DENDRITIC CELLS IN PIGMENTED HUMAN SKIN

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INTRODUCTION

It has long been known that the characteristic coloration of the skin of the pigmented human races is due to the presence of the pigment melanin. This occurs in the epidermis where it is most abundant at the level of the deepest or basal layer of cells. As to the source of origin of this pigment and the intimately connected problem of the exact cellular composition of the tissue that contains it, complete unanimity of opinion has not yet been reached, some authors still maintaining that the Malpighian cells are the site of melanogenesis (see reviews by Hoepke, 1927; Bloch, 1929; Percival & Stewart, 1930; Laterjet, 1938; Meirowsky, 1940; Masson, 1948).

In guinea-pig epidermis, a compound tissue in whose structure two anatomically distinct types of cell participate—Malpighian cells with their specialized derivatives, and dendritic cells—melanogenesis has recently been shown to be an exclusive property of the latter (Billingham, 1948). Two distinct 'true breeding' races of these branched cells exist: the 'pigmented' dendritic cells which occur in the black epidermis of the spotted black-and-white guinea-pig and which are responsible for its pigment formation, and the anatomically identical 'white' dendritic cells which are found exclusively in the white epidermal regions. 'Pigmented' and 'white' dendritic cells differ only in that the former are endowed with the power of melanogenesis. The epidermis of ordinary white human skin has a similar twofold composition, but so far as pigmentary activity is concerned it resembles the skin of the so-called albino guinea-pig in that it has a latent capacity for pigment formation. In this animal the epidermis is normally white, probably because of the presence of an inhibitor (Onslow, 1915; Ginsburg, 1944), but it may be caused to blacken by such a mild stimulus as cold weather. It may be added that in the white skin of the spotted guinea-pig no merely physical stimulus is known which will initiate even the slightest degree of melanogenesis (Lewin & Peck, 1941; Ginsburg, 1944).

In view of these considerations there seemed to be the strongest indirect evidence that the epidermis of pigmented human skin would have a functional and anatomical dendritic cell system closely resembling that present in pigmented guinea-pig skin. The object of this study has been to obtain direct evidence in support of this analogy.

In this paper the term 'pigmented human skin' refers only to that of Indian or Negro and no attempt has been made to differentiate between the two types. The two main techniques used, although briefly described in a previous paper (Billingham, 1948), are here reported in some detail, in the hope that they will be of use to other students of cutaneous pigmentation.

MATERIAL AND METHODS

Pieces of normal pigmented skin (made available through the kindness of Prof. T. Pomfret Kilner, Dr H. M. Hanschell and Mr Dallas Ross) from various sites on the bodies of Indians and Negroes have been used in this study. The bulk of the material

consisted of trimmings from Thiersch grafts (i.e. very thin sheets of skin comprising the epidermis and only the superficial part of the dermis).

Sections. The fresh material was fixed in formol-mercuric chloride, dehydrated in an ethyl alcohol series and, after the use of cedarwood oil followed by ligroin as antemedia, the tissue was embedded in paraffin wax. Sections were cut at 8–10 μ and were lightly stained with Mayer's carmalum or Ehrlich's haematoxylin and eosin.

Whole mounts of 'Split' skin. The skin-splitting technique adopted (Medawar, 1941) depends upon the enzymic dissolution of the fine elastic fibres that unite the epidermis to the dermis. Freshly obtained Thiersch graft shavings which had previously been vaselined on their cuticular surface were cut into fragments of approximately 0·5 cm.^{2} These were floated on to a Seitz-filtered 0·5% solution of commercial trypsin powder in Ringer-bicarbonate containing 1:100,000 phenol red and adjusted thereby to pH 7·8, and digested for about 30 min., or longer if necessary (depending on the thickness of the material), at 38° C. The fragments were then rinsed in unbuffered Ringer's solution, blotted free from excess fluid, and then carefully flattened out on a dry slide, cuticular surface lowermost. The thin layer of dermal tissue was then lifted off with fine forceps leaving the epidermis behind as a thin intact sheet. The sheets of 'pure' epidermis so obtained (see Pl. 1, figs. 3–5) and referred to hereafter as 'split' skin were either fixed directly in formal-calcium (Baker, 1944), dehydrated in an ethyl alcohol series, cleared in clove oil and mounted in balsam, or immediately after fixation were treated with the 'Dopa' reagent.

