DENDRITIC CELLS

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INTRODUCTION

It has been known for many years that in pigmented mammalian epidermis and in the matrices of the hairs, branching cells containing melanin granules (variously described as melanoblasts, melanophores, dendritic cells, stellate cells, chromatophores, *cellules amboceptrices*, etc.) can sometimes be distinguished in addition to the ordinary, rounded, epidermal cells. None of the several theories which have been put forward at various times concerning the origin and functional significance of these branching cells has, however, so far attained general acceptance (see reviews by Hoepke, 1927; Percival & Stewart, 1930; Meirowsky, 1940; Laterjet, 1938), though most authorities would agree with Meirowsky's (1940) statement that 'the central problem in pigment research is the origin and significance of the dendritic cells'. The 'cell of Langerhans', a branched cellular component of the epidermis originally described by Langerhans (1868) in normal human skin that had been impregnated with gold chloride, although regarded by some authorities (Woollard, 1935; Cowdry, 1938; Carleton, 1938) as identical with the branching, pigmented cells for which numerous synonyms have already been given above, will be considered separately here, since Langerhans specifically denied that this element had anything to do with pigmentation and regarded it as a nervous element—a view subsequently supported by Bloch (1929). This view implies, of course, that these cells are distributed generally throughout the epidermis and are not restricted to the pigmented regions.

It is probably true to say that in spite of all that has been written on dendritic cells the great majority of histologists regard the non-pigmented epidermis (and in many cases the pigmented epidermis also) as a homogeneous tissue in the composition of which structure only one type of cell participates.

In the present study a re-investigation of the anatomical basis of pigmentation of the mammalian epidermis has been undertaken (Part I of this paper) and the results of this investigation have been applied to an analysis of the striking system of branching elements observed in non-pigmented epidermis (Part II). Following Becker (1927) the term 'dendritic cell' will be used as a purely morphological description in preference to any of the other synonyms, since it begs no question about the origin and functional significance of the cell.

PART I. THE DENDRITIC CELLS IN PIGMENTED EPIDERMIS

MATERIAL AND METHODS

In this study black and white guinea-pigs have been used throughout. These animals were particularly suitable since in the areas that bear black hairs the superficial epidermis itself is deeply pigmented while in the white hair-bearing

regions the skin is non-pigmented. In other laboratory mammals, by contrast, the superficial epidermis of the skin that bears pigmented hairs is not pigmented. This is so in the case of the body skin of the rabbit; but in the skin of the dorsum of the ear in which the hair density is relatively low, some epidermal pigmentation does occur.

The epidermis of the ear of the guinea-pig, although differing in no essential details from that of the body skin with respect to pigmentation, proved more favourable for this investigation on account of its relatively hairless nature and also because it was much thicker than the body epidermis.

Fixation and staining. Formol-mercuric chloride, prepared by mixing saturated mercuric chloride solution, 40% formaldehyde, and distilled water in the ratio of 50:15:35 parts by volume, was adopted as a standard fixative. Paraffin sections were cut at 8–10 μ and lightly stained with Ehrlich's haematoxylin and eosin. To study the distribution of melanin sections were either mounted unstained or were lightly stained with Orange G.

Studies with living cells. These were carried out upon sheets of 'pure', pigmented epidermis—i.e. epidermis which had been completely freed from dermal material (Pl. 1, figs. 1, 2) and upon emulsions consisting of isolated cells and undissociated clumps of cells. These were prepared by the tryptic digestion of thin skin slices and the subsequent separation of the epidermis from its underlying collagen pad as described by Medawar (1941). This method is referred to hereafter as 'skin splitting'. It will be shown in another publication (Billingham & Medawar, 1948) that the epidermal sheet remains alive after this treatment.

The Dopa reaction. When freshly excised tissue is incubated at 37° C. with a 1:1000 solution in phosphate buffer at pH 7·4 of *l*-dioxyphenylalanine ('Dopa'), a likely precursor of melanin (Raper, 1927), certain cells, including those known to be capable of forming melanin, bring about the rapid intracellular oxidation of this substance thereby becoming blackened by the resultant Dopa-melanin. These cells are said to be 'Dopa positive'. The standard procedure of Laidlaw & Blackberg (1932) was carried out upon frozen sections of full thickness skin and upon sheets of pure epithelium. Counter-staining was found to be unnecessary, but it was found advisable to fix the fresh tissue, e.g. in 'formal calcium' (Baker, 1944) for 2 hr. before cutting in order to harden it.

