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

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The sweat glands of the sheep and goat have been shown to respond to heat and to the injection of adrenaline and noradrenaline (Brook & Short, 1960; Waites & Voglmayr, 1963; Kimura & Aoki, 1962). Waites & Voglmayr (1963) were of the opinion that the expulsion of sweat from the sweat glands of the scrotum of the ram in response to heat is controlled by adrenergic sympathetic nerves, but that adrenal medullary hormones may also play a part, and Kimura & Aoki (1962) demonstrated that thermal sweating in the goat is abolished or much reduced by sympathectomy, indicating that the sympathetic nervous system is involved in the control of sweating in the goat. These observations have been verified by O. Robertshaw (personal communication) using specific autonomic blocking agents. It appears, therefore, that in the sheep and goat as in the ox (Findlay & Robertshaw, 1965) an intact sympathetic nerve supply is necessary for heat-induced sweat gland activity. Jenkinson, Sengupta & Blackburn (1966), however, could find no evidence of a nerve supply to the sweat glands of cattle and suggested that the controlling mechanism may also involve a local component, perhaps of a humoral nature. As a similar mechanism may exist in the sheep and goat the present work was undertaken to investigate the innervation and distribution of monoamine oxidase and cholinesterase in the skin of the sheep and goat with particular reference to the sweat glands.

MATERIALS AND METHODS

Specimens of skin (15 × 5 mm) were taken immediately after slaughter from the necks of a total of forty-four adult sheep (female and castrated) of varying ages and breeds (Blackface, Suffolk, Cheviot and crosses of unknown parentage). Skin samples were also obtained by biopsy using the technique of Findlay & Jenkinson (1960) from fourteen 3-year-old castrated sheep (Kent, Suffolk, Hampshire, Welsh Mountain, Cheviot and Scottish Blackface) and from thirteen British Saanen goats (males, females and castrates) aged from 1 to 10 years. Four different techniques were used in the preparation of the various skin specimens for microscopic study.

Methylene blue

Sections 400–600 μ thick were cut from the skin samples and pretreated for 15–20 min at 37 °C in normal saline. They were then stained in methylene blue and fixed in ammonium molybdate according to the method of Arthur & Shelley (1959). Sheep and goat skin required 45–60 min in the staining bath and 18–24 h for fixation. The most satisfactory results were obtained at pH 3·0 with the addition of hyaluronidase (0·6 ml. of a 0·1 % solution) to the methylene-blue solution.

Localization of cholinesterase

Specimens were treated for specific and non-specific cholinesterase activity using the histochemical method of Naik (1963).

Localization of monoamine oxidase

Specimens were collected in normal saline at 37 °C and sections from them were incubated for 1–2 h according to the procedure of Glenner, Burtner & Brown (1957). Control sections were obtained by the incorporation of 2×10^{-1} M marsalid phosphate into the incubation solution.

Localization of monoamine oxidase and specific cholinesterase in the same section

Specimens were collected in normal saline at 37 °C and frozen sections, 25–30 μ thick, were cut and incubated both for monoamine oxidase and specific cholinesterase as described by Jenkinson *et al.* (1966). For comparison, sections were also incubated for non-specific cholinesterase after incubation for monoamine oxidase.

RESULTS

Innervation

(*a*) *Methylene blue*

The distribution of nerves in the skin of the sheep and goat was found to be the same. The hairy skin is innervated by nerve trunks, containing at least six fibres, in the deeper layers of the stratum reticulare. These nerve trunks and the nerves, containing two to five fibres, which arise from them are closely associated with the cutaneous blood vessels and run alongside them. Branches from the nerve trunks ramify upwards and form a branching plexus. Nerves from this plexus supply the components of the hair follicle unit, the epidermis and the blood vessels. Although some of the fibres in the large nerve trunks were seen to be myelinated the technique did not permit unequivocal determination of the nature of the fibres in the smaller nerves.

