THE MELANOCYTE SYSTEM OF CATTLE SKIN

II.* MELANOTIC MELANOCYTES OF EPIDERMIS AND DERMIS

By A. V. Schleger†‡ and K. G. Bean†

[Manuscript received 21 June 1972]

Abstract

The distribution of melanotic melanocytes has been studied throughout the epidermis and dermis of cattle skin. Characteristic differences occur between animals according to pattern of epidermal amelanotic dendritic cells and there is a marked contrast between pigmented and non-pigmented areas of the one animal.

The two major types of melanocyte observed in mice are apparent in cattle epidermis and epidermal melanocyte morphology appears to be related to size and distribution of dopa-positive dermal cells.

Active melanocytes, as indicated by the presence of peroxidase, have been demonstrated in all layers of the skin. Melanophages, rich in acid phosphatase, are more pronounced in the subepidermal portion of the dermal layer.

The melanocyte system is highly sensitive to external stimuli. Melanogenic response to surgical biopsy, parasitic exposure, and the topical application of agents increasing or decreasing pigment formation has been investigated.

It is postulated that the melanocyte system might have an important surveillance role in the defence mechanisms of the skin.

I. INTRODUCTION

The amelanotic dendritic cells of cattle skin show characteristic differences between animals in morphology and distribution throughout the epidermis (Schleger and Bean 1973). They respond to experimental treatment such as change of nutrition or the topical application of agents which increase pigmentation. There is a reciprocal relationship in cattle epidermis between the incidence of amelanotic dendritic cells and the degree of pigmentation, presumably an index of melanocyte activity.

This investigation centres on four main topics: (1) the morphology of the epidermal melanocyte and its possible relationship to melanosome formation in both the epidermis and dermis. This is based on the relationship between melanocyte morphology and cytocrine activity or melanosome transfer in the mouse as reported by Markert and Silvers (1959) and Sweet and Quevedo (1968); (2) the difference in

† Division of Animal Genetics, CSIRO, Cattle Research Laboratory, Rockhampton, Qld. 4700.
‡ Present address: Division of Animal Health, CSIRO, Long Pocket Laboratories, Private Bag No. 3, Indooroopilly, Qld. 4068.

melanotic melanocytes, both epidermal and dermal, between animals differing in dendritic cells and between areas differing in pigmentation; (3) the distinction between melanocytes and melanophages, that is, melanin formation and phagocytosis, in the dermis; and (4) the response of the melanotic melanocyte system to experimental treatment.

II. METHODS AND MATERIALS

(a) Treatment of Animals

The animals sampled in this investigation were from the herd at the National Cattle Breeding Station (Kennedy and Turner 1959). The trial involving the topical application of pigmented agents has already been described (Schlegel and Bean 1973).

Since the animals studied were sometimes involved in experiment, treatments likely to affect melanocyte morphology have been described. For example, heavy artificial tick infestation or initial exposure to tick larvae (ticks are endemic in the animals studied) is likely to produce an inflammatory response. Inflammatory conditions of the skin in humans produce large perikarya and elongate dendrites (Pinkus et al. 1959). Other skin characters which may be associated with the same melanocyte morphology are a high degree of pigmentation and a low melanocyte density (Breathnach 1957).

To produce increased melanogenesis as in the distinction between melanocytes and melanophages, animals on a high nutritional plane were studied, or selected areas of the skin were exposed to tick larvae for 24 hr. There is increased melanogenesis at the site of attachment of tick larvae [Section III(d)(iv)]. Because of the possible relationship between an animal's tick resistance and its melanogenic response, now being investigated, the relative tick resistance of an animal is stated where it may be relevant.

The level of tick resistance of animals equally exposed to larvae was assessed by counting females 4·5–8·0 mm in length on one side of each animal (Wharton and Utech 1970).

(b) Histology

Skin biopsies were taken with a 1 cm trephine and fixed in 10% neutral formalin for 4–6 hr. Sections of 16 μm thickness were cut in a cryostat at −20°C while sections of 100 μm were cut with a sledge microtome using a freezing stage.

Histochemical methods used were based on the reactions for alkaline phosphatase by Gomori (1952), dopa-oxidase by Becker et al. (1935), cholinesterase by Koelle and Friedenwald (1949), acid phosphatase by Barka and Anderson (1962), and peroxidase by Graham et al. (1965).