The 'Dopa' reaction. (See Bloch, 1929.) So far as the epidermis is concerned the Dopa reaction affords a perfectly valid method of selectively revealing those cells which possess an active melanogenic system or enzyme complex (Russell, 1939; Ginsburg, 1944). It is based upon the fact that when fresh or freshly formal-fixed epidermal tissue is placed in a suitably buffered solution of *l*-3,4-dihydroxyphenyl-alanine (Dopa), a likely precursor of melanin (Raper, 1927), certain cells are able to bring about its intracellular oxidation to Dopa-melanin, becoming intensely blackened in the process; i.e. they are Dopa-positive.

Sheets of pure epidermis immediately after splitting were fixed for about half an hour in formal-calcium, rinsed in distilled water and transferred to the Dopa substrate freshly prepared by adding 2 ml. of Sörensen M/15 potassium dihydrogen phosphate solution (KH_2PO_4) and 8 ml. of Sörensen M/15 disodium phosphate solution (Na_2HPO_4) to 25 ml. of the stock 1:1000 Dopa solution in distilled water. The tissue, placed in an open vessel to allow free access of air, was incubated in this substrate at 38° C. for half an hour, after which the substrate was replaced by a freshly made-up solution and the incubation was continued for a further 2½–3 hr. The reaction was at an end when the solution had taken on a sepia-brown coloration. After rinsing in distilled water the standard fixation and mounting procedure was carried out.

OBSERVATIONS AND CONCLUSIONS

Study of transverse sections of full thickness pigmented skin, irrespective of the site on the body from which it was derived, shows that although melanin granules occur at all levels throughout the epidermis, and in small quantities in the superficial dermis, it is always at about the level of the deepest Malpighian cells that the pigment

Tabular summary of the epidermal glial system in man and guinea-pig

Type of cell	Occurrence	Normal 'colorimetric' description	Pigmentary activity	Notes
Pigmentary dendritic cell	Coloured human skin, e.g. Indian or Negro	Pigmented or black dendritic cell	Has a high level of melanogenesis which is intrinsically maintained	Cannot be distinguished in unstained or un-Dopa'd preparations because melanin granules are golden-brown in colour and relatively large. Dopa-reaction is essential
	Black guinea-pig skin	Ditto	Ditto	Easily visible in unstained 'split' skin preparations (Billingham, 1948) since melanin granules are black and very minute in size
	White human skin	'White' or non-pigmented dendritic cell	Normally very slight or absent	Possesses necessary enzyme system for pigment formation but an inhibitor is present (Rothman *et al.* 1946). Suitable stimuli, e.g. ultra-violet light, will initiate limited degree of melanogenesis (e.g. sun-tan) probably because of destruction or removal of 'inhibitor'
	White skin of albino guinea-pig	Ditto	Can be evoked by appropriate stimuli	Can be caused to blacken, e.g. after exposure to cold. Dendritic cells can then be seen in 'split' skin preparations (Billingham & Medawar, 1948). Possess necessary enzyme system for melanogenesis but an inhibitor normally present. (Onslow, 1915; Ginsburg, 1944)
Non-pigmentary dendritic cell	White skin of spotted black-and-white guinea-pig ? wherever there is 'recessive' spotting in rodents	'White' or non-pigmented dendritic cell	Absent and cannot be evoked	No form of physical stimulus known which will initiate any trace of pigmentation (Lewin & Peck, 1941). *Do not* possess necessary enzyme system for pigment formation

Synonyms: melanophore, melanoblast, chromatophore, stellate cell, clear cell, 'cellule amboceptrice'.

is found at its greatest concentration (Pl. 1, figs. 1, 3; Pl. 2, figs. 9, 10). Here it may be so abundant as to obscure the boundaries of the cells (Pl. 2, figs. 9, 10). The pigment granules are spherical, uniform in size and of a deep golden brown colour in transmitted light, so differing markedly from those of black guinea-pig epidermis, which are black. The capping and partial enveloping of the basal-layer cells by dense masses of these pigment granules is as prominent here (Pl. 1, fig. 3; Pl. 2, fig. 9) as in the guinea-pig. In such preparations absolutely no indication of the presence of dendritic cells can be found.

In sections of 'split' skin, in the preparation of which the tryptic digestion process had been prolonged to the point at which there was a tendency for individual epidermal cells to break away from the epidermal sheet, dendritic cell processes can often be seen still applied to it and apparently anchoring isolated basal-layer cells to the sheet (Pl. 1, fig. 6; Pl. 2, figs. 10, 11).

