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The distribution of nerves, monoamine oxidase and cholinesterase in the skin of cattle

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The sweat glands in cattle are active organs (McDowell, McDaniel, Barrada & Lee, 1961; McLean, 1963). Taneja (1959) suggested on indirect evidence that they were supplied by adrenergic nerves and Findlay & Robertshaw (1964) showed that thermal sweating in the bovine requires an intact sympathetic nerve supply. There appear to be no histological studies of the innervation of the skin of cattle apart from investigations on the nerve endings in the muzzle (Nisbet, 1956) and in the hoof (Wagai & Tohara, 1962). The present work was therefore undertaken to investigate histologically the innervation of the skin of cattle and the distribution of monoamine oxidase and cholinesterase within it with particular reference to the sweat glands.

MATERIALS AND METHODS

Specimens of skin (15 × 15 mm) were taken from the neck or dewlap immediately after slaughter from a total of seventy-five adult cattle (bulls, bullocks and cows) of varying ages (2–12 years) and breeds (Aberdeen-Angus, Ayrshire, Dairy Shorthorn, Friesian, Galloway and Hereford). Skin samples were also obtained by biopsy, using the technique of Findlay & Jenkinson (1960), from ten Ayrshire bull calves (aged 6–12 months) and from areas of skin on four Ayrshire bullocks (aged 5–11 months) 6–10 weeks after they had been sympathectomized as described by Findlay & Robertshaw (1965). Five different techniques were used in the preparation of the various skin specimens for microscopic study.

Silver impregnation

Specimens of skin from fifteen adult animals, five bull calves and from sympathectomized areas on two bullocks were fixed in 10 % neutral formalin. Frozen sections were cut at 25–30 μm, some parallel and others perpendicular to the skin surface, and processed by the method of Garven & Gairns (1952).

Methylene blue

Skin samples from twenty animals and biopsy specimens (15 × 5 mm) cut with a scalpel from the sympathectomized area on 4 bullocks were placed in normal saline at 37 °C. The biopsy specimens were taken while the bullocks were under anaesthesia (Brietal Sodium—Elanco Products Ltd.). Specimens taken from areas of skin anaesthetized locally with 5 ml of a 2 % solution of procaine hydrochloride gave less satisfactory staining results. Within 30 min of collection, horizontal and vertical

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sections 400–600 μm thick were cut with a razor blade. Of these sections some were pretreated for 15–20 min at 37 °C in normal saline and some in normal saline containing hyaluronidase. The sections were then stained in methylene blue and fixed in ammonium molybdate according to the method of Arthur & Shelley (1959). Cattle skin required 45–60 min in the staining bath and 18–24 h fixation. Good results were obtained even when 4–6 sections were processed simultaneously. Methylene blue (Gurr, Merck and BDH Vital) all gave satisfactory results but with methylene blue thiocyanate the nerve staining was less specific. After fixation the sections were washed in normal saline at 4 °C, dehydrated in acetone, cleared in xylol and mounted in DPX. Sometimes before dehydration, thinner frozen sections 25–30 μm were cut from the thick 400–600 μm pieces to allow more detailed examination.

An attempt was made, using the intra-vital staining technique of Weddell & Pallie (1954), to obtain *in vivo* staining of cutaneous nerves in the thoracic region of the back. Methylene blue in concentrations from 0·02–0·2 % was injected, sometimes after pretreatment with hyaluronidase, and biopsy specimens taken at intervals thereafter. The results were unsatisfactory, specific nerve staining not being obtained.

Localization of cholinesterase

Specimens of skin from fifteen adults, five calves and from the sympathectomized area on four bullocks were used in this study. Some were placed in normal saline at 4 °C for up to 30 min, some in normal saline at 37 °C for a similar period and others in 10 % neutral formalin at 4 °C for 3–18 h. All the specimens were treated for cholinesterase activity using the histochemical method of Naik (1963). Sections from tissue collected in normal saline at 37 °C, and incubated for 4–5 h at pH 6·2 gave the best results. The reaction could be blocked by the concentration of eserine (3×10^{-5} M) recommended by Naik, but higher concentrations (10^{-3} M and 3×10^{-4} M respectively) of ethopropazine hydrochloride [10-(2-diethylaminopropyl) phenothiazine hydrochloride] and BW 284 C51 bromide [1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide] were required for selective inhibition of the reaction.

Localization of monoamine oxidase

Skin specimens were collected from twenty adult animals after slaughter, and biopsy specimens including control specimens from normal areas were taken from the sympathectomized area on four bullocks. Most were collected in normal saline at 37 °C or at 4 °C but some were fixed for 18–24 h in 4 % neutral formalin. Frozen sections 25–30 μm thick were cut and incubated according to the procedure of Glenner, Burtner & Brown (1957). The substrate mixture recommended by these workers (tryptamine hydrochloride and nitro-blue tetrazolium) also proved to be the most satisfactory combination for bovine skin. The incubation times ranged from 1–2 h for fresh frozen sections to 18 h for formalin-fixed material. Control sections were subjected to either pretreatment in a buffered 2×10^{-1} M marsalid phosphate (1-iso-nicotinyl-2-isopropyl hydrazine) solution at pH 7·6 or concomitant inhibition by incorporation of 2×10^{-1} M marsalid phosphate or alternatively 10^{-1} M marsalid phosphate with 10^{-3} M potassium cyanide (Yasuda & Montagna, 1960) into the incubation solution.

Localization of monoamine oxidase and specific cholinesterase in the same section

Samples of skin from five normal animals and from the sympathectomized area on four bullocks were collected in normal saline at 37 °C. Frozen sections, 25–30 μm thick, were cut, incubated for 1½ h in the solution recommended for the detection of monoamine oxidase and immediately transferred to the incubation medium (a mixture of acetylthiocholine and ethopropazine hydrochloride) used for the localization of specific cholinesterase. After incubation for 5 h in this solution the sections were treated with ammonium sulphide, dehydrated, cleared and mounted. Incubating for cholinesterase before incubating for monoamine oxidase gave less satisfactory results. For comparison, sections were also incubated for non-specific cholinesterase (in a mixture of acetylthiocholine and BW 284 C 51 bromide) after incubation for monoamine oxidase.

RESULTS

Innervation

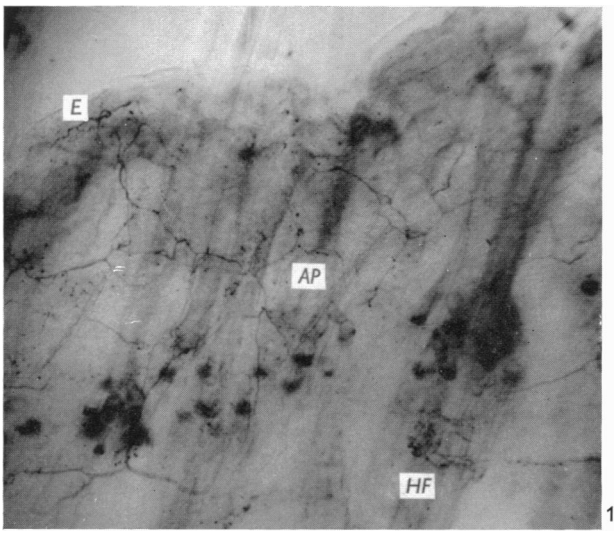
(*a*) *Methylene blue and silver techniques*

The pattern of innervation observed with both techniques was the same. Staining with methylene blue, however, both with and without pretreatment of the skin with hyaluronidase demonstrated much finer nerve fibres than was possible using silver impregnation.