(c) Combination Enzyme Reactions

A distinction between melanin synthesis (melanocyte) and melanin phagocytosis (melanophage) has been made by combination enzyme reactions involving acid phosphatase and peroxidase. Acid phosphatase, the principal enzyme of the lysosome (De Duve 1963; Novikoff 1963), is an index of phagocytosis. Its association with melanin or its precursor suggests a melanophage. The role of peroxidase in the catalysis of melanin synthesis has been documented (Mason et al. 1957; Okun 1967). Peroxidase has been shown to be associated with the formation of melanin granules in the intestines (Marsden 1966) and liver (Schlegel 1970) and it plays a part in initiating the in vivo synthesis of melanin and catecholamines (Okun et al. 1970).

The acid phosphatase-dopa reaction was based on that described by Mishima (1966). Similar washing procedures between enzyme reactions were used in a peroxidase-dopa reaction and a cholinesterase-dopa reaction. The latter combination reaction was used to demonstrate the α- and β-cholinergic nerve supply to the melanocytes. Through an alkaline phosphatase-dopa reaction using fast red TR as the diazonium salt, the dopa-positive cells surrounding capillaries were illustrated in the dermis and the melanotic cells of the epidermis could be distinguished from the amelanotic.
III. Results

(a) Melanotic Melanocytes of Epidermis

(i) Melanotic versus Amelanotic Melanocytes

A comparison between the melanotic melanocytes and amelanotic dendritic cells of cattle epidermis may be made when the reactions for alkaline phosphatase and dopa-oxidase are carried out on different sections of the one biopsy. When the dendritic score is low the melanocytes are distributed throughout the basal layer and appear in high concentration in the follicle infundibulum (Fig. 1). When the dendritic score is high the melanocytes are restricted to the lower infundibulum (Fig. 2).

Using an alkaline phosphatase–dopa-oxidase combination enzyme reaction with fast red TR as the diazonium salt, the phosphatase-positive amelanotic dendritic cells (red) can be easily distinguished from the dopa-positive melanotic melanocytes (black). This combination reaction, however, often results in loss of dendritic processes by the amelanotic cells.

(ii) Morphology

The epidermal melanotic melanocytes appear to conform to two major types comparable to those which Markert and Silvers (1959) have described in mice as nucleopetal (Fig. 3) and nucleofugal (Fig. 4). Morphology of epidermal melanocytes appears to be associated with number and size of dopa-positive cells in the adjacent dermis. The dermal cells are larger and more numerous when the epidermal melanocytes are predominantly of the simpler nucleopetal type.

Complex dendritic melanocytes have been observed in Hereford–Sahiwal animals exposed to tick larvae for the first time and in Hereford–Shorthorns on a high nutritional plane and under heavy artificial tick infestation. The dendritic processes may contain bead-like structures representing oscillatory melanin granules (Klaus and Snell 1967) or show secondary branching.

(iii) Pigmented versus Non-pigmented Areas

The melanocyte pattern differs widely between the pigmented and non-pigmented areas of skin in a Hereford or Hereford–Shorthorn animal. The pigmented areas generally contain a high concentration of melanocytes throughout the basal layer of the epidermis. The non-pigmented areas may differ in two ways. The epidermis may contain no melanocytes which are restricted to the upper follicle sheath or the epidermis may contain distended melanocytes which give no evidence of melanin transfer. That is, there is no association with the dopa-positive cells of the dermis.

(iv) Breed Comparison

The darkly pigmented (eumelanic) epidermis, typical of pure-bred Brahman animals and a proportion of their crossbreds, is discussed in Section III(d)(iii). Hereford–Shorthorn animals normally have a pheomelanic (yellow–red) epidermis but those showing high melanogenic activity may have dopa-positive granules distributed throughout a thickened epidermis.
Figs. 1 and 2.—The epidermal melanocyte pattern in an animal having a low amelanotic dendritic score (Fig. 1) and in one showing a high density of complex amelanotic cells (Fig. 2). In the latter case the dopa-positive cells of the epidermis are largely restricted to the follicle infundibulum. Dopa-oxidase. × 140.
(v) Hair Follicle

In cattle with pigmented epidermis melanotic melanocytes may be observed in the outer root sheath of the hair follicle. They are sometimes observed in the outer root sheath in the vicinity of the hair bulb and in the catagen phase, when the hair shaft has largely lost its pigment, the melanocytes can be seen capping the dermal papilla. In contrast to follicles in the anagen phase, follicles in the beginning of catagen often show an array of pigmented material entering the follicle sheath from the hair bulb.