Studies on hyperplastic skin. These were carried out upon pigmented ear skin which had been rendered hyperplastic by grafting it to a recipient area on the animal's own thorax (Medawar, 1944). About 12 days after transplantation (the most favourable time) thin shavings of the thickness of Thiersch grafts were cut from the graft and 'split'. Both the resultant sheet of epidermis and the collagen pad were then fixed, dehydrated, and cleared and mounted unstained.

OBSERVATIONS

General histology of the epidermis. The pigmented auricular epidermis consists of about six layers of cells and is penetrated by well-defined dermal papillae. The melanin granules are located mainly in the cells of the basal layer in contact with the corium. These cells are of cylindrical shape with their long axes disposed in the direction normal to the plane of the integument. The granules are generally heaped up over the poles, usually the superficial poles, of the nuclei. Other granules may be distributed more evenly throughout the cytoplasm of these cells. In the more superficial layers, in which the cell bodies are more rounded, the polar distribution gives way to a more even one. One gets the impression that as the cells move towards the stratum granulosum the quantity of melanin in each cell decreases. This is probably illusory: as the cells become more superficial the volume of the cytoplasm in relation to that of the nucleus increases and the original complement of melanin granules becomes diluted.

Melanin granules are to be seen also in the squamous cells of the stratum corneum.

In paraffin sections of pigmented skin dendritic cells can only rarely be distinguished. This may be due to the fact that they are obscured by the rather dense pigmentation of the basal layer. A few dendrites are occasionally visible by virtue of their pigment content, or a large, heavily pigmented cell body may be seen disposed horizontally and lying so deep in the basal layer as to touch the dermis.

In transverse sections of pigmented epidermis which had been prepared by 'splitting' the dendritic cells and some of their processes stand out more clearly (Pl. 1, figs. 2–4). This is probably attributable to the fact that as long as the epidermis is anchored down to the dermis, the individual cells are under slight lateral compression; but when the basal layer is freed from its substratum all the cells, particularly the basal, are loosened, thus permitting the dendritic cells, and the processes from them which run in the intercellular spaces, to become apparent. It is highly probable that these intercellular spaces in the deeper strata of the epidermis are all permeated by the dendrites of these branching cells. The so-called 'intercellular substance', so far as the epidermis is concerned, may be none other than the protoplasmic extensions of dendritic cells.

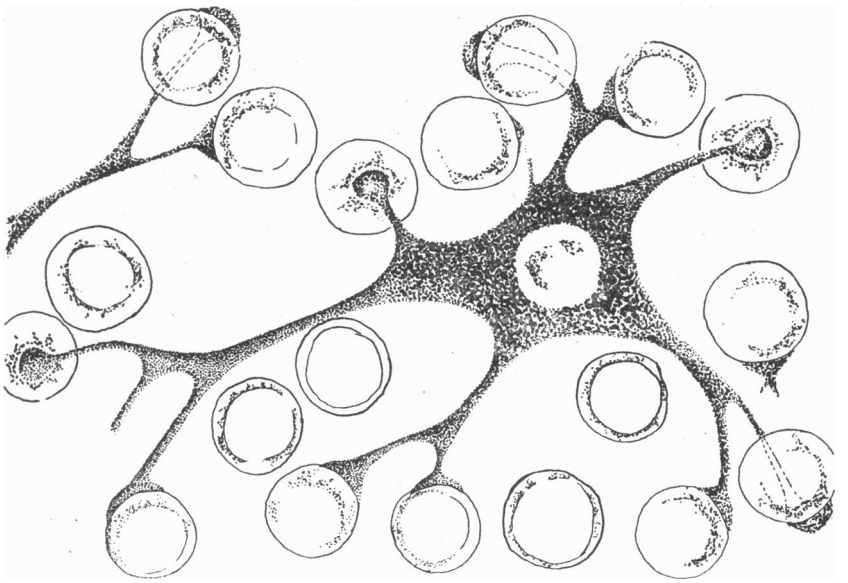
The epidermis of the body rarely exceeds in thickness two layers of cells including the basal layer, and dendritic cells or their processes are rarely seen in sections unless the skin has previously been rendered hyperplastic. The dermal papillae, which are feebly developed, are broad and shallow.