The hairy skin of cattle is innervated by nerve trunks (containing at least six fibres) which are closely associated with the cutaneous blood vessels. This is especially true of the large nerve trunks in the stratum reticulare which always accompany blood vessels. In the stratum papillare, too, nerves (usually containing from 2–5 fibres) follow the course of the larger blood vessels. Branches from large nerve trunks deep in the reticular layer of the dermis ramify upwards and give rise to a branching plexus (Fig. 1). Nerves from this plexus supply the components of the hair follicle unit and the epidermis. Nerves from a large nerve trunk may, for example, supply the epidermis directly or divide, part supplying hair pallisade endings and the remainder ramifying towards the epidermis. However, although numerous nerves were observed traversing the dermis no direct nerve supply to or endings in the connective tissue were seen. Some of the fibres in the large nerve trunks deep in the dermis were myelinated. The techniques employed, however, did not permit unequivocal determination of the nature of the fibres in the smaller nerves. No description of myelination is, therefore, given.

A nerve trunk was occasionally observed crossing the fundus of a sweat gland but no fibres were clearly seen to terminate on the gland. There was certainly no visible network of nerve fibres near the glands and no clear evidence for an innervation to either the secretory or the myoepithelial cells of these glands was obtained. A nerve was often observed running parallel to the duct of the sweat gland and ending in the epidermis at the upper part of the hair follicle. There were, however, no visible branches from this nerve to the duct.

Large nerves in their passage through the skin give rise to a series of side branches each of which terminates in a well-developed pallisade of nerve fibres surrounding the hair below the sebaceous gland (Figs. 2, 3). The branch nerve divides as it



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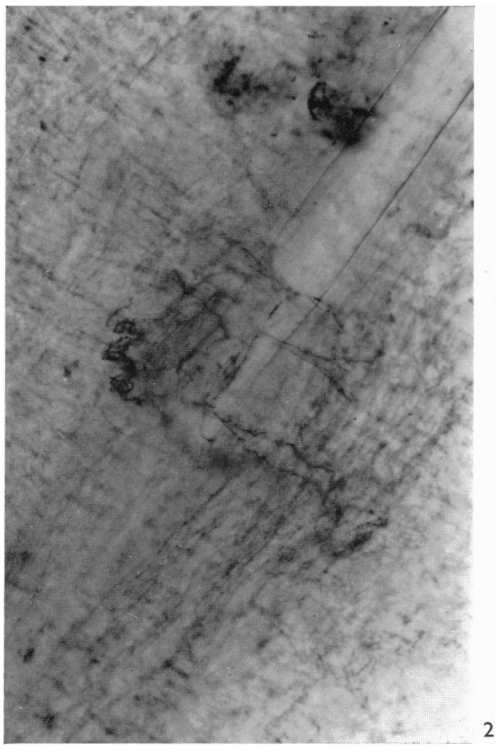
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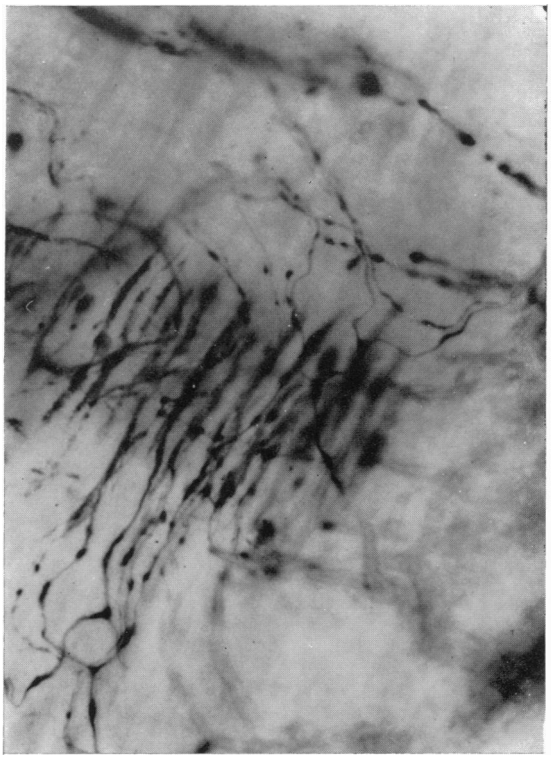
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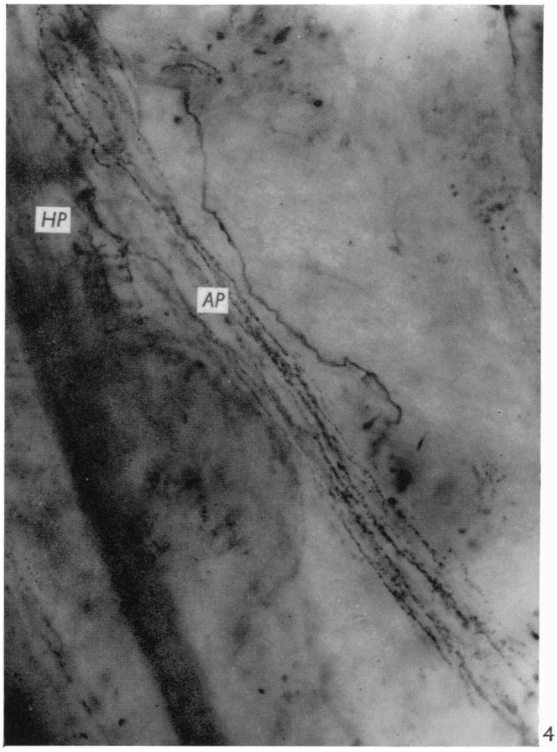

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approaches the hair follicle giving rise to two smaller branches, one on each side of the follicle. These branches further divide and give rise to two systems of fibres, an inner one longitudinal to the hair follicle and an outer circular one. A nerve fibre emerging from the system of fibres in the pallisade was sometimes seen to enter a neighbouring pallisade. In one section four pallisade endings were apparently connected in this way. Occasionally a nerve fibre was seen running between the hair pallisade ending and the parallel system of nerve fibres supplying the arrector pili muscle. It was impossible to determine with certainty, however, whether these were direct connexions or whether the nerve fibre merely passed through the muscle or pallisade nerve supply or both. Other than the nerve fibres composing the hair pallisade and fibres ending freely in the epidermis at the upper part of the hair follicle, the remaining nerves in close proximity to the hair follicle were apparently associated with the blood vessels supplying it.

The arrector pili muscle is innervated by a nerve which branches as it approaches the muscle and enters it midway along its length and sometimes near either end. Other branches of this nerve ramify and travel to other organs and to the epidermis. A number of parallel fibres which originate from the main nerve and are sometimes interconnected by short branches run along the length of the arrector pili muscle (Fig. 4). A nerve fibre leaving the nerve supply of an arrector pili muscle can be seen to disappear among the parallel nerve fibres in a neighbouring one, and as described above a nerve fibre from the hair pallisade ending can sometimes be seen to enter the muscle nerve supply. The nerve supplying the muscle could be demonstrated after silver impregnation but the system of fine parallel fibres was not so clearly defined with this technique.

Fine nerve fibres can be seen in close association with the sebaceous glands (Fig. 5), which appear to be innervated by freely ending nerve fibres. The epidermis is supplied by numerous single nerve fibres which occasionally intertwine underneath it but end freely in it. No encapsulated nerve endings were observed in the epidermis or in the dermis. The cutaneous arteries are innervated by a dense network of nerve fibres (Fig. 6) which appear to terminate in the tunica adventitia and outer tunica media. The veins do not have a similar nerve plexus and are served only by single nerve fibres.

Fig. 1. Photomicrograph of a section cut perpendicular to the skin surface illustrating the nerve supply to the skin. The branching plexus of nerves, the nerve supply of the arrector pili muscle (*AP*), the epidermis (*E*) and the hair follicle (*HF*) can be seen. Methylene blue, × 50.