Figs. 3 and 4.—The nucleopetal type of epidermal melanocyte (Fig. 3) is generally more dense and associated with more dopa-positive subepidermal cells than the nucleofugal type (Fig. 4). Dopaoxidase. ×160.
Figs. 5 and 6.—A comparison between an area of skin treated for 4 weeks with progesterone and oestrogen (Fig. 5) and another area of the same animal simultaneously treated with hydroquinone (Fig. 6). Small, well-defined dendritic cells are barely visible in the pigmented epidermis of the hormonally treated area while the cells of the depigmented epidermis appear somewhat pyknotic. Dopa-oxidase. × 225.
(vi) **Glandular Epithelia**

Melanotic melanocytes have not been observed in either the sweat gland or sebaceous gland wall of cattle skin. This is in contrast to the amelanotic dendritic cells which are occasionally found in the sebaceous gland wall (Schleger and Bean 1973). In animals with pigmented epidermis, melanotic melanocytes are sometimes observed in the sweat gland duct. On the other hand amelanotic dendritic cells are always observed.

(b) **Dopa-positive Dermal Cells**

(i) **Morphology**

In addition to the rounded cells of the papillary layer, cells of various shapes are found in the reticular layer in association with the blood vessels. The cells of the reticular layer, best seen under conditions of increased melanogenesis, are in general larger than those of the papillary layer.

(ii) **High versus Low Amelanotic Dendritic Score**

When the dopa reaction is carried out on animals of high and low epidermal dendritic score a different pattern of dermal cells is evident between the two types. The animal with the low amelanotic dendritic score has a uniform distribution of relatively large dopa positive dermal cells (Fig. 1). The dermal cells of the high-scoring animal are variable in size but mostly small (Fig. 2).

(iii) **Pigmented versus Non-pigmented Areas**

In pigmented areas the dopa-positive dermal cells are denser than in non-pigmented areas and are closely associated with the epidermal melanocytes. There is no association between dermal and epidermal melanocytes in the non-pigmented areas.

(iv) **Capillary and Nerve Supply**

The association between dermal melanocytes and capillaries is illustrated by the alkaline phosphatase–dopa reaction. Likewise the accumulation of dermal melanocytes round cholinergic nerves is seen in sections on which the dopa and cholinesterase reactions have been carried out. The dopa reaction is often shown by the larger nerve fibres, suggesting phagocytosis by the perineural cells.

(c) **Melanocytes and Melanophages of Dermis**

(i) **Melanocytes**

The peroxidase–dopa reaction showed that melanin formation takes place, not only in the epidermis but in both the papillary and reticular layers of the dermis. While the peroxidase reaction was best demonstrated in association with the smaller dopa-positive cells or granules, it was also evident in a proportion of the larger perivascular cells of the reticular layer.

(ii) **Melanophages**

In the papillary layer acid phosphatase was frequently observed in combination with the dopa-positive cells. The extent of the acid phosphatase reaction decreased
from the subepidermal to the deeper papillary layer. That is, enzyme activity decreased as size of the melanophage increased. This suggested that a high proportion of the dopa-positive cells of the papillary layer were melanophages and originated from the epidermis. Further evidence was given by an array of cells frequently observed linking the epidermis and the larger dopa-positive cells of the papillary layer.

In the reticular layer there was little evidence of acid phosphatase in the perivascular dopa-positive cells. This suggested that these cells were inert residual bodies (De Duve and Wattiaux 1966) but those containing peroxidase were assumed to have melanogenic potential.

No epidermal melanocytes were demonstrated by the acid phosphatase–dopa reaction, although they may be observed if the dopa reaction precedes the acid phosphatase reaction. This suggests that the epidermal melanocytes are specific for tyrosine, preincubation with the acid phosphatase substrate probably causing diazotization of tyrosine by the nitrous acid component.

(d) Experimental Treatment

(i) Physical Trauma

Increased melanogenesis, as indicated by an association between peroxidase and fine melanin granules, has been observed in the various skin layers towards the cut edge of skin biopsies. (The fixation procedure does not inhibit an increase in dopa-oxidase activity after the biopsy is taken.)

The daily application of wool fat to cattle skin for a period of 4 weeks produced a thickening and lightening of the epidermis as well as an increase in melanocyte dendritic processes.

This change of melanocyte morphology is an indication of increased melanin formation in mammals (Snell 1967) and the physical treatment of the skin, through metabolic stimulation of the keratinocytes, is the likely cause.

(ii) Nutrition

The effect of plane of nutrition on melanocyte morphology has not been critically examined, but animals on a high nutritional plane invariably have a pigmented epidermis, lengthy dendrites in their epidermal melanocytes, and a high concentration of dopa positive dermal cells.