In both types of pigmented skin small, round, shield-shaped conglomerations of melanin granules may occasionally be seen lying in the 'intercellular spaces' at about the level of the basal layer.

Studies with living cells. The isolated cells which can be observed in suspensions prepared from pure, pigmented epidermis can be divided into three groups

according to size and the relative volume of the cell occupied by the nucleus. Most of the cells in each group contain melanin granules.

Cells of the first group are those of the basal layer, and are the smallest. They are roughly spherical in shape and have very little cytoplasm round the nucleus. Pigment, when present, occupies a polar-capping position (Pl. 1, fig. 5, and Text-fig. 1). The melanin granules associated with these cells do not, however, always form a cap *within* the cell membrane, for careful study with the oil immersion lens reveals that there may be a cap-shaped aggregate of granules either within the cell or closely applied to, but definitely *outside*, the cell membrane.



Text-fig. 1. Illustrating rather diagrammatically the anatomical relationships between a pigmented dendritic cell and its ordinary basal-layer cell neighbours. Several cells have been figured in which the end-caps have broken away from their dendrites. The pigment granules, which are produced within the cytoplasm of the dendritic cell, pass across the boundaries of the ordinary epidermal cells via the end-caps and, in the majority of cells of the lower epidermal strata, remain in a localized 'polar-capping' position with respect to the nucleus. This distribution normally gives way to a more even one as the cells reach the more superficial layer of the epidermis.

These extracellular melanin caps nearly always appear incomplete, so giving the impression that they have broken away from some other structure of which they were once a part.

It seems fairly certain that the shield-shaped conglomerations of pigment mentioned above are identifiable with melanin caps of this nature that have broken away from the cells that 'wore' them.

The second group is composed of cells which are slightly larger than those of the basal layer and which possess relatively more cytoplasm. By dark ground

illumination 'prickle' remnants may frequently be seen projecting from their membranes. These cells lie between the basal layer and the stratum granulosum. Pigment, when present, is distributed evenly throughout their cytoplasm and the 'caps' are very rarely seen.

Cells of the third group are rarely present in suspensions prepared by *lightly* scraping the undersides of pure epidermal sheets. They are larger in surface area than those of the other groups, being normally flattened, and their cytoplasm is packed with semi-transparent globules of eleidin. They usually contain scattered melanin granules.

Intimately bound up with the undissociated clumps of basal layer cells present in these suspensions, there can be observed dark bodies which are more irregular in shape and of slightly larger size than the basal layer cells. These elements are so completely filled with melanin granules, or at least the periphery of their cytoplasm is so completely lined with pigment granules, that they appear homogeneously black and no internal structure can be distinguished. These are the cell bodies ('perikarya') of the dendritic cells, and from them stumpy processes arise and travel outwards to pass between the surrounding basal layer cells.

If the amount of fluid between slide and coverslip has been correctly adjusted these clumps of cells gradually flatten out to form a single but still united layer of cells which is highly suitable for the detailed study of dendritic cells. Under such conditions the cell-bodies of dendritic cells appear elongated, rarely spherical, and generally have a maximum diameter of 14–16μ and a minimum diameter of 8–12μ. The maximum diameters observed were $19 \times 18\mu$, but this degree of elongation is rare. (Ordinary basal layer cells vary in diameter between 11 and 14μ.) From the bodies of these cells a number of primary dendrites arise: they vary in number from 2–7, 5 and 6 being modal. These primary dendrites branch peripherally, mainly by dichotomy, and as they travel outwards there is a progressive diminution in their calibre. Proximally their width may be very variable. In the dendritic cells of ear skin it may be as much as 2·5μ at their point of origin from the cell body. Dendritic cells of body skin have longer processes—their maximum length may be as much as 100μ—but the primary dendrites at their point of origin are much smaller, having a diameter of between 1 and 1·5μ.

The finer 'twigs' of these branching dendrites can nearly always be seen to terminate in the vicinity of basal-layer cells in the form of a swelling which is button or cap-like in shape (Pl. 1, fig. 8). This terminal cap, like the finest ramification of the dendrite itself—conspicuous entirely on account of its melanin granule content—appears to be intimately applied to the membrane of the basal cell (Text-fig. 1; Pl. 1, fig. 8) and differs in no respect from the polar caps described above in relation to the isolated basal layer cells.