A nerve trunk was occasionally observed crossing the fundus of a sweat gland, which in the sheep and goat is found only in association with a primary hair follicle, but no fibres were observed to terminate on the gland. There was no visible network of nerve fibres near the glands and no sign of any stem fibres in their vicinity. A nerve was occasionally observed parallel to the duct and ending in the epidermis at the upper part of a primary hair follicle. There were, however, no visible branches from this nerve to the duct.

Hair palisade nerve endings containing both circular and longitudinal fibres were present around both primary and secondary hairs in the sheep and in the goat (Figs. 1, 2). In the sheep, however, the longitudinal fibres ran more closely together. A fine nerve fibre emerging from the system of fibres in the palisade around a large primary hair follicle was sometimes observed to enter the nerve system of a neighbouring primary hair follicle and, occasionally, a fine nerve fibre leaving the palisade of a primary hair ramified in close proximity to the palisade of a secondary hair follicle. A nerve fibre was also sometimes seen running between the palisade ending

of a large primary hair and the parallel system of nerve fibres supplying the arrector pili muscle (Fig. 3). Nerves were also prominent surrounding the hair follicle near the bulb, but these appeared to be associated with the numerous blood vessels supplying the hair rather than innervating the hair itself. No endings on the hair follicle were observed at this level.

The arrector pili muscle which in sheep and goat is found associated only with a primary hair follicle was innervated by a nerve which gave rise to a series of complexly inter-connected parallel nerve fibres (Fig. 4). Sometimes a nerve fibre leaving the nerve supply of an arrector pili muscle could be seen merging with the parallel nerve fibres of a neighbouring one. Fine nerve fibres were found in close association with and apparently ending on the sebaceous glands. The epidermis was supplied by numerous single nerve fibres. The fibres sometimes intertwined beneath the epidermis and some ended as unencapsulated spray endings. No encapsulated nerve endings were observed in either the epidermis or dermis. The cutaneous arteries were innervated by a dense network of nerve fibres which appeared to terminate in the tunica adventitia and outer tunica media. The veins did not possess a similar nerve plexus and were served only by single nerve fibres.

(*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 what may be nerves around the arteries. The pattern of nerve distribution observed in sections processed for both monoamine oxidase and specific cholinesterase together confirmed the results obtained using the two techniques separately, and the distribution of AChE-reactive and MAO-reactive nerve fibres was similar to that found in sections processed by the methylene blue technique. Several additional features, however, became apparent:

(1) Below the level of the sebaceous glands some fibres in the nerves reacted for specific cholinesterase while others in the same nerve reacted for monoamine oxidase. Above this level almost all of the fibres reacted for specific cholinesterase.

(2) Two intertwining but apparently independent systems of nerve fibres, one reactive for specific cholinesterase and the other for monoamine oxidase, were seen in the palisade endings around both the primary and the secondary hairs. The first consisted of fibres, both circular and longitudinal to the hair follicle, which reacted for monoamine oxidase and the second of fibres circular to the hair follicle reactive for specific cholinesterase. AChE-reactive nerve fibres longitudinal to the follicle were also seen in the larger primary hairs, especially in the goat, but were not prominent in the endings around the smaller hairs.

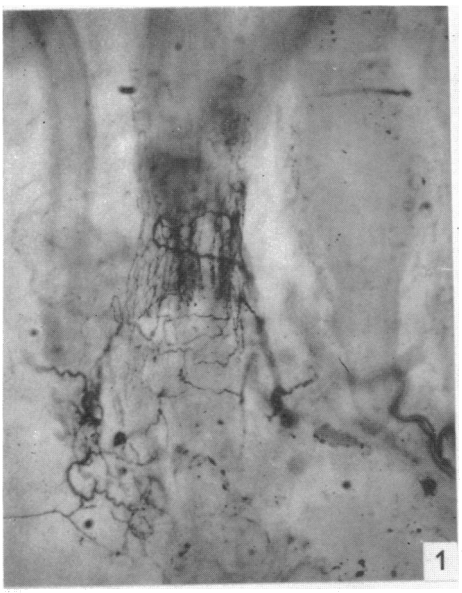
(3) The fine nerves between palisades, from palisades to arrectores pilorum muscles and between these muscles were reactive for specific cholinesterase.