(iii) Pigmentation and Depigmentation

If a comparison is made between the normal epidermis of a Brahman crossbred heifer and that to which a combination of progesterone (0·5% ethisterone) and oestrogen (0·05% ethinyl oestradiol) in wool fat had been applied for 4 weeks, the treated epidermis (Fig. 5) is thicker, slightly less pigmented, and the melanocytes are larger and more dendritic than normal. The control area is intermediate in epidermal thickness. This must be a consequence of the physical application of the wool fat. There was a systemic effect of the female sex hormones, pronounced mammary development being observed, and this would have affected both the untreated and control areas.
The area which had been treated for the same time with 5% hydroquinone in wool fat showed complete loss of pigment in the epidermis. This made more visible the melanocytes which had lost their dendritic processes and appeared quite pyknotic (Fig. 6). Only the melanocytes of the infundibulum showed any length of dendrite.

The dermal melanocytes of cattle skin appear refractory to these topical treatments.

(iv) Reaction to Tick Larvae

Examination of skin areas following exposure to tick larvae gave evidence of increased melanogenesis. The epidermal melanocytes became much more reactive and the number of reacting cells increased in the vicinity of the tick lesion. The dopa-positive cells of the dermis appeared larger, more numerous, and more reactive than normal. The response of the phosphatase-positive, amelanotic dendritic cells was quite the opposite. The amelanotic dendritic cells almost completely disappeared from the epidermis and upper follicle sheath.

IV. DISCUSSION

A number of observations have been made on the melanocyte system of cattle skin which corroborate the findings made on a number of other species. Such observations are listed in Table 1 for the epidermal melanocyte system while the dermal melanocytes are featured in Table 2. The characters listed in section B of Table 2 are those which differ in cattle skin.
(a) Melanotic and Amelanotic Cell Systems

Although the melanotic and amelanotic cell systems have been described as distinct, self-maintaining, and functionally independent (Wolff and Winkelmann 1967) there appears a reciprocal relationship between the two systems in cattle skin. When the enzyme activity or cell number of one system is high, that of the other system appears low. This inverse relationship is brought out in comparisons between animals, and in the contrasting behaviour of the two systems in response to parasitic attachment, or the topical application of agents which either increase or decrease pigmentation.

<table>
<thead>
<tr>
<th>Character</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Features common to cattle and other species</td>
<td>Melanophages beneath dermal–epidermal junction</td>
<td>Man</td>
</tr>
<tr>
<td>Small and rounded in papillary layer</td>
<td>Small and rounded in papillary layer</td>
<td>Primates</td>
</tr>
<tr>
<td>Various forms in reticular layer</td>
<td>Various forms in reticular layer</td>
<td>Rhesus monkey</td>
</tr>
<tr>
<td>Distributed round nerves and blood vessels</td>
<td>Distributed round nerves and blood vessels</td>
<td>Primates</td>
</tr>
<tr>
<td>Peroxidase associated with melanin granules</td>
<td>Sheep</td>
<td>Sheep</td>
</tr>
<tr>
<td>Refractory to hormonal treatment</td>
<td>Rodent intestine</td>
<td>Rodent intestine</td>
</tr>
<tr>
<td>Surgical biopsy stimulates melanogenesis</td>
<td>Mouse</td>
<td>Mouse</td>
</tr>
<tr>
<td>B: Features found in other species but not cattle</td>
<td>Melanocytes present only in pathological conditions</td>
<td>Man</td>
</tr>
<tr>
<td>Melanocytes inactive metabolically</td>
<td>Rhesus monkey</td>
<td>Rhesus monkey</td>
</tr>
<tr>
<td>Melanocytes in sebaceous glands</td>
<td>Primates</td>
<td>Primates</td>
</tr>
<tr>
<td>Melanocytes in sebaceous glands and sweat glands</td>
<td>Sheep</td>
<td>Sheep</td>
</tr>
</tbody>
</table>

There is evidence that phosphatase-positive cells are not melanotic and that melanotic melanocytes do not react for alkaline phosphatase. Animals may contain no phosphatase-positive cells in the epidermis yet have a uniform distribution of dopa-positive melanocytes. The non-pigmented areas of skin contain a high density of phosphatase-positive epidermal dendritic cells (Schleger and Bean 1973). They contain no dopa-positive cells, other than in the hair follicle sheath, or a small number of apparently non-functional cells in the epidermal basal layer.

The amelanotic and melanotic cells systems of cattle skin are therefore specific in their enzyme content as has been shown for primates (Montagna and Ellis 1959; Montagna and Yun 1962; Wolff and Winkelmann 1967).

(b) Melanocytes and Melanophages

Under conditions of increased melanogenesis the distribution of melanocytes and melanophages becomes quite apparent. Melanin formation may take place at
all levels in the skin. The melanogenic enzyme marker, peroxidase, is generally associated with the finer dopa-positive granules but it also occurs in at least a proportion of the larger perivascular cells of the reticular layer.

