THE MELANOCYTE SYSTEM OF CATTLE SKIN

I. AMELANOTIC DENDRITIC CELLS OF EPIDERMIS

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Abstract

The amelanotic dendritic cells of cattle skin have been studied using the histochemical reactions for non-specific alkaline phosphatase and specific cholinesterase. These epidermal cells in cattle skin show no reaction for adenosine triphosphatase. Their distribution throughout the epidermis, hair follicle sheath, and glandular epithelium has been compared with that reported for other species.

The difference between animals in pattern of amelanotic dendritic cells may be expressed as a score depending on number and size of cells and the length of the dendritic processes. This score has a good repeatability and heritability.

The dendritic score varies inversely with the plane of nutrition. The cells are unaffected when the epidermis is depigmented with hydroquinone. On the other hand they virtually disappear when pigmentation is increased by the joint application of progesterone and oestrogen.

The complexity of the amelanotic dendritic cell pattern of cattle skin varies inversely with (1) keratinocyte activity as expressed in hair growth phase or coat type, a reflection of follicle activity; and (2) the production of melanin which might vary between different hair growth phases, between different body regions of the one animal, between animals subject to the same treatment, between different levels of nutrition, or in response to topically applied hormones.

I. INTRODUCTION

Mammalian epidermis contains distinctive "dendritic" cells in addition to the "ordinary" epithelial cells (Malpighian cells and keratinocytes). Niebauer (1968) has divided the dendritic cells into (1) melanocytes, between the ordinary basal cells, able to form pigment, and so having a positive dopa-oxidase reaction, and (2) Langerhans cells, in the middle and upper epidermis, displaying no function in pigment formation, and so having a negative dopa reaction. This distinction between the two cell systems appears an oversimplification and will be discussed further in Section IV.

Basically, the amelanotic dendritic cells are defined as lacking the capacity to form melanin (dopa-negative) but they are positively identified and visualized by their dendritic form and their content of several hydrolytic enzymes. The enzymes present vary according to species (Niebauer 1968) but may include non-specific alkaline

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phosphatase, specific cholinesterase, or adenosine triphosphatase. Acid phosphatase has been claimed as a component of the dendritic cells (Jarrett 1967) but this enzyme has not been demonstrated in primates (Bell 1967). The amelanotic dendritic cells of cattle skin were demonstrated by their specific cholinesterase activity (Jenkinson et al. 1966). While we have used this technique, we have found the non-specific alkaline phosphatase reaction more satisfactory for routine use in this species.

The object of this investigation was fourfold: (1) to carry out on cattle skin the various enzyme reactions used to demonstrate the amelanotic dendritic cells in other species; (2) to study the distribution of these cells throughout the epidermis and its appendages; (3) to investigate genetic differences in cell pattern; and (4) to study the response of the amelanotic dendritic cells to experimental treatment.

II. Materials and Methods

All animals used in this investigation were from the herds of the National Cattle Breeding Station, Belmont, Rockhampton (Kennedy and Turner 1959). The repeatability and heritability of epidermal patterns were examined in breeding cows and growing stock of various breeds.

The effect of plane of nutrition was examined in two Shorthorn–Hereford steers from a long-term nutritional experiment (Springell 1968). These steers, when first sampled, had been on a low plane of nutrition for 15 months, during which time they had lost 10 kg in body weight. They were then transferred to a high plane of nutrition and after 5 months had gained 112 kg, when they were again sampled.

Two heifers, a Shorthorn–Hereford and a Brahman crossbred, were subjected to treatments designed to increase or decrease epidermal pigmentation. For 4 weeks before sampling, wool fat containing 5% hydroquinone was applied daily to an area of about 40 cm² on the right flank, wool fat containing 0.5% ethinyl and 0.05% ethinyl oestradiol was applied similarly on the left flank, and wool fat alone was applied to a control area on the right side.

The histochemical methods used were based on the reaction for alkaline phosphatase by Gomori (1952), adenosine triphosphatase by Wachstein and Meisel (1957), acid phosphatase by Barka and Anderson (1962), and specific cholinesterase by Koelle and Friedenwald (1949) with the modification by Henderson (1967).