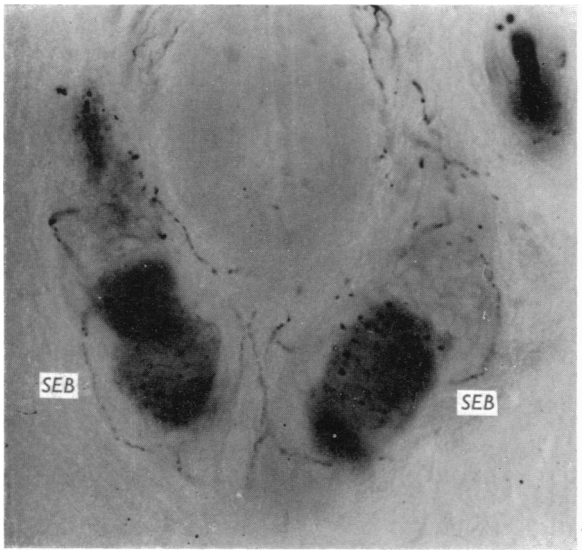
Fig. 2. Photomicrograph illustrating nerve fibres both circular and longitudinal to the hair follicle, forming a typical pallisade nerve ending around the follicle. Methylene blue, × 400.

Fig. 3. Photomicrograph illustrating nerve fibres ending in close relationship with the hair follicle in the hair pallisade ending. Silver, × 600.

Fig. 4. Photomicrograph of a section cut perpendicular to the skin surface illustrating the nerve supply to the arrector pili muscle (*AP*). Note the fine nerve fibres running parallel to the length of the muscle. Part of a hair pallisade ending (*HP*) is also shown and a nerve fibre emerging from it and disappearing amid the nerve fibres supplying the muscle can be seen. Methylene blue, × 150.

(*b*) *Monoamine oxidase and cholinesterase*

Monoamine oxidase (MAO) and specific cholinesterase (AChE) were present in nerve fibres. Non-specific cholinesterase (ChE) was not present in the cutaneous nerves, with the possible exception of its presence in a network of what may be nerves around the arteries. The pattern of nerve distribution observed in histological sections processed for both monoamine oxidase and specific cholinesterase together confirmed the results obtained using the two techniques separately. In



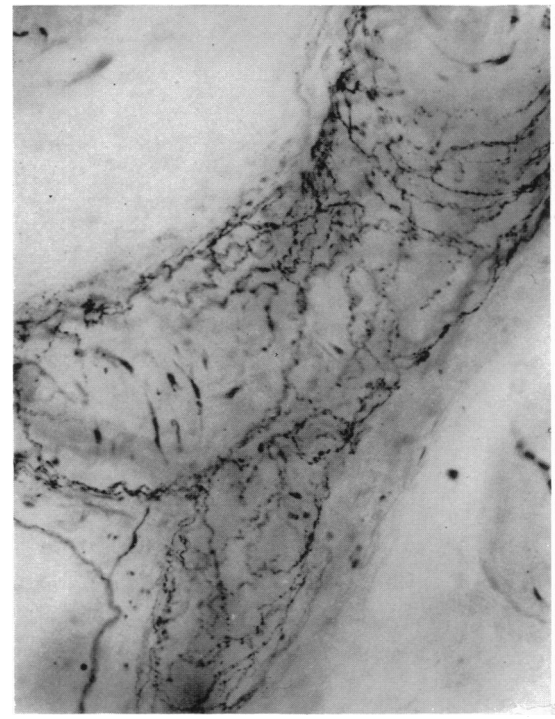


Fig. 5. Photomicrograph of a section cut parallel to the skin surface illustrating nerve fibres around and apparently supplying the sebaceous glands (*SEB*). Methylene blue, × 150.

Fig. 6. Photomicrograph illustrating a plexus of nerve fibres around an artery in the stratum reticulare of the dermis. Methylene blue, × 150.

general, the nerve distribution was the same as that observed after treatment of the skin with methylene blue and with silver. Several additional features of this nerve plexus, however, became apparent. Below the level of the sebaceous glands some of the fibres in the cutaneous nerves and nerve trunks contained specific cholinesterase while other fibres in the same nerves and nerve trunks contained monoamine oxidase (Fig. 7). Above this level all the fibres in the nerves generally contained specific cholinesterase.

Most of the fibres in the occasional nerve trunks found crossing the fundus of the

sweat gland reacted for monoamine oxidase (Fig. 8) and only a few for specific cholinesterase. The nerve observed running parallel to the duct of the sweat gland contained AChE-reactive and also MAO-reactive fibres but no branches to the duct were observed. There was no evidence of an innervation to either the fundus or the duct of the gland.

The hair pallisade endings were again clearly observed and the nerves supplying them were found to contain AChE-reactive and also MAO-reactive fibres. The MAO-reactive fibres gave rise to fine fibres also reactive for monoamine oxidase which

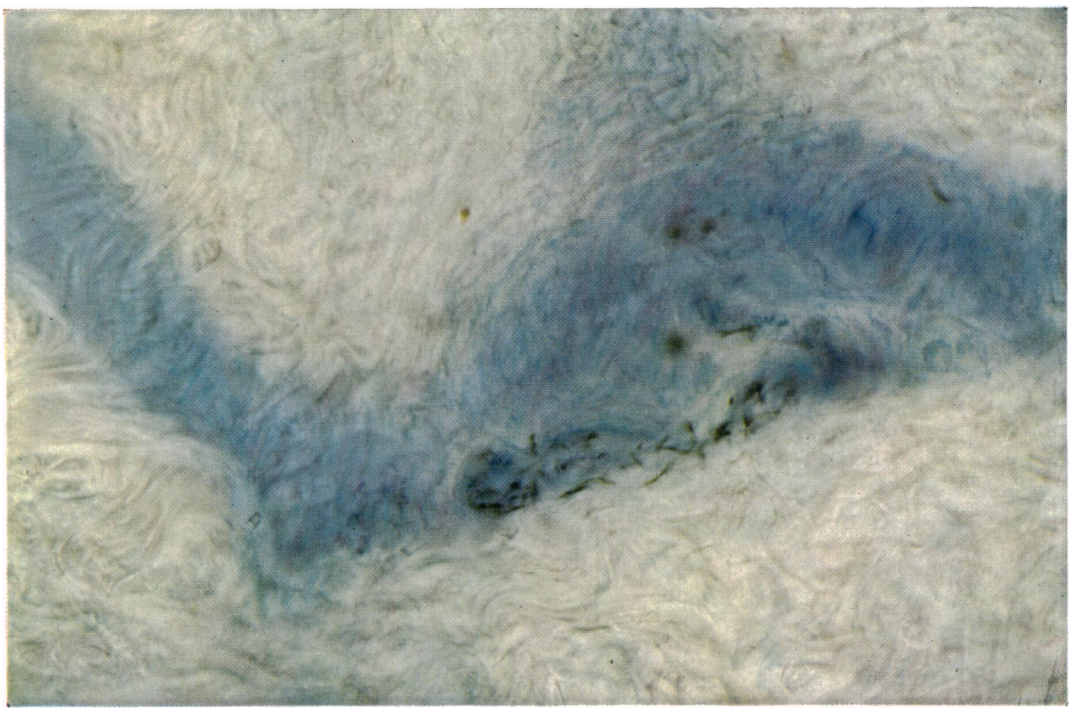
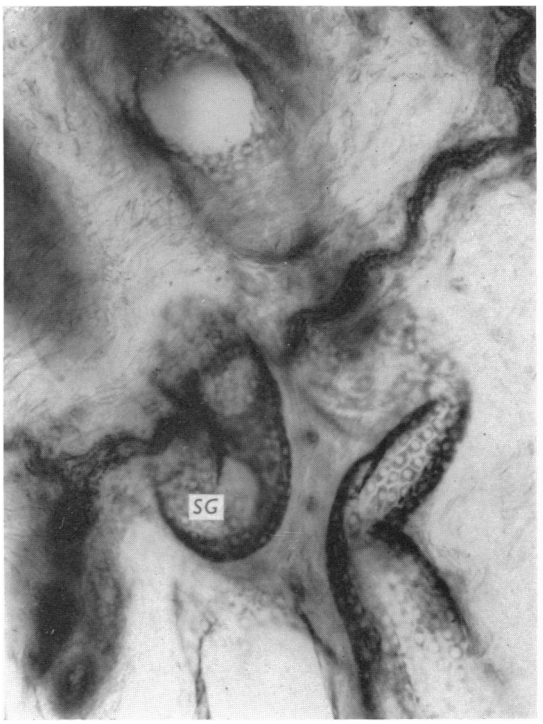