In unstained whole mounts of 'split' skin, examined with their cuticular surfaces lowermost, careful search rarely if ever reveals a trace of a dendritic cell (see Pl. 1, fig. 4). This is in contrast to similar preparations of guinea-pig skin in which the pigmented dendritic cells stand out boldly by virtue of their content of opaque melanin granules which also enable the processes arising from their perikarya to be followed out to their finest terminal twigs.

The Dopa-treated 'split' skin preparations have been the mainstay of this study. Examination of these reveals that dendritic cells are present in exactly the same position and abundance as are their 'white' homologues in white human skin from corresponding regions. Their perikarya occur at about the same level as the basal-layer cells (Pl. 1, figs. 2, 7, 8) and, as a single layer of discretely scattered cells among the latter, they follow faithfully the complex 'hill and valley' relief pattern which the lower surface of the separated epidermal sheet presents (Pl. 1, figs. 4, 5; Pl. 2, fig. 12). In this pattern the 'hills' are the downward prolongations of the epidermis into the dermis while the 'valleys' are really the spaces which were formerly occupied by the upward projection of the dermal papillae which, especially in thigh skin, tend to have flattened tops above which the epidermis is at its minimum thickness. The dendritic cells are found in highest concentration along the summits and sides of the downwardly directed epidermal ridges, while along the 'valleys' corresponding to the shallowest and thinnest regions of the epidermis they are more sparsely distributed. In these latter regions the branching systems arising from the individual dendritic cells tend to ramify more in the horizontal plane, thus enabling their relationships to neighbouring Malpighian cells and to cells of their own type to be studied (Pl. 2, figs. 12, 14). These preparations also reveal that a proportional relationship exists between the degree of epidermal pigmentation and abundance of dendritic cells.

The epidermis of pigmented human skin is thus identical in its anatomical constitution to nonpigmented epidermis (see Pl. 2, fig. 13), being composed of two types of cell: Malpighian cells and dendritic cells. Functionally, of course, the dendritic cells in pigmented skin differ from those in white skin in that they maintain in the epidermis a constant and relatively high concentration of pigment, while the non-pigmented dendritic cells in white skin can only effect a perceptible pigmentation of the epidermis as a result of some form of external stimulus, e.g. ultraviolet light, irritants, etc. This pigmentation never reaches a very high level of concentration and for its indefinite duration is dependent upon the continued application of the external stimulus. Rothman, Krysa & Smiljanic (1946) have suggested that white human epidermis contains a factor, probably sulphydryl compounds, with an inhibitory action on melanogenesis. On the basis of this evidence they have suggested that in melanoblasts (dendritic cells) both substrate and active enzyme are present but no reaction takes place between them because of the presence of the inhibitor. Melanogenic stimuli act by oxidizing or otherwise destroying the inhibitor, thus allowing

melanogenesis to take place. This would account for the similarity, with respect to pigmentary activity, between white human skin and that of the albino guinea-pig which has previously been referred to.

These pigmented dendritic cells in human material are very similar both anatomically and functionally to the dendritic cells found in the black skin of the guinea-pig. They differ from the latter in that they cannot normally be distinguished by virtue of their own melanin content, either in sections or in 'split' skin preparations; the use of the Dopa reaction, which is a sensitive colorimetric test for their melanogenic system, is essential. This difference is probably due in large part to the intense black colour and fine grain of guinea-pig melanin compared with the golden-brown colour of the human pigment granules.

As in the case of the pigmented skin of the guinea-pig there is ample evidence that melanogenesis is an exclusive property of these pigmented dendritic cells and that the pigment granules present within the ordinary basal-layer or Malpighian cells, often in considerable numbers, are derived at second hand from the dendritic cells by a process which may almost be compared to injection. As Masson (1948) has pointed out, these cells are both secretory and excretory in function and may therefore be considered as glandular cells. Unlike endocrine and exocrine glands, however, their product, melanin, is secreted into other cells across the terminal end-caps of the dendritic cell branches. Masson has called this 'cytocrine' activity.

In mammalian epidermis dendritic cells comprise a definite cell system as definite and specific in its own right as the reticulo-endothelial system. Billingham & Medawar (1948) have called it the *epidermal glial system*. To avoid any confusion which may have arisen with respect to terminology, a comparative summary of the 'epidermal glial system' in man and the guinea-pig has been included (see p. 111).

SUMMARY

1. It is shown that the pigmented epidermis of Indian and Negro skin is exactly similar in its anatomy to that of the white races, being composed of the more or less rounded Malpighian cells with their derivatives and the branched dendritic cells.