III. Results
(a) Alkaline Phosphatase
(i) Epidermis

Cattle epidermis may show a high density of alkaline phosphatase-positive cells situated both basally and suprabasally. These cells extend their dendritic processes towards the stratum corneum (Fig. 1). On the other hand, the epidermis may be free of dendritic processes although a positive reaction is shown by the endothelial lining of capillaries and other cells of the dermal layer. Depending on the density of cell bodies and the length of dendrites, it is possible to score the epidermis for dendritic cell pattern. A score ranging from 1 to 7 has been used.

Two estimates of the repeatability of dendritic scores on individual animals were made. For a group of 19 heifers, sampled twice at an interval of 12 months, the repeatability was 0.771 (P<0.01). For a second group of 36 heifers, sampled at 20 months of age and again at 36 months when dropping their first calves, the repeatability of the dendritic score was 0.530 (P<0.01).

The heritability of the score based on the correlation between dam and 3-year-old heifer offspring was 78% (r = 0.391, d.f. = 20), calculated within breed groups.
(ii) **Hair Follicle**

Dendritic cells extend down to approximately the same level in the active follicles of cattle as has been reported in primates, viz. the region of the bulge. They are not found in the zone of cell growth and differentiation nor in the pigment-forming region of the hair bulb. In quiescent follicles where hair growth and pigment formation has ceased, dendritic cells occur right to the level of the dermal papilla. The distribution of dendritic cells in active and quiescent hair follicles is illustrated in Figures 2 and 3. In both active and quiescent follicles there is an increase in dendritic cell complexity towards the follicle infundibulum, covering the range of morphology described by Shukla (1966). In the upper or permanent region of the hair follicle the dendritic cells appear more numerous in the catagen phase than in the anagen phase. While dendritic cells in the follicles do not attain the complexity that may be present in the epidermis, they are still found there even in sections where the epidermis is devoid of dendritic processes.

![Image](https://via.placeholder.com/150)

**Fig. 1.**—A vertical section of skin, cut against the lay of the hair follicles, to show dendritic cells in the epidermis and follicle sheath. Alkaline phosphatase. \( \times 150 \).

(iii) **Skin Glands**

Alkaline phosphatase-positive dendritic cells have been found in the sweat gland duct to the level of the sebaceous gland. They are sometimes seen on the periphery of sebaceous glands.

(b) **Specific Cholinesterase**

The reaction for specific cholinesterase shows the terminal nerve endings and the dendritic cells of the epidermis, follicle infundibulum, and upper follicle sheath.
Figs. 2 and 3.—A comparison between active (Fig. 2) and quiescent (Fig. 3) follicles in distribution of dendritic cells. In active follicles the dendritic cells are not found in the zone of cellular activity (keratin or pigment formation) while in quiescent follicles they extend to the level of the dermal papilla. Alkaline phosphatase. × 225.
Figs. 4 and 5.—The dendritic cells in the epidermis of a Hereford–Shorthorn steer on a low plane of nutrition in autumn are relatively complex (Fig. 4). But if the steer is on a high nutritional plane in spring no dendritic cells are apparent and the epidermis is quite pigmented (Fig. 5). Alkaline phosphatase. ×240.
Figs. 6 and 7.—A comparison between an area treated with progesterone and oestrogen (Fig. 6) and an area simultaneously treated with hydroquinone (Fig. 7) in a Hereford-Shorthorn heifer. The hormonally treated epidermis has lost its dendritic cells and is quite pigmented. In the depigmented epidermis, which shows acanthosis, the dendritic cells are largely intact. Alkaline phosphatase. × 225.
(c) Adenosine Triphosphatase

A positive reaction for adenosine triphosphatase was given by the endothelial lining of blood vessels, the arrector pili muscles, and the orifice of sebaceous glands. No reaction was given by the epidermal dendritic cells.

(d) Acid Phosphatase

The dendritic cells did not react positively for acid phosphatase. A reaction, continuous from the sebaceous gland orifice to the epidermis, could be seen while a positive reaction was given by the intra-epidermal portion of the sweat gland duct.

The secretory cells of the sweat glands reacted positively for acid phosphatase and a strong reaction was given by the regressing papillary cells of the catagen follicles.

(e) Experimental Treatment

(i) Effect of Nutrition

The alkaline phosphatase reaction was carried out on skin sections from two Hereford–Shorthorn steers in April when they had been on a low plane of nutrition, and again in October when they had been on a high nutritional plane for 5 months. For both animals, the amelanotic cells were complex in April when the epidermis was relatively unpigmented. In October phosphatase-positive cells were virtually absent from the epidermis which had become quite pigmented. The change in dendritic cell pattern is shown in Figures 4 and 5.