Fusion between processes originating from the same dendritic cell has not been observed, but instances of fusion between processes originating from *different* dendritic cells can be found by careful examination of these flattened

cell suspensions (Pl. 1, fig. 9). Moreover, it may be established that an end-button from one dendritic cell may be applied to the body of another.

The melanin granules contained within dendritic cells are approximately constant in size and are in all respects similar to those seen elsewhere within the epidermis.

At the centre of the body of a dendritic cell a pellucid sphere, presumably the nucleus, can usually be distinguished (Pl. 1, figs. 6–8). When the pellucid sphere of a suitably flattened dendritic cell body is examined under an oil immersion objective by phase contrast microscopy a faint indication of the existence of a nuclear membrane and nucleolus is obtained. If a drop of acidified methyl green is then allowed to run in under the edge of the coverslip and the same dendritic cells examined *without* the use of phase contrast, the nuclear membrane and contained nucleolus can be identified with certainty.

The Dopa reaction. In sections Dopa-positive cells were found only in pigmented skin (see p. 104, footnote). They were invariably dendritic in form; their bodies, slightly larger than the basal layer cells, were usually disposed horizontally and so deep in the basal layer as frequently to touch the dermis. From their bodies, which were deeply blackened by Dopa melanin, equally blackened processes arose laterally and apically, and branched among cells of their own and superficial layers. In control sections which had not been treated with Dopa these dendritic elements could only very rarely and with difficulty be identified and then only by virtue of their natural melanin content. There could be no doubt that the elements made clearly visible in pigmented skin by means of the Dopa reaction were identical with the dendritic cells from living material described above.

Since dendritic cells branch principally in the plane of the epidermis transverse sections are obviously unfavourable material for studies of their numerical incidence and the extent of the 'zones of influence' of their branches. This can be carried out best on whole mounts of pure pigmented epidermis mounted upside down. In Dopa and non-Dopa material the picture was essentially the same save that in the Dopa material there was more contrast between the dendrites and other cells and the final terminations of the dendrites were more readily visible (Pl. 2, fig. 10). Under the high power the terminal buttons are seen to be strongly Dopa-positive; the basal layer cells with which they are in contact are however scarcely visible, since they are Dopa-negative and such natural melanin as they possess lies normally very close to the cap-like endings of the dendrites.

A comparison of the dendritic cell content of black body skin and of pigmented ear skin, based on the study of both living and Dopa-treated material, reveals that in both there is but a single layer of dendritic cells, interspersed among the basal layer cells. In ear epidermis the concentration of dendritic cells is much higher and they are found mainly in the epidermal re-entrants between the dermal papillae (Pl. 1, fig. 1) while in body epidermis they are most highly concentrated at the bases of its broad and shallow epidermal ridges.

Studies on hyperplastic skin. In the week or two that follow the transplantation of a skin graft the mitotic activity of its epidermal cells is greatly increased. If dendritic cells do in fact enjoy a cell lineage of their own, i.e. if they breed true to type instead of originating as induced modifications of basal layer cells, it seemed reasonable to hope that specific division stages would be found in hyperplastic skin.

Examination of pure hyperplastic epidermis (from 'split skin') revealed that its melanin content was sub-normal. The majority of the basal layer cells were completely devoid of melanin, and the dendritic cells, although no less numerous than in normal skin, contained only about half the expected quantity of pigment granules.* Moreover, many of them were undergoing division. From the various stages illustrated (Pl. 2, figs. 11–14) it is clear that in crude outline the mode of division resembles that of an amoeboid cell in the sense that the dividing dendritic cell withdraws its processes and forms a pigmented, rounded sphere about 12μ in diameter (measured from fixed material) (Pl. 2, fig. 11). This becomes dumb-bell-shaped by constriction in an equatorial plane (Pl. 2, fig. 12). At this stage daughter nuclei become visible. The two lobes of the dumb-bell draw apart and thick, blunt processes appear (Pl. 2, fig. 13). Even after the bodies of the daughter cells have separated, and each has developed a fairly complex system of dendrites, daughter dendritic cells can often be seen linked together by a common process (Pl. 2, fig. 14).