Fig. 7. Photomicrograph illustrating a nerve accompanying a blood vessel in the stratum reticulare of the dermis. Some of the fibres in this nerve (those which are brown) react for specific cholinesterase and others (blue) react for monoamine oxidase. This appearance is typical of all the nerves and nerve trunks found below the level of the sebaceous glands. MAO and AChE, $\times 100$.

ran both circularly and longitudinally in the pallisade (Fig. 9). The AChE-reactive fibres also gave rise to fine fibres which circled the hair follicle (Fig. 10) and intertwined with the MAO-reactive fibres. There was no reaction for specific cholinesterase in fibres running in the longitudinal system around the hair follicle. The AChE-reactive and the MAO-reactive fibres in the pallisade were apparently independent although supplied by the same nerve. A nerve fibre reactive for specific cholinesterase was sometimes observed leaving the network of fibres composing the pallisade and entering the arrector pili muscle nerve supply. An AChE-reactive nerve fibre apparently connecting neighbouring pallisades was also occasionally observed. Again

the exact site of termination of these fibres could not be traced in the complex endings. There was no evidence of any additional nerve supply to the hair follicle apart from AChE-containing nerve fibres ending freely in the epidermis at the upper part of the follicle.



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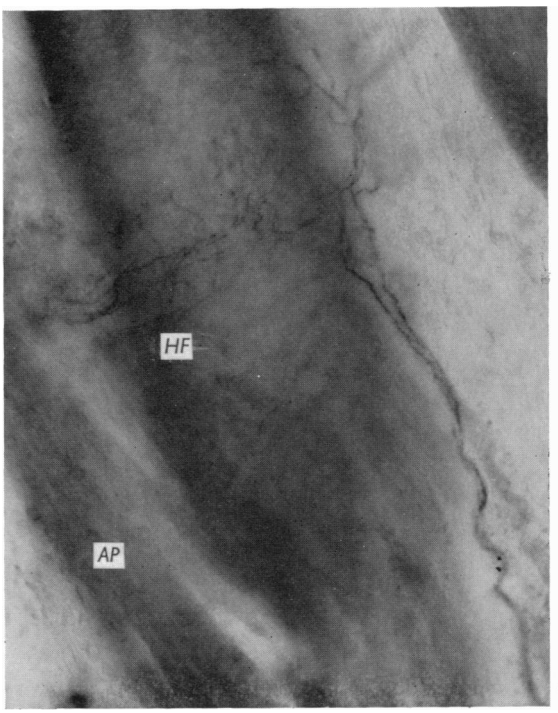

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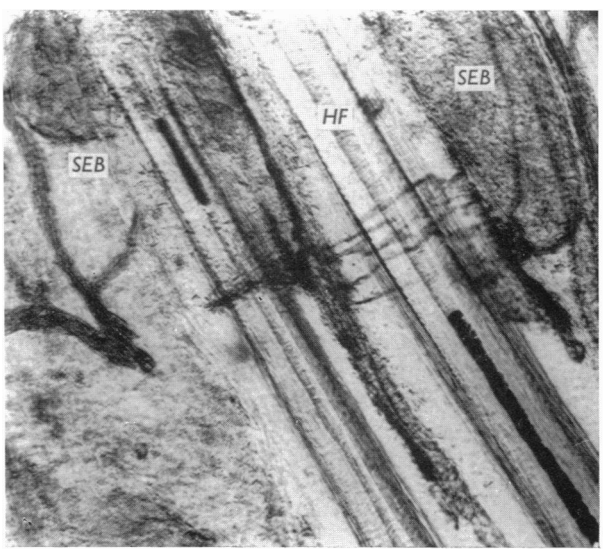

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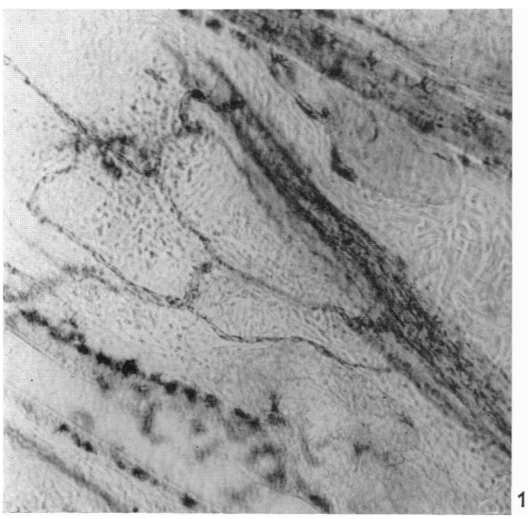

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The parallel system of nerve fibres in the arrector pili muscle reacted only for specific cholinesterase (Fig. 11). A few fibres containing monoamine oxidase were sometimes seen near the muscle apparently entering it but no widespread MAO-reactive innervation was observed. The fibres in the nerve supplying the muscle react predominantly for specific cholinesterase and only a few fibres react for monoamine oxidase. A nerve fibre reactive for specific cholinesterase from an arrector pili muscle nerve supply can on occasion be seen entering a neighbouring muscle nerve supply. The terminations of these fibres could not, however, be traced within the complex system of fibres in the muscle.

Most of the fibres in the neighbourhood of the sebaceous glands contained specific cholinesterase (Fig. 10) and some appeared to end freely in them although some fibres containing monoamine oxidase were seen in this region (Fig. 12). The freely ending nerve fibres supplying the epidermis reacted only for specific cholinesterase (Fig. 13) and again no encapsulated nerve endings were found at the junction of the epidermis and dermis or elsewhere in the skin.

In contrast to the nerve supply to the arrector pili muscle where AChE-reactive fibres predominated, a high proportion of the fibres in the dense plexus around the arteries contained monoamine oxidase. However, some fibres reactive for specific cholinesterase were also observed (Fig. 14). MAO-containing and occasionally AChE-containing fibres were seen in close relationship with the veins but no plexus was observed.

Distribution of cholinesterase and monoamine oxidase

The relative distributions of specific cholinesterase, monoamine oxidase and non-specific cholinesterase in cattle skin are illustrated in Figs. 15–17. The results obtained with the combined monoamine oxidase and cholinesterase techniques confirmed the findings with the individual methods.

In addition to its presence in nerve fibres, specific cholinesterase was also found in the sweat glands, the epidermis and to a slight extent in the sebaceous glands. The duct of the sweat gland exhibited a stronger reaction for this enzyme than did the fundus. In the latter, the specific cholinesterase (Fig. 18) appeared to be distributed along its length and may be associated with the myoepithelial cells which are

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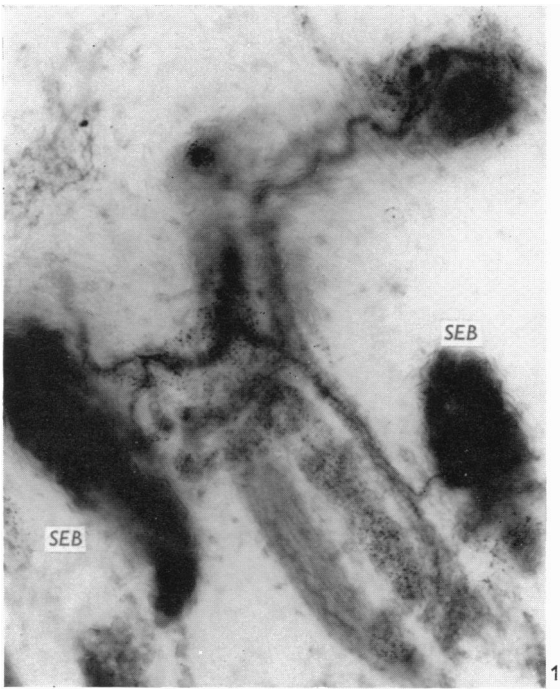
Fig. 8. Photomicrograph of a section cut obliquely, illustrating a nerve trunk crossing the fundus of a sweat gland (SG). The MAO-reactive fibres in the nerve are visible with this technique. The cells of the sweat glands exhibit a reaction for monoamine oxidase although in this case the distribution of the enzyme is less uniform than usual. MAO, × 150.