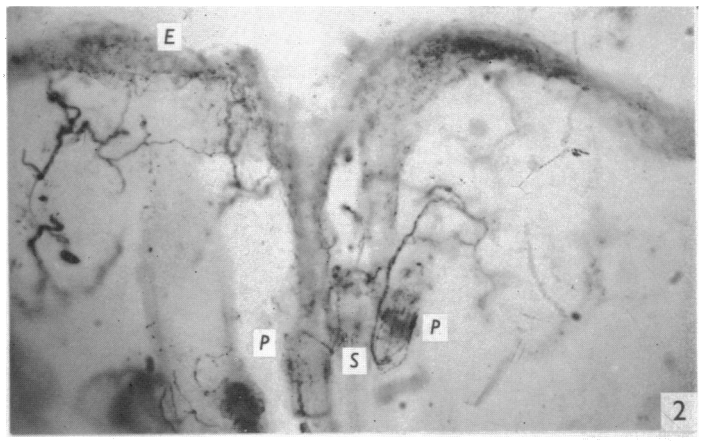
(4) Although the nerve supplying the arrector pili muscle contained an occasional MAO-reactive fibre, the parallel system of fibres within it reacted only for specific cholinesterase.

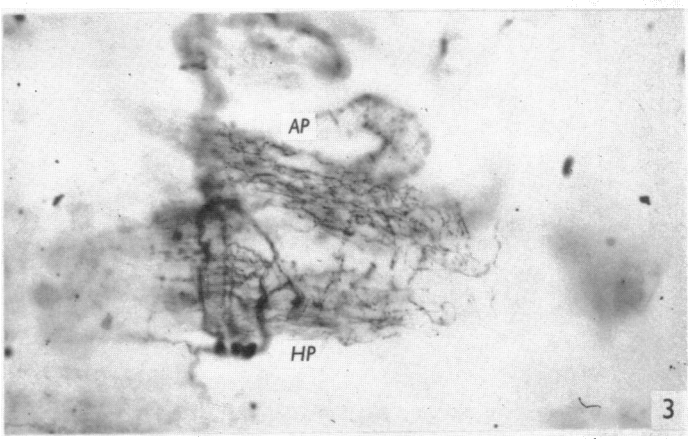
(5) Most of the nerve fibres around the follicle bulbs and in and around the sebaceous glands reacted for specific cholinesterase although MAO-reactive fibres

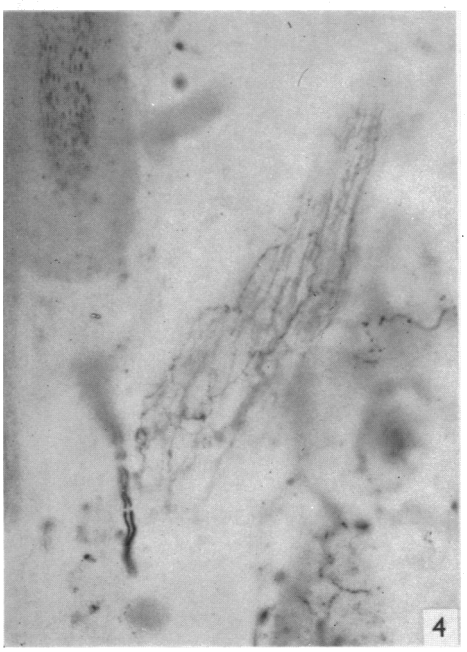
were also present. The nerve fibres supplying the epidermis, however, reacted only for specific cholinesterase. No nerves were observed near the sweat gland save for an occasional nerve trunk crossing it.