PART II. DENDRITIC CELLS IN NON-PIGMENTED EPIDERMIS

MATERIAL AND METHODS

In this study the non-pigmented skin of spotted guinea-pigs, of rabbits and of man has been used. In the case of the rodents the epidermis of the ear proved more favourable for reasons which have already been given. The human material consisted chiefly of trimmings from Thiersch grafts made available through the kindness of Prof. T. Pomfret Kilner (i.e. very thin grafts comprising epidermis and the superficial part only of the dermis), which had been cut from the forearm.

Gold impregnation. The technique adopted was essentially that described by Gairns (1930) as a reliable method for demonstrating the nerve endings in muscle. The human Thiersch graft material, or in the case of the rodents pieces of skin cut as thin as possible (in either case the material must be fresh) was placed in a mixture of one part pure formic acid and three parts filtered lemon juice and left in the dark for 10 min. or until it had cleared. The material was then removed from the liquid and lightly blotted on a filter-paper. It was then transferred to a 1 % aqueous solution of gold chloride and returned to the dark

* These observations are in conformity with the fact that a graft of pigmented skin, studied microscopically, seems to lose its colour temporarily following transplantation, taking on a pinkish colour at the end of the first week and gradually darkening thereafter until its former level of pigmentation is regained after about 3 weeks (see Billingham & Medawar, 1948).

for a further period of 10 min. after which it was again blotted and subsequently placed in a 25 % aqueous solution of formic acid and left in total darkness for 24 hr. Finally, the tissue was again blotted and placed in pure glycerine and left until it had cleared. The epidermis with most of its deeper strata intact was then fairly easily stripped from the dermis with the aid of fine forceps. It was then mounted in glycerine, basal layer uppermost, together with any epidermal material which could be obtained by lightly scraping the upper (i.e. surface which was applied to the epidermis) surface of the stripped collagen pad. This latter part of the technique is similar, manipulatively, to that previously described for skin splitting. When the shavings of skin were very thin they were teased and mounted directly after impregnation. (This technique, although probably reliable for the purpose for which Gairns described it, has not invariably been successful as a method of staining white dendritic cells.)

Methylene blue staining. Thin shavings of living skin were used with as little of the dermis left adherent as possible. These shavings were mounted on slides, dermal side uppermost, in a 0·02 % solution of methylene blue in Ringer's solution (B.D.H. methylene blue, batch number 519420, has given satisfactory results). After several minutes a few drops of a 0·2 % solution of the dye in Ringer were drawn under the coverslip and the preparation was examined periodically with the microscope. After 1–2 hr. it was observed that the tissue as a whole had remained relatively unstained but in the peripheral region of the epidermal sheet certain elements, which appeared to have a branching form, had taken up the stain selectively. At this stage the tissue was dehydrated, cleared and mounted in Canada balsam after treatment with saturated ammonium picrate solution and Bethe's fluid as described by Carleton (1938).

Azan staining. Full thickness non-pigmented skin was fixed in formol-mercuric-chloride, dehydrated and cleared by passage through the alcohols, cedarwood oil and ligroin. After paraffin embedding, sections were cut at 10μ and at 15μ and stained by Heidenhain's 'Azan' method as described by Pantin (1946).

OBSERVATIONS

Gold impregnation. The superficial layers of the epidermis, comprising the stratum granulosum and stratum corneum, were found to be stained a deep purplish black by the presence of fairly coarse particles of reduced gold. The deeper layers of cells, including the basal layer, were found to vary in colour from pink to deep red. Only the nuclei of the cells in this case were stained and whatever the nature of the colouring matter, it was not visibly particulate. The actual boundaries of these cells could be made out only with difficulty. The dermis rarely took on more than a deep pink colour. In successful preparations, at about the level of the basal cells of the epidermis, dark reddish purple bodies of slightly fusiform but irregular shape could be seen (Pl. 2, fig. 15) which were, on the average, slightly larger than their ordinary epidermal neighbours. These bodies were made visible by the presence of very

fine granules of metallic gold within their substance and only with difficulty could a nuclear outline be distinguished. This latter fact is not very surprising since, in effect, one was trying to distinguish a pale reddish body surrounded by a mass of dark coloured particles.

