. *Journal of Bone and Joint Surgery* **52A**, 1351–1356.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology* **4**, 475–478.

WHITEAR, M. (1960). An electron microscope study of the cornea in mice, with special reference to the innervation. *Journal of Anatomy* **94**, 387–409.