While the coat was relatively long, pale, and woolly in April when the animals were on a low nutritional plane, it was short, sleek, and rich in colour in October when the nutritional level was relatively high. This transformation was of an order of magnitude much greater than the normal seasonal change between autumn and spring.

(ii) Pigmentation and Depigmentation

Increased melanogenesis occurred in the skin of the two heifers treated topically with a mixture of 0·05% ethinyl oestraadiol and 0·5% ethisterone. The changes in the amelanotic dendritic cells were comparable to those brought about by an increased plane of nutrition. While the phosphatase-positive cells were still evident in the follicle sheath, they had virtually disappeared from the epidermis which had become quite pigmented (Fig. 6).

After 4 weeks treatment with 5·0% hydroquinone in wool fat the epidermis was completely depigmented (Fig. 7) although there was no noticeable bleaching of the hair. The dendritic cells were not affected. Acanthosis of the epidermis was evident and the treated areas were somewhat tender to the touch.

A comparison between areas having pigmented and non-pigmented hairs showed differences comparable to those produced by change of nutritional plane or by topical treatment. The density of dendritic cells in the epidermis, follicle sheath, and infundibulum of the non-pigmented skin was much higher than in the pigmented skin.

IV. Discussion

The amelanotic dendritic cells, which are positive for alkaline phosphatase in cattle skin, are distributed throughout the epidermis, hair follicle sheath, and glandular
epithelia, as reported for primates (Montagna 1967) and sheep (Lyne and Hollis 1968). An apparently unique feature of these cells in cattle skin is their negative reaction for adenosine triphosphatase. This enzyme has been claimed by Wolff and Winkelmann (1967a, 1967b) as a specific enzyme for the Langerhans cell although this is disputed by Zelickson and Mottaz (1968). A comparison is made between the amelanotic dendritic cells of cattle skin and those of other species in Table 1.

Table 1
Comparison between the Characteristics of Amelanotic Dendritic Cells of Cattle Skin and Those Reported for Other Species

<table>
<thead>
<tr>
<th>Character</th>
<th>Cattle (this paper)</th>
<th>Observations for other species</th>
<th>References</th>
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<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+ (african primates)</td>
<td>Montagna and Yun (1962); Bell (1967)</td>
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<tr>
<td>Specific cholinesterase</td>
<td>+*</td>
<td>+ (bat)</td>
<td>Bourland et al. (unpublished data)</td>
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<tr>
<td></td>
<td></td>
<td>+ (sheep)</td>
<td>Lyne and Chase (1966)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>– (african primates)</td>
<td>Bell (1967)</td>
</tr>
<tr>
<td>Adenosine triphosphatase</td>
<td>–</td>
<td>+ (man)</td>
<td>Riley (1966)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ (rhesus monkey)</td>
<td>Im and Montagna (1965)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ (albino mouse)</td>
<td>Riley (1966, 1967)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ (guinea pig)</td>
<td>Wolff and Winkelmann (1967b); Riley (1966, 1967)</td>
</tr>
<tr>
<td>Presence in keratogenous zone</td>
<td>–</td>
<td>– (primates)</td>
<td>Montagna (1967)</td>
</tr>
<tr>
<td>Abundance where pigmentation is scarce or absent</td>
<td>+</td>
<td>+ (potto)</td>
<td>Montagna and Yun (1962)</td>
</tr>
<tr>
<td>Presence in sweat gland duct</td>
<td>+</td>
<td>+ (bat)</td>
<td>Wolff and Winkelmann (1967c)</td>
</tr>
<tr>
<td>Presence in sebaceous gland wall</td>
<td>+†</td>
<td>+ (primates)</td>
<td>Montagna (1967)</td>
</tr>
<tr>
<td>Association with dermal nerves</td>
<td>+</td>
<td>+ (guinea pig)</td>
<td>Niebauer (1968)</td>
</tr>
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<td></td>
<td></td>
<td>+ (human foetus)</td>
<td>Serri and Cerimele (1967)</td>
</tr>
<tr>
<td>Effect of depigmenting agent</td>
<td>–</td>
<td>– (guinea pig)</td>
<td>Bleehen et al. (1968); Bleehen (1970)</td>
</tr>
</tbody>
</table>

* Also observed by Jenkinson et al. (1966). † Infrequent occurrence.