From these bodies a variable number of branches or dendrites arose (Pl. 2, figs. 16, 17). On an average there were about five of these but any number between two and ten have been observed. When there were but few branches, their initial diameter tended to be greater than in the case of the multi-branched elements. The mode of branching of these dendrites differed in no respect from that of the pigmented dendritic cells which have already been described. The processes likewise occupied the intercellular spaces between the basal and more superficial cells and some exceeded 70μ in length although the majority varied between 30 and 40μ. The finer twigs of the branches seemed to be rendered rather brittle by gold impregnation and were often found to have broken away from the parent cell. In spite of this, it could be established beyond doubt that they terminated as small, button-shaped bodies in close proximity to the boundaries of the basal-layer cells. These processes travelled both horizontally and towards the surface but not deeply. By careful searching, cases could be found in which end-buttons from these gold-impregnated dendritic bodies ended, not on an ordinary neighbouring epidermal cell but on the perikaryon of an element of its own type. Further, in several instances, two of these branched bodies were observed to be united by a common dendrite.

From the above description of the branching elements which can be shown to exist in non-pigmented epidermis by gold impregnation it will be seen that they closely resembled pigmented dendritic cells with respect to their size, shape, position, number, mode of branching, number and length of branches, the disposition and mode of termination of the branches or dendrites, the relations which may exist between two such dendritic cells and finally their nucleate nature. Merely on superficial inspection the branched elements of non-pigmented skin qualify to be called 'white' dendritic cells and to describe them more fully would result only in a repetition of what has already been said about pigmented dendritic cells.

In spotted guinea-pigs the non-pigmented epidermis of the sole of the foot (hairless and heavily cornified as in man), of body skin and of ear skin have all been found to contain white dendritic cells.

Apart from the fact that white dendritic cell bodies, as revealed by metallic impregnation, are of slightly more irregular shape and that their processes are, on an average, of finer diameter than in the case of the pigmented dendritic cells there are no other purely morphological grounds upon which pigmented and non-pigmented dendritic cells can be separated. As a critical test of the possibility that the very slight differences in form might be fixation artefacts, the pigmented skin from a guinea-pig's ear was treated by the full gold chloride impregnation method. In the resulting preparations there was no difficulty in distinguishing between granules of melanin and reduced gold chloride, because

the former appear brownish in transmitted light. In very heavily pigmented dendritic cells the body remained rounded in shape, probably on account of the mechanical resistance to fixation-induced shrinkage offered by the densely packed melanin granules. On the other hand, those dendritic cells which were more lightly pigmented assumed a form similar in all respects to that of the white dendritic cells, and their finer twigs were revealed with even greater clarity than in vital preparations of pigmented dendritic cells.

The white dendritic cells of human skin. Non-pigmented* human skin from the face, the arms and the abdomen was examined. White dendritic cells were found in the epidermis in each case and were similar in all respects to those which have been described in the case of the non-pigmented skin of the guinea-pig (cf. Pl. 2, figs. 16, 17).

Methylene blue staining was applied to skin from the human forearm, from the dorsum of the ear of albino and black-and-white rabbits and to the white ear skin of spotted guinea-pigs.

In each case elongated and irregularly shaped bodies could be seen in the epidermis at about the level of the very feebly stained ordinary basal layer cells. They were slightly larger than the neighbouring epidermal cells and a variable number of branches arose from them (Pl. 2, figs. 18, 19). Cell bodies and branches were rendered visible by the presence of numerous, brightly stained blue granules within their substance. It is unfortunate that no cytological detail could be distinguished within these branching bodies. With respect to their size, shape, numerical incidence, location and number of branches to which they gave rise, they were strikingly similar to the white dendritic cells revealed in control material which had been treated with gold chloride. In the methylene blue stained material, however, the cytoplasmic bridges, which are said to traverse the intercellular spaces and unite the deeper epidermal cells, took up the stain strongly (Pl. 2, fig. 19), particularly at their nodes, which made it impossible to trace the branches very far from their points of origin or to investigate their mode of termination. On many occasions it was found that the finer ramifications of the cutaneous nerves had stained particularly well but in no case was there any indication that these were related to the branching cells (Woollard, 1935).