2. There is, however, a difference in functional activity between dendritic cells in the two types of epidermis, in that those present in pigmented tissue, like those of the black guinea-pig, have a high rate of melanogenesis which is intrinsically maintained, while those in white epidermis are capable only of a relatively slight degree of pigmentary activity as a consequence of the application of suitable external stimuli.

3. Attention is drawn to the glandular or 'cytocrine' role of pigmented dendritic cells.

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EXPLANATION OF PLATES

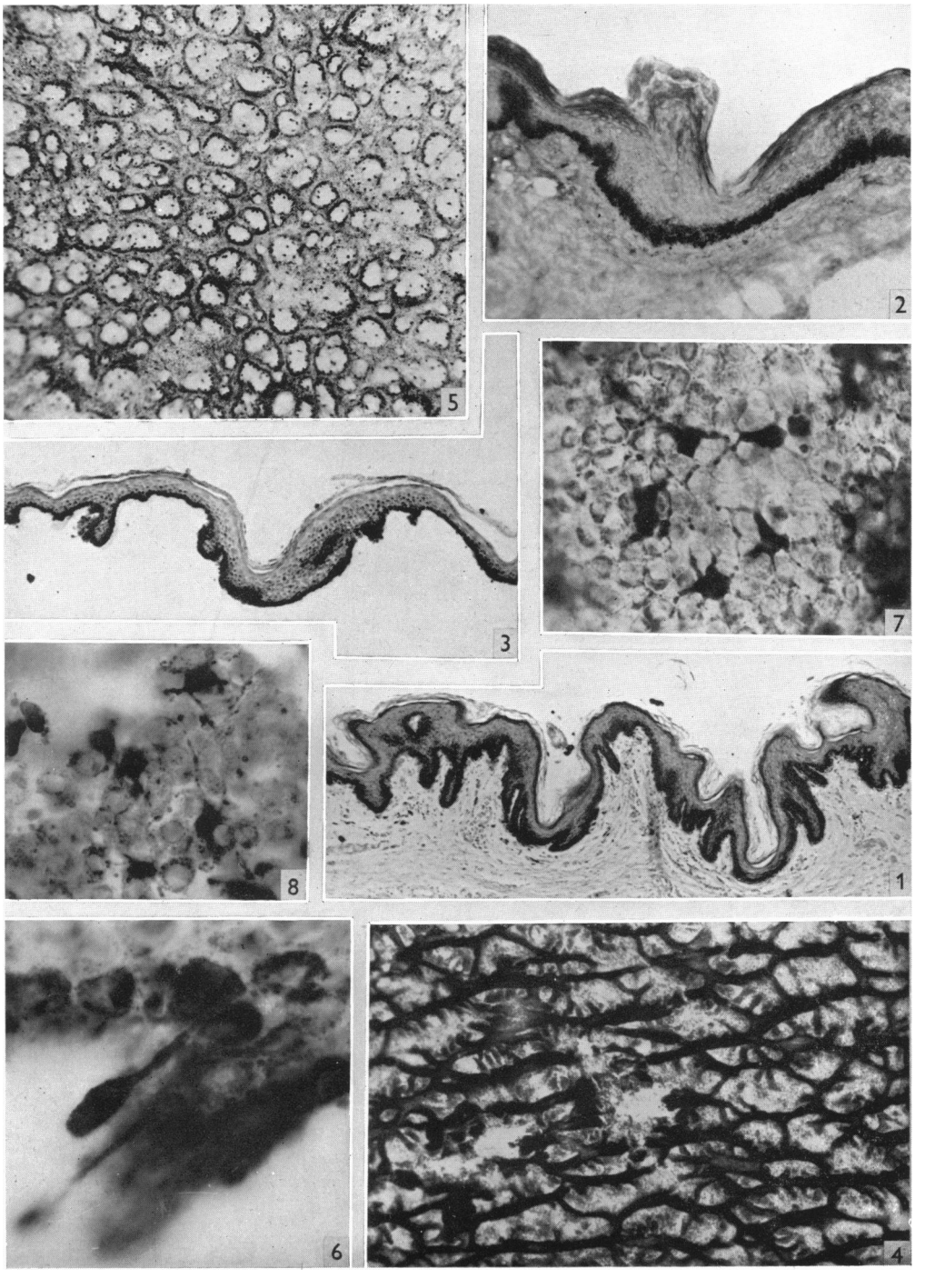
PLATE 1

Fig. 1. Vertical section through pigmented skin of Negro's scrotum. Observe the complex corrugation pattern characteristic of scrotal skin. The epidermal ridges penetrate rather deeply into the underlying dermis. Pigment density is greatest at the level of the lowermost cells of the epidermis. The dermis is almost devoid of melanin. Lightly stained with Ehrlich's haematoxylin and eosin. ×58.