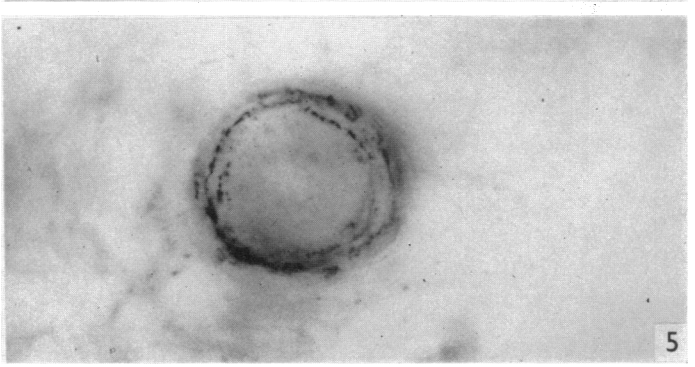
(6) A high proportion of the fibres in the dense plexus around the arteries were MAO-reactive although AChE-reactive fibres were also observed (Fig. 5).











Distribution of cholinesterase and monoamine oxidase

In addition to their presence in nerves, specific cholinesterase, non-specific cholinesterase, and monoamine oxidase were found in other organs in the skin of the sheep and goat. Their relative distributions are illustrated in Figs. 6–8. Dendritic cells which could often be seen in the epidermis and in the upper parts of the hair follicles in unstained sections, especially in the sheep, reacted strongly for specific cholinesterase.

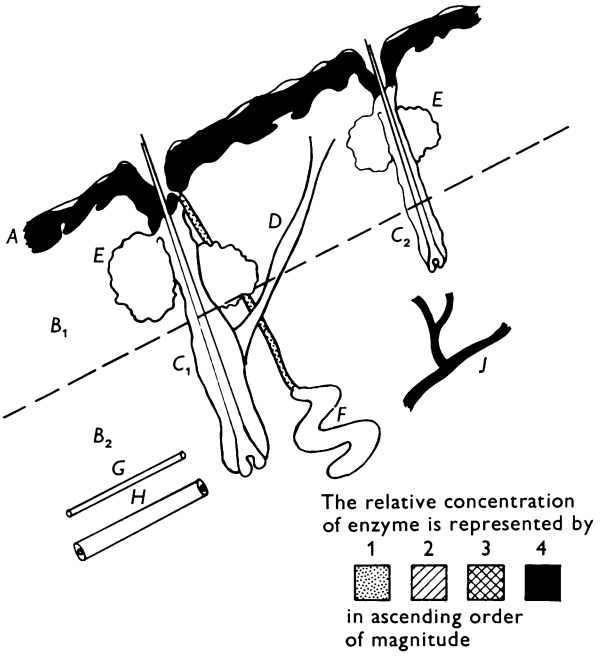


Fig. 6. A diagram illustrating the relative distribution of specific cholinesterase in the skin of the sheep and goat. Key to lettering of Figs. 6–8: *A*, Epidermis; B_1, stratum papillare dermis; B_2, stratum reticulare dermis; C_1, primary hair follicle; C_2, secondary hair follicle; *D*, arrector pili muscle; *E*, sebaceous gland; *F*, sweat gland; *G*, venule; *H*, arteriole; *J*, nerve trunks and fibres.

Fig. 1. Photomicrograph illustrating a hair palisade nerve ending around a primary hair follicle in the skin of a goat. Methylene blue, ×250.

Fig. 2. Photomicrograph of a section of goat skin cut perpendicular to the skin surface illustrating palisade nerve endings around both a primary (*P*) and secondary hair follicle (*S*). Nerve fibres supplying the epidermis (*E*) can also be seen. Methylene blue, ×50.

Fig. 3. Photomicrograph illustrating a hair palisade ending (*HP*) and part of the nerve supply to the arrector pili muscle (*AP*) in the skin of a sheep. Nerve fibres which have emerged from the complex muscle nerve supply can be seen in close asociation with the palisade ending. (Skin surface to left of photograph.) Methylene blue, ×130.