Azan staining. In sections of full thickness, of non-pigmented human skin stained by this method and examined with the one-sixth and one-twelfth objectives it was observed that certain cells in the basal layer of the epidermis were conspicuous by their larger size and by often being slightly elongated in the horizontal plane. A much thicker layer of cytoplasm surrounded their nuclei than in the case of the neighbouring epidermal cells (Pl. 2, fig. 22); and although clearly defined 'prickles' were present around the boundaries of most of the latter (Pl. 2, fig. 22), in no instance were they found around the former

* Through the particular kindness of Dr H. M. Hanschell I have now been able to study the pigmented dendritic cells in negro skin. These are essentially similar to those found in pigmented guinea-pig skin.

(Pl. 2, figs. 20, 22). In fact the lack of prickles constituted the most obvious diagnostic character of these large cells. It could be established that fairly large protoplasmic branches arose from the lateral and distal boundaries of these cells, and these, by careful focusing, could be followed for a short distance as they entered the adjacent intercellular spaces between the ordinary epidermal cells (Pl. 2, figs. 20, 21). Unfortunately these processes neither took up the stain selectively enough nor differed sufficiently in refractive index from the cytoplasm of the neighbouring cells for them to be traced any farther. None of these cells were undergoing mitosis.

These prickle-less, giant basal-layer cells suggest very strongly that the Azan technique provides us with yet another method of demonstrating at least the cell-bodies of non-pigmented, branched cells in white epidermis.

The cells described in this section are almost certainly the 'clear cells' mentioned and figured by Cowdry (1944).

CONCLUSIONS

The facts presented in Part I of this paper concerning the pigmentation of the epidermis support the theory that melanogenesis takes place exclusively within the cytoplasm of dendritic cells which have been shown to be constantly present with approximately the same numerical incidence in all the samples of pigmented epidermis examined. The nucleate nature of these branching elements, questioned by some authorities (e.g. Cowdry, 1938), has been established, and it has been shown that as a result of certain stimuli, e.g. transplantation, they undergo cell division and maintain their type-specific cell lineage. In no case was there any doubt as to whether a division stage belonged to a dendritic cell or to an ordinary epidermal cell. These findings conflict with the so-called 'transition theory' supported by Bloch (1927, 1929), according to which the dendritic cells are derived from ordinary basal layer cells as a result of functional stimulation and represent an active phase in the pigment-forming function of the latter. The numerical incidence of these cells in conjunction with the numerous processes to which each gives rise provides a system whereby almost every basal layer cell is 'capped' by the button-like end organ of a dendritic cell process. It has been shown that whenever melanin granules are present within the cytoplasm of a basal-layer cell they are invariably located close to the point on the cell wall at which the end-cap of a dendritic cell is applied, although as these cells are traced towards the surface this highly localized distribution is normally lost.

Dendritic cells are the only cells in pigmented epidermis to give a positive Dopa reaction. No instance of an ordinary basal-layer cell giving a positive reaction has been encountered, though the end-caps which make contact with them are often strongly Dopa-positive.

The value of the Dopa reaction in pigment research has frequently been criticized since it was first described by Bloch (1927), on the grounds that a specific 'Dopa oxidase' does not exist and that such cells as leucocytes,

which do not play any part in melanogenesis, give a strong positive reaction. The present study is only concerned with the epidermis and within this tissue positive Dopa reactions have only been obtained from dendritic cells.* The end-buttons of these cells gave a particularly strong reaction. Under these circumstances it seems justifiable to regard a positive Dopa reaction as perfectly valid histochemical evidence of the existence of a melanogenic system even though it offers no further information about it. The melanin granules which have been formed within the cytoplasm of the dendritic cells are probably secreted from them by way of the end-buttons and are thus brought immediately into intimate contact with the basal layer cells which may possibly ingest them, a hypothesis supported by the results of tissue culture experiments in which melanin granules have actively been taken up by epithelial cells (Matsumoto, 1918; Smith, 1921, 1925).

The occurrence of extracellular melanin in the intercellular spaces of the epidermis which ultimately is absorbed by, and appears within, the reticulo-endothelial elements of the dermis is explained by the fact that the end-caps of the dendritic cells probably break away from their embraced basal layer cells and disintegrate, thus liberating the contained melanin granules, as the latter travel towards the surface.

The elements so far non-committally designated 'white dendritic cells' have been shown by three different techniques to occur in non-pigmented skin. They so closely resemble the pigmented dendritic cells in all respects, save that of melanogenesis, that they must be regarded as true-breeding variants of the same race of cells. Among all the true-breeding races of cells known to histology it is difficult to imagine two which are obviously so closely related and yet so visibly distinct as are those of the pigmented and non-pigmented dendritic cells.