Fig. 9. Photomicrograph of a section cut perpendicular to the skin surface illustrating nerve fibres reactive for monoamine oxidase both circular and longitudinal to the hair follicle (*HF*) in the hair pallisade ending. A positive reaction for monoamine oxidase can be seen in the accompanying arrector pili muscle (*AP*). MAO, × 150.

Fig. 10. Photomicrograph of a section cut perpendicular to the skin surface illustrating nerve fibres reactive for specific cholinesterase circular to the hair follicle (*HF*) in the hair pallisade ending. Fine nerve fibres can be seen around the sebaceous glands (*SEB*) in this section. AChE, × 100.

Fig. 11. Photomicrograph of a section cut perpendicular to the skin surface illustrating nerve fibres reactive for specific cholinesterase in the nerve supplying the arrector pili muscle. The parallel system of AChE-reactive nerve fibres in the muscle is also shown. AChE, × 100.

orientated in the same direction. The distribution of specific cholinesterase in the epidermis was not uniform. The strongest reaction was present in the stratum germinativum, diminishing in amount to the stratum corneum which contained none at all. On short incubation specific cholinesterase was seen only in a small number of



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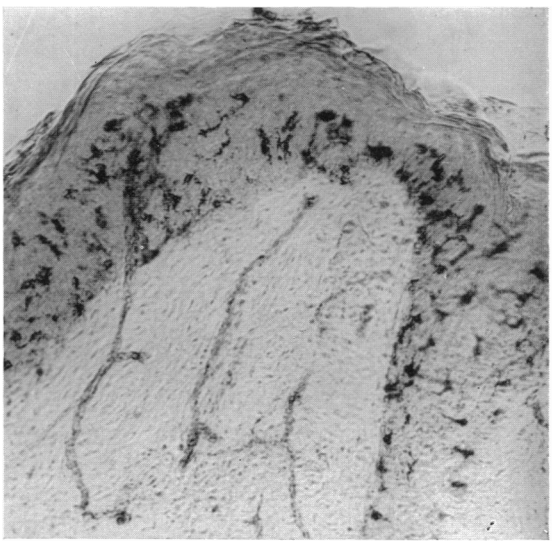

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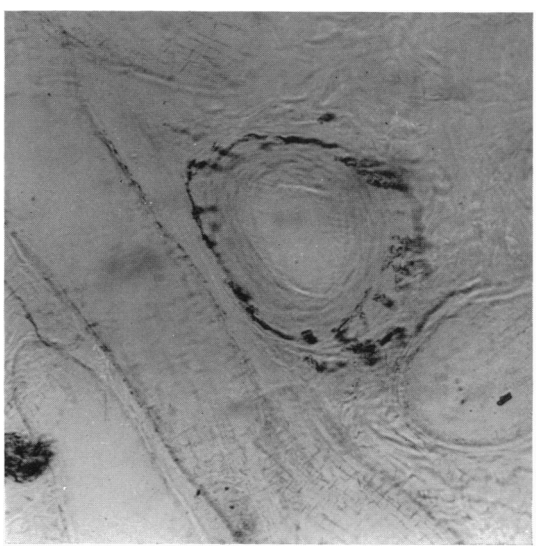

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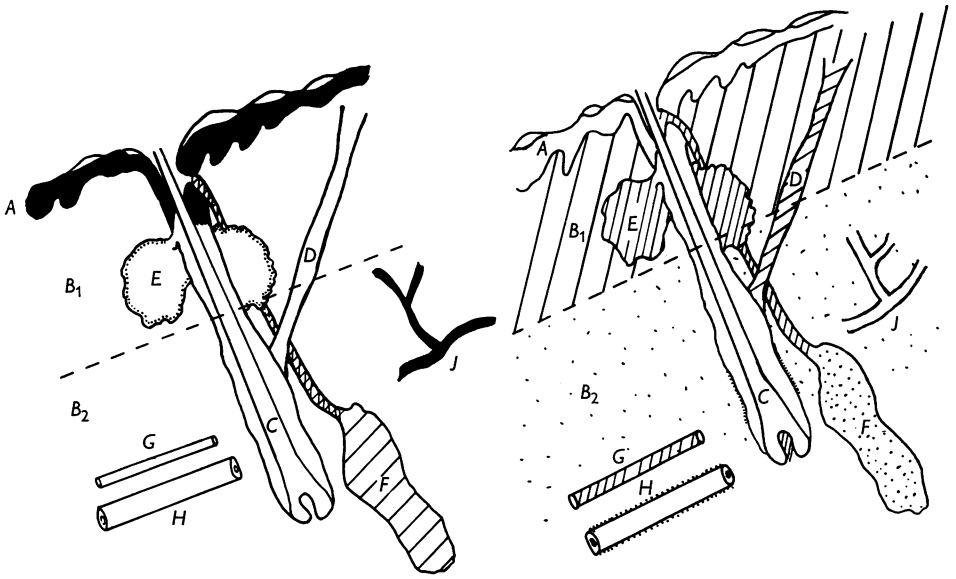
cells within the epidermis (Fig. 13) but on longer incubation it was seen to be distributed throughout the epidermis. This enzyme was also observed in the upper part of the hair follicle close to the epidermis. Small amounts were detected in the basal cells at the periphery of the sebaceous glands after prolonged incubation. This



The relative concentration of enzyme is represented by

1 2 3 4

in ascending order of magnitude

Fig. 15

The relative concentration of enzyme is represented by

1 2 3 4

in ascending order of magnitude

Fig. 16

Fig. 15. A diagram illustrating the relative distribution of specific cholinesterase in bovine skin. *A*, Epidermis; B_1, stratum papillare (dermis); B_2, stratum reticulare (dermis); *C*, hair follicle; *D*, arrector pili muscle; *E*, sebaceous gland; *F*, sweat gland; *G*, venule; *H*, arteriole; *J*, nerve trunks and fibres.

Fig. 16. A diagram illustrating the relative distribution of non-specific cholinesterase in bovine skin. Key as for Fig. 15.

Fig. 12. Photomicrograph illustrating nerve fibres reactive for monoamine oxidase in the vicinity of the sebaceous glands (*SEB*) and apparently supplying them. The sebaceous glands react intensively for monoamine oxidase. MAO, × 150.

Fig. 13. Photomicrograph of a section cut perpendicular to the skin surface illustrating nerve fibres reactive for specific cholinesterase supplying the epidermis. A reaction for specific cholinesterase can also be seen in numerous cells within the epidermis which have a dendritic appearance. On longer incubation, however, a more uniform distribution of the enzyme is seen. AChE, × 100.

Fig. 14. Photomicrograph illustrating nerve fibres reactive for specific cholinesterase around an artery in the stratum reticulare of the dermis. AChE, × 150.

reaction for specific cholinesterase in the sebaceous glands was completely masked by that for monoamine oxidase in sections incubated for both enzymes.