Acid phosphatase is most evident in the smaller subepidermal dopa-positive aggregates. It decreases in the deeper papillary layer where the size of the dopa-positive cells increases. Virtually no acid phosphatase was found in the relatively large aggregates of the reticular layer. This suggests a transition from phagosomes to inert residual bodies (De Duve and Wattiaux 1966) but at least a proportion of the reticular cells have a melanogenic function and must be melanocytes.

(c) Activity of Dermal Melanocytes

It is apparent that increased pigmentation of the dermal region of cattle skin can occur from increased activity of the dermal melanocytes as has been shown for the Mongolian gerbils (McDonald et al. 1970). In man, melanocytes are not normally found in the dermis (Breathnach 1969) and Montagna (1967) concluded that the dermal melanocytes of adult primates were static non-functioning cells like the pigment cells of the retina. More critical work is necessary to distinguish between true dermal melanocytes and other dermal cells having dopa-oxidase activity.

An example of epidermal–dermal independence in melanin synthesis is provided by the non-pigmented areas of Hereford skin. There is no uptake of melanosomes by the malpighian cells of the epidermis in such areas; the transfer of pigment from the epidermis to the dermis seems doubtful whereas dermal melanocyte activity is suggested by the presence of fine pigment granules in association with the larger pigment aggregates or flocculations.

(d) Epidermal Melanocyte Morphology

The relationship between melanocyte morphology and pigment production does not appear to be a simple one. As a general rule, the simple rounded nucleopetal type of melanocyte is associated with a higher concentration of dopa-positive dermal cells. Complex dendritic melanocytes on the other hand, have been observed in a number of circumstances:

(1) The sparse melanocytes in the pigmented epidermis of a Sahiwal–Hereford cross-bred animal after initial exposure to tick larvae had elongated processes.

(2) Hereford–Shorthorn steers on a high nutritional plane and receiving heavy infestations of larvae had very long dendritic processes which featured secondary branching.

(3) Stimulation to melanogenesis by the topical application of sex hormones promoted the complexity of the melanocytes also.

(4) In non-pigmented areas of Hereford animals epidermal melanocytes may occur. Although these yield no pigment to the epidermis and probably none to the dermis they are distended in a somewhat grotesque fashion. So complexity of melanocytes per se does not necessarily mean high melanocyte activity or a high rate of pigment transfer.
(e) Significance of the Keratinocyte

Evidence that keratinocyte proliferation precedes or outstrips increased melanocyte activity is given by the decreased pigmentation of the hormonally treated epidermis, the hypopigmentation representing a dilution effect. Fitzpatrick (1965) considers that the low melanin content of keratinocytes in psoriasis and verruca vulgaris results from the short duration of contact with the melanocyte. Increased epidermal thickness accompanies increased melanin synthesis in cattle skin just as occurs in radiation-induced tanning (Quevedo and Smith 1963).

It has been claimed by Cruickshank and Harcourt (1964) that the keratinocyte is more active than the melanocyte in pigment transfer. Klaus (1970) listed rate of epidermal cell turnover among the factors affecting his "transfer index". Prunieras (1969) believes that keratinocyte multiplication exerts some inductive effect upon melanocyte morphology while Hadley and Quevedo (1966) claim that the melanogenic stimulus of ultraviolet light is effected indirectly by inducing proliferation of keratinocytes.

(f) Melanocyte System in Skin Adaptation

The melanocyte system of cattle skin is highly responsive to trauma. Treatments such as surgical biopsy, infestation with tick larvae or the topical application of a chemical sensitizer (Schlegel, unpublished data) produce increased melanogenesis. Papa and Kligman (1965) refer to the epidermal melanocyte system as a sensitive barometer of inflammatory conditions of the skin. This suggests a passive role whereas there is an active melanogenic response to external stimulation of cattle epidermis. Wasserman (1967) stresses the importance of leucocytic melanin transport and brackets this phenomenon with the epidermal melanin unit (Fitzpatrick and Breathnach 1963). Dopa is known to stimulate the immunogenic response [Devoino and Eliseeva (Korovina) 1970]. It is possible that the melanocyte system has an important surveillance role in the defence mechanisms of the skin.

V. ACKNOWLEDGMENTS

We appreciate the co-operation of Mr. J. F. Kennedy and staff of the National Cattle Breeding Station. The assistance of Mrs. J. Frisch is acknowledged. We thank Dr. V. J. McGovern and Mr. H. G. Turner for their criticism of the manuscript.

VI. REFERENCES


GOMORI, G. (1952).—“Microscopic Histochemistry.” (Chicago Univ. Press.)