What are the specific cells comprising the alkaline phosphatase-positive dendritic cells of cattle epidermis? Using ultramicroscopic techniques, Bell (1967) confirmed the earlier hypothesis of Montagna and Yun (1962) and Quevedo and Montagna (1962) that the alkaline phosphatase-positive cells of the potto were identical with the Langerhans cells of other species. The Langerhans cells are the only dendritic cells occurring in the middle and upper epidermal layer and so they constitute the most striking feature of the phosphatase-positive dendritic pattern in cattle skin. But the Langerhans cells are not necessarily confined to the middle and upper layers of the epidermis. They occur normally in the basal layer of the potto (Bell 1967) while they are found in the basal layer of human epidermis in vitiligo (Birbeck et al. 1961) and in piebaldism (Breathnach et al. 1965).
From their studies on the hairless mouse, Wolff and Winkelmann (1967c) concluded that a proportion of the adenosine triphosphatase cells were amelanotic melanocytes. This appears to be the case also with the alkaline phosphatase cells of cattle skin where a reciprocal relationship between dendritic cell score and melanogenic activity occurs in a number of instances. While the dopa-positive cells of primates apparently lack alkaline phosphatase (Montagna and Ellis 1959; Montagna and Yun 1962), the dopa-negative cells, which include young and old melanocytes (Hu 1968), appear to be phosphatase-positive.

The total evidence indicates that in cattle skin the alkaline phosphatase-positive cells of the middle and upper epidermis are Langerhans cells. The phosphatase-positive cells of the basal epidermal layer may be a mixture of Langerhans cells and inactive or amelanotic melanocytes, whether immature or effete.

The morphology, distribution, and density of the amelanotic cells confer a characteristic pattern on cattle epidermis. This pattern may be expressed as a score the repeatability of which was highly significant in the two groups of animals studied. For a group of 24 cows and their 3-year-old heifer offspring the heritability was 78%, calculated within each of the three breed groups.

The experimental treatment of animals showed there was a relationship between dendritic cell pattern and both the rate of keratinization and degree of pigmentation. This phenomenon was well illustrated in a comparison between different growth phases within the hair follicle sheath. In the anagen phase, when keratinocyte activity and hair pigmentation or melanotic melanocyte activity in the hair bulb is a maximum, no dendritic cells were found in the zone of cellular activity and those approaching this zone had a simple morphology. In the catagen and telogen phases when hair growth and pigment production in the hair follicle has ceased, complex dendritic cells may be found right to the level of the dermal papilla. In the same way, animals on a low nutritional plane had a straw-coloured woolly coat and their non-pigmented epidermis contained complex cells with large dendritic processes. After several months on a high nutritional plane, the woolly coat was shed, new richly pigmented hairs were produced, and the complex dendritic cells largely disappeared from the epidermis which had become quite pigmented.

The distribution of dendritic cells in relation to keratinization has been observed in laboratory species (Montagna 1956; Lessard et al. 1966; Riley 1966, 1967) while a relationship has been observed under pathological conditions in humans. The density increases in ichthyosis where there is an excess scaliness and decreases in psoriasis where there is an increase in mitotic activity and abnormal keratinization (Giacometti 1968).

The observations of Riley showed an inverse relationship between dendritic cell concentration and tyrosinase activity in comparisons of the scale and interscale regions of mouse tail and an increase in the number of suprabasal dendritic cells in depigmented human skin. The dendritic cells of cattle skin are denser, the processes longer, and the cell bodies larger in non-pigmented areas than they are in pigmented areas. This applies whether the differences in pigmentation are genetic or a result of experimental treatment.

There is a functional and close biologic relationship between the Langerhans cells and the epidermal cells (Wolff and Winkelmann 1967c) and Prunieras (1969) has
shown that tissue cultures of epidermal cells need dendritic cells for organized growth. The esterase activity of the Langerhans cell suggests active metabolism and its ultrastructural appearance indicates that it is engaged in protein metabolism (Breathnach et al. 1963; Breathnach 1964; Zelickson 1965). In cattle skin, as has been reported in other species, there is a close association between dendritic cell pattern, keratin production, and pigment formation, and it is possible that the role of the Langerhans cell as in tissue cultures is an organizational one.

V. Acknowledgments

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A. V. SCHLEGER AND K. G. BEAN
MELANOCYTE SYSTEM OF CATTLE SKIN. I