Fig. 4. Photomicrograph illustrating part of the nerve supply to the arrector pili muscle in the skin of a goat. Methylene blue, ×200.

Fig. 5. Photomicrograph illustrating nerve fibres reactive for specific cholinesterase around an artery in the dermis of the skin of a sheep. AChE, ×150.

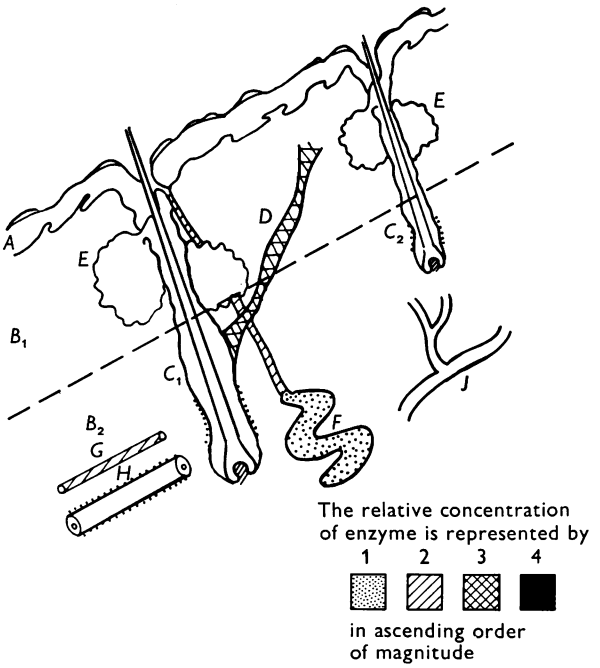


Fig. 7. A diagram illustrating the relative distribution of non-specific cholinesterase in the skin of the sheep and goat. See Fig. 6 for key to lettering.

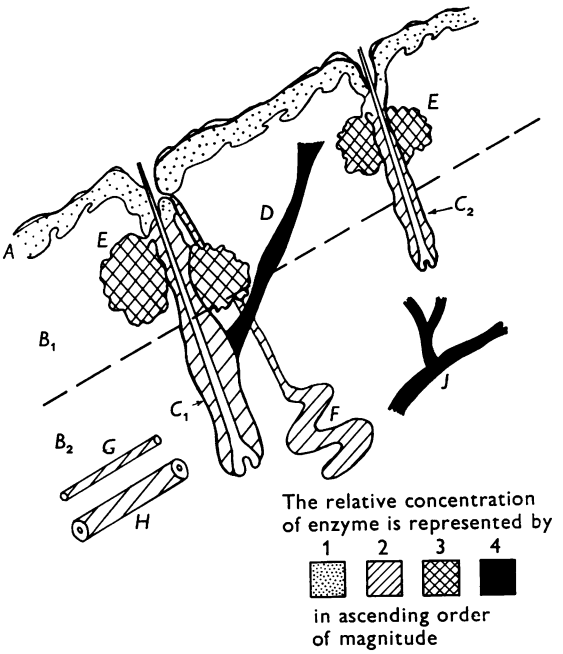


Fig. 8. A diagram illustrating the relative distribution of monoamine oxidase in the skin of the sheep and goat. See Fig. 6. for key to lettering.