Monoamine oxidase was distributed widely throughout the skin. It was absent only from the matrix of the dermis. The most intense reaction was found in the sebaceous glands (Fig. 12) and it was present also in large amounts in the epidermis, hair follicles and blood vessels. Small amounts were found in the sweat glands and

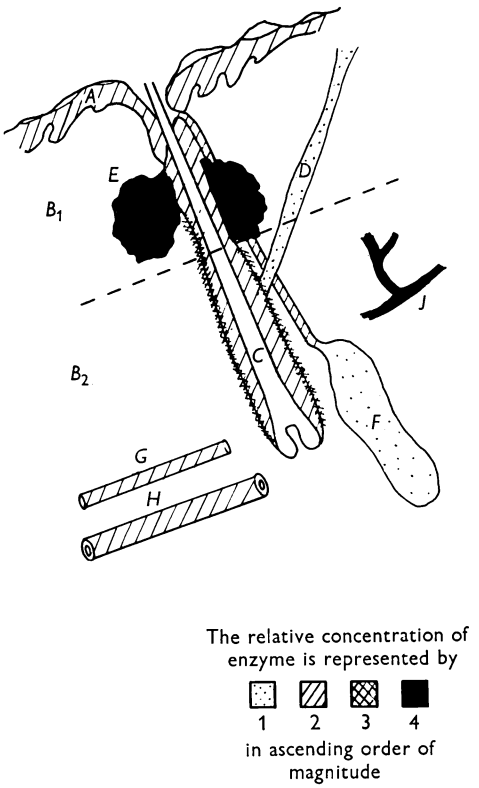
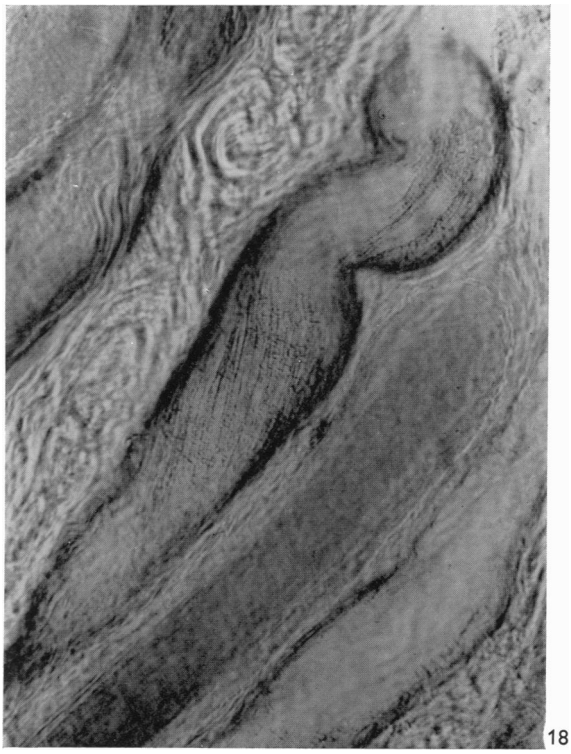


Fig. 17. A diagram illustrating the relative distribution of monoamine oxidase in bovine skin. Key as for Fig. 15.

arrectores pilorum muscles. Small amounts of monoamine oxidase were distributed throughout the sweat gland fundus (Fig. 8). The sweat-gland duct, however, reacted more intensely for this enzyme. In sections incubated for both monoamine oxidase and specific cholinesterase the reaction for both enzymes was clearly seen in the sweat glands. Monoamine oxidase was uniformly distributed throughout the arrector pili muscle (Fig. 9). The most intense reaction for this enzyme was found in the sebaceous gland, the amount at the neck being considerably smaller than that at the periphery of the gland. In sections incubated for monoamine oxidase and either specific cholinesterase or non-specific cholinesterase, the intense blue coloration denoting the site of monoamine oxidase activity in the sebaceous glands completely masked the brown precipitate of copper sulphide which develops at the site of

cholinesterase activity. The entire hair follicle, apart from the hair, contained monoamine oxidase although the reaction for this enzyme was more intense in the outer root sheath. Monoamine oxidase was present in arteries and veins. It was uniformly distributed over the walls of the arteries except in the tunica intima which had a higher concentration.

Non-specific cholinesterase was also distributed widely throughout the skin, being found in the arrectores pilorum muscles, sebaceous glands, blood vessels, dermis and to a slight degree in the sweat glands. The hair follicles did not react for



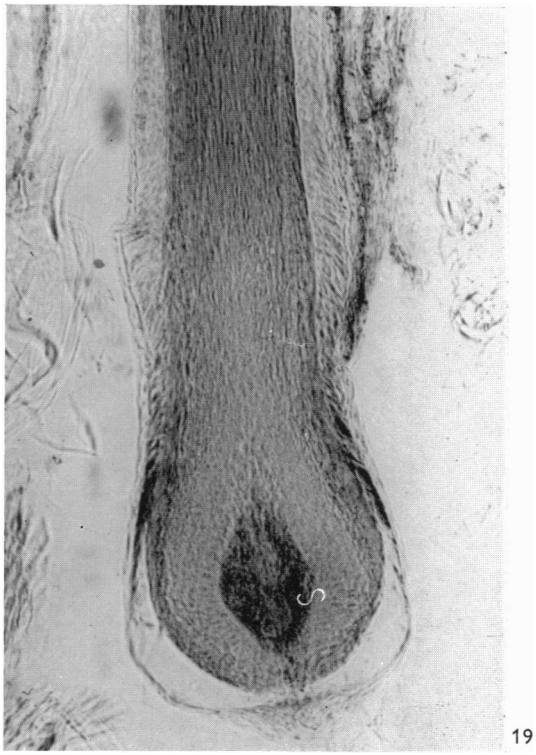


Fig. 18. Photomicrograph illustrating a positive reaction for specific cholinesterase in the fundus of a sweat gland. The enzyme appears to be distributed in strands orientated along the length of the gland. There are, however, no nerve fibres supplying the gland. AChE, $\times 150$.

Fig. 19. Photomicrograph illustrating a positive reaction for non-specific cholinesterase in the papilla and to a slight degree in the outer root sheath of a hair follicle. ChE, $\times 250$.

non-specific cholinesterase except faintly at the periphery of the outer root sheaths in some of the larger follicles. The papillae of the hair bulbs, however, exhibited a positive reaction (Fig. 19). The epidermis did not contain non-specific cholinesterase. The fundus of the sweat gland did not show a marked reaction for non-specific cholinesterase, although a positive reaction was generally obtained. The duct, on the other hand, reacted intensively. The matrix of the dermis, especially the stratum

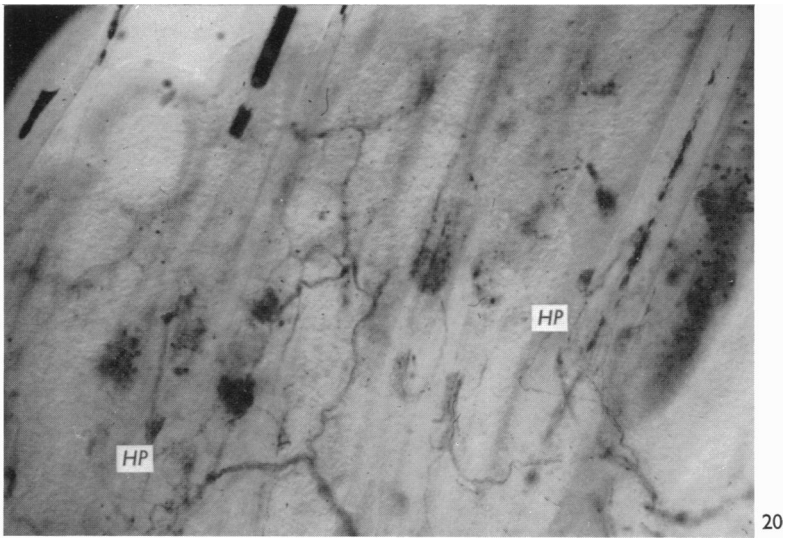


Fig. 20. Photomicrograph of a section cut perpendicular to the skin surface, illustrating the general appearance of the nerve supply to sympathectomized skin. Fewer than normal nerves are present. The nerve supply to the arrector pili muscle is no longer visible and that to the hair pallisade (*HP*) has been disrupted. The nerve fibres supplying the epidermis appear to be unaltered. Methylene blue, × 90.