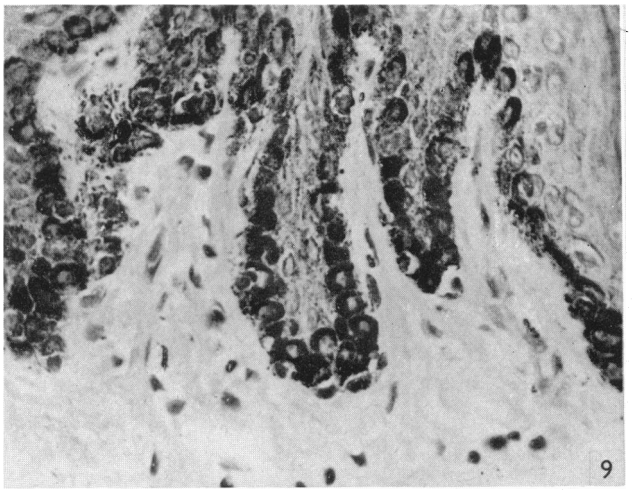
Fig. 2. Vertical section of pigmented scrotal skin of Negro cut at 40 μ on the freezing microtome and treated with Dopa. The bodies of the dendritic cells and their processes can just be distinguished due to the intracellular formation of black Dopa-melanin. In the epidermis Dopa-positive cells are only found at the level of the Malpighian layer. ×75.

Fig. 3. Vertical section of 'split' pigmented scrotal epidermis. The epidermis has been separated from the dermis by tryptic digestion and the sections prepared from it lightly stained with Ehrlich's haematoxylin and eosin (compare with fig. 1). Although pigment is mainly restricted to the level of the basal-layer cells, many of the epidermal cells at higher levels are 'capped' with pigment in a highly characteristic manner. ×67.

Fig. 4. Whole mount preparation of a sheet of pure pigmented epidermis ('split' skin) of Negro's scrotum, unstained and viewed from the underside. Note the complex 'hill and valley' contour or ridging pattern which the lower surface of the epidermis makes with the dermis. This is often characteristic and specific for the different types of epidermal tissue found throughout the body (compare with that of thigh skin, figs. 5 and 12). The 'hills' are the epidermal ridges while the 'valleys' are the spaces originally occupied by the dermal papillae. In this preparation no indications of dendritic cells can be distinguished. Pigment is mainly concentrated along the crests and sides of the epidermal ridges. ×27.