DISCUSSION

The distribution of nerves in the skin of the sheep and goat was the same. Compared with the ox (Jenkinson *et al.* 1966) differences were observed particularly in the innervation of the hair follicle, the arrector pili muscle and the epidermis. The hair palisade nerve endings, although individually similar to those described in other species (Weddell, Pallie & Palmer, 1954; Jenkinson *et al.* 1966) were much more variable in size due to the marked differences in size between the primary and secondary hair follicles. The overall size of the palisade ending appears to be related to the size of the hair follicle. The palisade endings around the primary hairs appear to be not only inter-connected, but also connected with those around the secondary hairs by nerve fibres reactive for specific cholinesterase. This suggests that at least part of the palisade ending may function in conjunction with its neighbours as part of an organized unit. The presence of at least two types of nerve fibre, one reactive for specific cholinesterase and the other for monoamine oxidase, within the palisade endings, suggests that as in the ox there may be more than one type of nerve ending present in this complex organ. The presence of what appear to be AChE-reactive nerve fibre connexions between the palisade endings around the primary hairs and the nerve supplies to the arrectores pilorum muscles leads to the speculation that the hair palisade endings may have not only a sensory but also a motor function, perhaps neurosecretory. The disappearance after sympathectomy of what appeared to be nerve fibre connexions between the palisades and arrectores pilorum muscle nerve supplies in the ox (Jenkinson *et al.* 1966) lends support to such a hypothesis. In the sheep and goat, unlike the ox, fibres were observed in the region of the hair follicle bulb, although these appeared to be associated with the numerous blood vessels supplying the follicle rather than with the follicle itself, since no nerve fibres were observed terminating on the bulb.

The nerve supply to the arrector pili muscle was similar to that observed in the ox, but in the sheep and goat the AchE-reactive nerve fibres in the muscle appeared to be more complexly interconnected. The presence of spray nerve endings in the epidermis contrasts with the observation in the ox in which none were found. The presence of AChE-reactive dendritic cells, which were particularly prominent in the sheep and which could be seen in unstained sections, contrasts also with the findings in the ox in which similar cells could be observed only after short incubation for specific cholinesterase.

The failure to detect any nerve fibres to the sweat glands of either the sheep or the goat, or any stem fibres in their vicinity leads to the conclusion that in the sheep and goat, as in the ox, the mechanism of sweat secretion involves not only the sympathetic nervous system but also a local component.

The presence in the skin of two types of nerve fibre, one reactive for specific cholinesterase, and the other for monoamine oxidase, confirms the observations in the ox. It is possible, however, that as in the ox there may be present in the sheep and goat, fibres which react for methylene blue but not for specific cholinesterase or monoamine oxidase although this could not be established in this case.

The distribution of specific cholinesterase, non-specific cholinesterase and monoamine oxidase in the skin of the sheep and goat was similar to that found in the ox.

The only differences were: (1) the general absence in sheep and goat skin of a reaction for specific cholinesterase in the fundus of the sweat gland and in the sebaceous gland, (2) the absence of a reaction for non-specific cholinesterase in the sebaceous glands and in general from the matrix of the dermis, and (3) that the reaction for monoamine oxidase in the sebaceous glands was not uniform, the glands having a stippled appearance. The relative concentrations of these enzymes in the different organs within the skin, however, varied from those in cattle skin. Compared with the ox there was relatively more non-specific cholinesterase and monoamine oxidase in the arrectores pilorum muscles and more monoamine oxidase in the fundus of the sweat glands. A relatively weaker reaction for monoamine oxidase was present in the epidermis and sebaceous glands and the relative amount of specific cholinesterase in the sweat gland duct was diminished. The variability in the distributions and relative concentrations of these enzymes between the sheep and goat and the ox is not unexpected since species differences in the distribution of these enzymes have previously been reported in other animals (Pospíšil, 1959; Winkelmann & Schmit, 1959).

SUMMARY

The distribution of nerves, monoamine oxidase and cholinesterase in sheep and goat skin has been studied. The distributions of specific cholinesterase, non-specific cholinesterase and monoamine oxidase are illustrated. The innervation of the skin of the sheep was similar to that of the goat. Large nerves in the stratum reticulare, which are situated alongside blood vessels, branch to innervate the arteries, veins, arrectores pilorum muscles, epidermis, sebaceous glands and nerve palisade endings around both primary and secondary hairs. No nerve supply to the sweat glands was found. Some of the fibres in the nerves below the level of the sebaceous gland reacted for monoamine oxidase and others for specific cholinesterase. Above this level the nerve fibres generally reacted only for specific cholinesterase. Non-specific cholinesterase was not observed in nerve fibres except in what may be nerve fibres around the arteries.

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