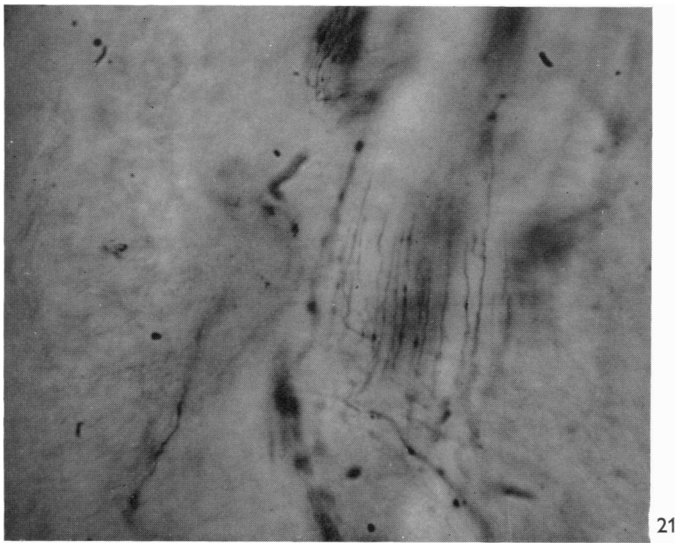


Fig. 21. Photomicrograph illustrating the appearance of the hair pallisade, ending in sympathectomized skin. Only fibres longitudinal to the hair follicle are present. These fibres, were, however, not seen in sympathectomized skin using the histochemical techniques. Methylene blue, × 400.

papillare, exhibited a diffuse background reaction for non-specific cholinesterase, and fat cells deep in the stratum reticulare also reacted positively for this enzyme. The non-specific cholinesterase was distributed diffusely and uniformly in the arrector pili muscle and could be detected simultaneously with monoamine oxidase in sections processed for both enzymes. Non-specific cholinesterase was distributed diffusely throughout the sebaceous glands, except at the periphery where a more intense reaction occurred. This reaction was completely masked by the blue reaction for monoamine oxidase in sections treated for both enzymes simultaneously. The walls of the veins reacted positively and intensively for non-specific cholinesterase but the arterial walls failed to do so. At the extreme periphery of the arteries, however, a positive reaction for non-specific cholinesterase was obtained in fibrous structures. This may have been a reaction in the adventitia, in associated nerve fibres or in small vaso vasorum blood vessels supplying the arteries. The reaction for non-specific cholinesterase in the veins was almost completely masked by simultaneous treatment for monoamine oxidase.

Sympathectomized skin

(*a*) *Methylene blue and silver techniques*

In sympathectomized skin, with the exception of the nerve supply to it, no difference in the morphological appearance of the skin or any of the organs within it was detected. When compared with normal skin fewer nerves were seen, the nerve supplies to the arrectores pilorum muscles and arteries were no longer seen and that to the hairs was noticeably changed (Fig. 20).

In the vicinity of the sweat glands an occasional nerve trunk traversing the fundus of a gland was still observed, but the nerve observed alongside the duct in normal skin was not present. No intact hair pallisade endings were present. Fibres circling the hair follicle were not observed and their absence made the longitudinal fibres appear more prominent even in histological sections examined at low magnifications (Fig. 21). No nerve fibres were seen in or around the arrector pili muscle but nerve fibres could still be seen in the vicinity of the sebaceous glands although there were fewer than in normal skin. The epidermis was still supplied by nerves which ramified towards it and appeared morphologically normal. Nerves running alongside the blood vessels were still observed but there was little evidence of an innervation to either the arteries or veins. After sympathectomy the peri arterial plexus was lost and only a few isolated fibres remained intact.

(*b*) *Monoamine oxidase and cholinesterase techniques*

In sympathectomized skin MAO-containing fibres were generally absent from the remaining nerves but otherwise no apparent change in the distribution of specific cholinesterase, non-specific cholinesterase or monoamine oxidase was detected.

The only change from normal in the vicinity of the sweat glands was the absence of nerves running alongside the ducts and the absence of MAO-containing fibres in the occasional nerve trunks traversing the fundus of the gland. Again no complete pallisade ending could be detected around the hairs in sympathectomized skin. In this case, however, in contrast with the findings using the methylene blue and silver techniques no fibres longitudinal to the hair were observed. Only fragments of the

disrupted pallisade ending persisted in the form of a few circular fibres, some reactive for specific cholinesterase and a few for monoamine oxidase. Using these techniques there was nothing to suggest that even part of the pallisade nerve-ending previously observed in normal skin had remained intact. MAO-containing fibres were also absent from the vicinity of the sebaceous glands but a few fibres reacting for specific cholinesterase were still observed in close proximity to these glands. The complete denervation of the arrector pili muscle was confirmed. The parallel arrangement of AChE-containing nerve fibres could not be demonstrated and fibres containing monoamine oxidase were not observed near the muscle. Again no apparent change in the nature of the nerve supply to the epidermis could be detected but the characteristic plexus of nerve fibres supplying the arteries was no longer visible. Only a few isolated fibres were observed around the arteries. Most were reactive for specific cholinesterase, but occasionally an MAO-reactive fibre was observed. There was no evidence of a nerve supply to the veins apart from an occasional AChE-containing fibre which remained in their vicinity after sympathectomy.

DISCUSSION

The results demonstrate that in the cutaneous nerves of cattle there are fibres which contain monoamine oxidase and others which contain specific cholinesterase. It might be argued that all the fibres of the nerve react for monoamine oxidase, the resulting blue pigment being removed from some fibres by the treatment required to demonstrate specific cholinesterase and replaced by the brown copper-sulphide deposit indicative of the latter. This possibility is unlikely, however, for the following reasons:

(1) The cutaneous nerves above the level of the sebaceous glands do not contain MAO-reactive fibres.

(2) Reactions for both enzymes can be simultaneously demonstrated in single organs of the skin such as the sweat glands.

(3) In sympathectomized skin the fibres of the cutaneous nerves generally react only for specific cholinesterase.

It may be concluded, therefore, that two types of fibre, one reacting for specific cholinesterase and the other reacting for monoamine oxidase, are present in the cutaneous nerves and nerve trunks.

The results also show that the techniques used in the present study are adequate for illustrating fine nerve fibres in the skin, yet no convincing evidence for a nerve supply to the sweat glands was obtained. Further, since in some cases fresh frozen material was used for processing by the histochemical techniques the failure to observe fine nerve fibres is unlikely to be due to a fixation effect. No network of fine nerve fibres around the sweat gland such as that described for the sweat glands of man (Weddell, Palmer & Pallie, 1955; Montagna & Ellis, 1960) and the horse (Takagi & Tagawa, 1961) was present, and the absence of any stem fibres which could form such a network makes the existence of a nerve supply to the sweat glands doubtful. This evidence suggests, therefore, that the sweat glands in the skin of the general body surface of cattle have no nerve supply. This conclusion differs from that of Wagai & Tohara (1962), who state in their summary that nerve fibres in the

vascular lamina of the bovine hoof are distributed around a follicular gland or a sweat gland, and from the conclusion of Taneja (1959) that cattle sweat glands have an adrenergic innervation. The results of Findlay & Robertshaw (1965) indicate that the sympathetic nervous system is involved in the control of thermal sweating in cattle. It is possible that the mechanism controlling the activity of cattle sweat glands involves not only the activity of the sympathetic nervous system but also a local component perhaps of a humoral nature.

Yasuda & Montagna (1960) describe a nerve reactive for monoamine oxidase running alongside the straight portion of the duct of the sweat glands in man. This is similar to the nerve which accompanies the long straight duct of cattle sweat glands. The function of this nerve is unknown but in cattle it does not appear to supply the duct in spite of close proximity to it. The presence of monoamine oxidase in the cells of the sweat gland fundus and its duct has been reported in man (Yasuda & Montagna, 1960) and the horse (Hellman, 1955). In cattle it may be involved in the metabolism of adrenaline, which is known to activate the sweat glands in this species (Taneja, 1959; Findlay & Jenkinson, 1964). Specific cholinesterase has also been reported to be present in the cells of the sweat glands in the paw of the dog (Winkelmann & Schmit, 1959) and around the sweat glands in the skin of man (Aavik, 1955).