BILLINGHAM—Dendritic cells in pigmented human skin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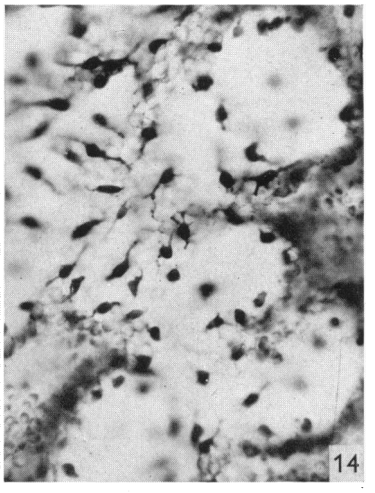
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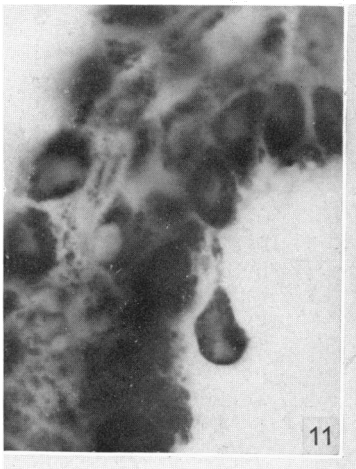
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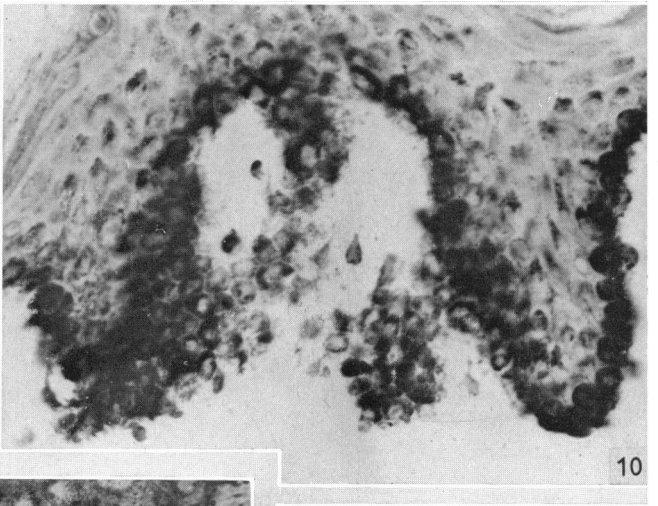
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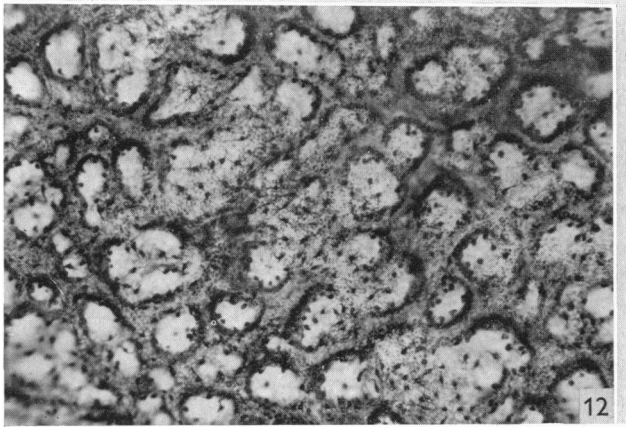
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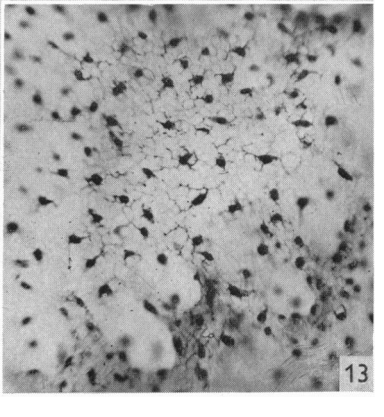
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Fig. 5. Whole mount of 'split' thigh skin of Indian which has been treated with Dopa. The preparation is viewed from the underside. Observe and compare the ridging pattern with that of scrotal epidermis (fig. 4). The dendritic cell bodies have been 'stained' black by the Dopa and can be seen clearly. They are more closely distributed in the epidermal ridges which are also the regions of maximum depth of pigmentation. ×50.

Fig. 6. Vertical section of 'split' pigmented scrotal skin. One of the ordinary basal-layer cells has almost broken away from the epidermal sheet, being retained only by a process from a dendritic cell which ends upon it as an intimately-applied cap or end-button. Unstained. ×1080.

Figs. 7, 8. Pigmented dendritic cells in a sheet of pure epidermis from Negro's scrotum which has been 'stained' with Dopa. Their perikarya and the processes which arise from them are intensely blackened by Dopa melanin. Note the melanin granules in the cytoplasm of the Malpighian cells in fig. 8. ×383.

PLATE 2

Fig. 9. Vertical section of pigmented skin of negro's scrotum to show the highly characteristic 'capping' distribution of melanin in relation to the basal-layer cells of the epidermis. Lightly stained with Ehrlich's haematoxylin. ×383.

Figs. 10, 11. Vertical sections of 'split' pigmented scrotal skin lightly stained with Ehrlich's haematoxylin and eosin. The enzymic splitting process was deliberately prolonged to the point at which cells had begun to separate from the epidermal sheet. Dendritic cell processes can be seen still adherent to some of the Malpighian cells which are breaking away. Note also the almost complete localization of pigmentation to the level of the basal-layer cells of the epidermis. Fig. 10, ×383; fig. 11, ×383.

Figs. 12, 14. Pigmented dendritic cells in pure pigmented epidermis of Indian's thigh skin stained by the Dopa method. At the bottoms of the epidermal 'valleys' the dendritic cells, though more sparsely distributed, tend to have their branches spread out more in the horizontal plane. Fig. 12, ×80; fig. 14, ×120.

Fig. 13. White dendritic cells of 'split' white (Caucasian) human skin. The preparation has been stained supravitally with methylene blue solution in Ringer. Note that the distribution, size, number and mode of branching of the dendritic cells is just the same as with pigmented skin (compare with figs. 12 and 14). ×150.