The structure of the hair pallisade nerve ending is similar to that described by Weddell, Pallie & Palmer (1954) in man, the rabbit and the monkey. In cattle the pallisade ending is a complex unit which appears to be connected with its neighbours and possibly with the nerve supply to the arrector pili muscle. However, since the exact site of termination of the fine nerve fibres within the system of nerve fibres in the muscle and in the pallisade could not be traced, it was not possible to determine whether these were direct connexions. Yasuda & Montagna (1960) concluded that in human skin some of the nerves around hair follicles and beneath the epidermis may contain both specific cholinesterase and monoamine oxidase. The present findings illustrate that in the skin of cattle these enzymes are located in different nerve fibres around the hair and that beneath the epidermis the nerve fibres react only for specific cholinesterase. The presence of two histochemically different types of nerve fibre in the pallisade suggests that there may be more than one type of nerve ending present within it. Such a conclusion is supported by the continued appearance in sympathectomized skin of nerve fibres longitudinal to the hair after methylene blue and silver treatment but not after incubation for monoamine oxidase or specific cholinesterase. This suggests that there may also be nerve fibres in the pallisade which do not react for either monoamine oxidase or specific cholinesterase. At least part of the pallisade is associated with the sympathetic nervous system since, although it is not completely eliminated by sympathectomy, some of its component fibres are affected by this procedure. The finding of a single zone of innervation of bovine hair follicles supports the observations of Weddell *et al.* (1954) who failed to find nerve fibres or terminals specifically related to the tissues of the hair bulb in certain body areas of man, in the monkey and in the rabbit, but contrasts with observations on the hair follicles of the human scalp, which have two plexuses related to two different regions of the hair (Montagna & Ellis, 1967).

The well-developed system of fine parallel fibres observed in the arrector pili

muscle and reactive for specific cholinesterase resembles the 'fibril-like dark structures' described in man by Aavik (1955). In cattle these fibres are not visible in sympathectomized skin and it would appear, therefore, that the arrector pili muscle has a sympathetic nerve supply. This conclusion is supported by the fact that piloerection does not occur on an area of cattle skin which has been sympathectomized (Findlay & Robertshaw, 1965). Burn & Rand (1962) concluded that in the cat the action of acetylcholine in causing piloerection was due to the release of noradrenaline and that this action was much reduced as a result of the degeneration of the sympathetic nervous system. The above evidence in cattle skin together with the presence of monoamine oxidase in the muscle itself is not inconsistent with this hypothesis. Findlay & Robertshaw (1965), however, showed that piloerection can be elicited in cattle by intradermal injection of noradrenaline but that there is no visible response to acetylcholine. The relatively few MAO-reactive nerve fibres associated with the arrector pili muscle in normal skin but which are not present in sympathectomized skin are probably associated with the blood supply to the muscle, but the possibility of an additional nerve supply to the muscle cannot be excluded.

The present finding of nerve fibres some of which react for monoamine oxidase and others which react for specific cholinesterase around and apparently entering the sebaceous glands is comparable with the results of Yasuda & Montagna (1960) and Montagna & Ellis (1957) for the nerve supply to the sebaceous glands of man. Since in sympathectomized skin nerve fibres containing monoamine oxidase were absent and those containing specific cholinesterase were greatly reduced in number, it would appear that the sebaceous glands are supplied mainly by sympathetic nerve fibres. The reaction for monoamine oxidase in the cells of the sebaceous gland is more intense than in any other organ in the skin. The presence of this enzyme has also been reported in the sebaceous glands of man and of the chimpanzee (Yasuda & Montagna, 1960; Montagna & Yun, 1963) but its physiological significance at this site is unknown. The presence of only small amounts of specific cholinesterase at the periphery of the gland contrasts with what is found for the sebaceous glands of the chimpanzee, rat and mouse which contain fairly large amounts of this enzyme (Montagna & Yun, 1963; Winkelmann & Schmit, 1959; Pospišil, 1959).

The nerves ramifying towards and supplying the epidermis do not appear to be part of the sympathetic nervous system because they are still present in sympathectomized skin. The absence of MAO-reactive nerve fibres in this region contrasts with the findings of Yasuda & Montagna (1960) who reported the presence of MAO-reactive nerve fibres to the human epidermis. Weddell *et al.* (1954) concluded from a study of human, monkey and rabbit skin that encapsulated nerve endings are seen in large numbers in mucous membranes and in skin devoid of hairs. They are not seen in skin covered with hairs but are found in skin where hairs are sparse. The absence of encapsulated nerve endings in the skin of the body surface of cattle but their presence in the skin of the muzzle (Nisbet, 1956) confirms this conclusion. In containing specific cholinesterase the epidermal cells of the skin of cattle resemble those of the mouse and rat but differ from those of the dog, cat and rabbit (Pospíšil, 1959; Winkelmann & Schmit, 1959). Monoamine oxidase has previously been described in the cells of the epidermis in man (Yasuda & Montagna, 1960) but the physiological significance of its presence is still unknown.

The plexus of nerve fibres around the arteries is similar to that described in the cat by Polley (1955) and in a review by Weddell *et al.* (1955). Most of the nerve fibres supplying the arteries are reactive for monoamine oxidase and are not present in sympathectomized skin. The disappearance of the nerve plexus after sympathectomy is consistent with the findings of Polley (1955) for the blood vessels in the reticular layer of the skin of the cat. It appears, therefore, that the arteries in cattle skin are supplied mainly by sympathetic nerve fibres most of which are reactive for monoamine oxidase. This contrasts with the nerve supply to the arrector pili muscle, which is supplied by sympathetic fibres that react for specific cholinesterase. Complete cutaneous sympathectomy is very difficult to achieve and the continued presence of occasional MAO-reactive nerve fibres around the arteries in sympathectomized skin may be due to incomplete sympathectomy. They may, however, be afferent nerve fibres. The nature of the fibres around the arteries which react for non-specific cholinesterase is unknown.

SUMMARY

1. A study was made of the innervation of the skin of cattle and the distribution of cholinesterase and monoamine oxidase within it with special reference to the sweat glands. Five different techniques, namely, silver impregnation, methylene-blue staining, incubation for monoamine oxidase, for cholinesterase and for both together were used on normal and sympathectomized skin.

2. The skin of cattle was found to be innervated by a plexus of nerves and nerve trunks which accompany the cutaneous blood vessels. Large nerve trunks in the stratum reticulare branch and innervate the arteries, veins, arrectores pilorum muscles, sebaceous glands and nerve pallisade endings around the hair follicle. The hair pallisade endings appear to be interconnected by fine nerve fibres and are possibly also connected with the nerve supplies to the arrectores pilorum muscles again by fine fibres. The nerve supplies to the arrectores pilorum muscles also appear to have fine nerve fibre interconnexions. No encapsulated nerve endings were observed and no convincing evidence for a nerve supply to the sweat glands was obtained.

3. Below the level of the sebaceous glands some of the fibres in the cutaneous nerve trunks contained specific cholinesterase while other fibres in the same nerve trunks contained monoamine oxidase. All the fibres in the nerves above this level generally contained specific cholinesterase. Non-specific cholinesterase was not present in nerve fibres with the possible exception of a network of fibres around the arteries which may be nerves.

4. Specific cholinesterase, non-specific cholinesterase and monoamine oxidase were found in other structures within the skin and their distribution is illustrated.

5. Fewer nerves were observed in sympathectomized skin. The nerve supplies to the arteries, veins and arrectores pilorum muscles found in normal skin were not seen in sympathectomized skin and those to the sebaceous gland and hair pallisade endings were disorganized. The nerve supply to the epidermis, however, was not visibly different.

6. Fibres reactive for monoamine oxidase were almost completely absent from nerves observed in sympathectomized skin, and fibres reactive for specific cholinesterase appeared to be reduced in number. The distribution of specific cholin-

esterase, non-specific cholinesterase and monoamine oxidase in the rest of the skin was, however, the same as that observed in skin from normal animals.

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