The dendritic cell must be regarded as a cellular element constantly present within the epidermis, which is therefore a compound tissue composed of cells of at least two distinct races.

Their origin is no longer in doubt. From embryological studies it has been established that in the case of amphibia (Du Shane, 1935; Twitty, 1936) and birds (Dorris, 1939; Eastlick, 1939; Hamilton, 1941) the pigment cells are derived from the neural crest region. More recently and, for the present discussion, decisively, Rawles (1940, 1947) has established that in the mouse too the melanophores (as she calls them) are derived from the same region. Thus the theory that the origin of the dendritic cells is related to that of nervous elements, supported by the work of Masson (1926), has been confirmed.

* In the *white* epidermis of the black and white spotted guinea-pig a positive Dopa reaction is never obtained even after traumatic or actinic stimulation (Lewin & Peck, 1941; Ginsburg, 1944). In the case of the so-called albino guinea-pig, however, the white epidermis of the extremities such as the ears and the soles of the feet may be blackened by such a mild form of stimulation as that provided by cold weather; its dendritic cells will then give a positive Dopa reaction (Ginsburg, 1944), and they may be seen in ordinary split skin preparations. Ordinary white human epidermis would appear to resemble that of the albino guinea-pig with respect to its potentiality for melanogenesis and consequent positive Dopa reaction of its dendritic cells.

Sewall Wright (1942) reviewing the work which has been done on the origin and mechanism of distribution of vertebrate pigment cells, suggests that the spotted pattern in guinea-pigs is in part a pattern of arrested migration. This theory was apparently based on the assumption that only the pigmented type of dendritic cell existed. Now that dendritic cells have been shown to be integral components of the guinea-pig's epidermis, irrespective of whether it is pigmented or not, one is entitled to ask whether the future colour pattern of the developing guinea-pig is not determined in the epidermis before the dendritic cells have begun their migration from their presumptive to their definitive positions, since it would appear that they are not melanogenic during the initial stages of the journey (Rawles, 1947).

Apart from the melanogenic role played by the one variant of the dendritic cell race, no evidence can be offered at present concerning the function performed by these cells taken as a whole. The claim of Pautrier, Lévy & Diss (1928), based on a study of the pigmented dendritic cells, that these form a connecting link in a nutritive syncytial system operating between the dermis and the epidermis is certainly very attractive. Most authorities, however, have failed to observe a single downwardly directed dendritic process actually passing into the dermis and no indication of one has been found in the present study. Certainly the dendritic cell system is ideally suited, anatomically, to mediate the spread of a virus type of infection through the epidermis once an infection has started.

Preparations of both types of dendritic cell were demonstrated to Dr W. Holmes and to Dr O. L. Thomas and each, independently, drew the author's attention to their amazing similarity to certain of the glial elements of the central nervous system. This resemblance, which has also been observed by Becker (1927), is particularly striking if we compare white dendritic cells with protoplasmic astrocytes. The latter can only be demonstrated by means of special techniques, most of which involve metallic impregnation, and some of their branches terminate in the form of specific end-organs known as perivascular feet or podics (Penfield, 1932) which are at least superficially similar to the end-caps of dendritic cells. A nutritive function has been ascribed to certain of these glial elements by some histologists (see Ingleby, 1925). Certainly it would appear that both these types of cell are closely related by virtue of their common embryological derivation from the neural crest region.

The relationship which exists between the 'cell of Langerhans' and the dendritic cells described in this paper can only be determined by comparing the white dendritic cells revealed by the gold chloride technique with the 'dark staining bodies' described by Langerhans in his original paper (1868). The account presented in this paper is in almost complete agreement with that of Langerhans with respect to the numerical incidence, size and shape of body and in number of dendritic processes to which the latter gives rise. Moreover, Langerhans clearly describes and figures the terminal buttons. He does not, however, seem to have observed any relationship between the latter

and epidermal cells of the basal layer. His cells are described as occurring at a relatively more superficial level in the epidermis than do the dendritic cells. The existence of a centrally directed process passing to the dermis, which he described upon slender evidence (on his own admission) has not been confirmed in the present investigation and, finally, even in cases where a rich pigmentation of the epidermis occurred he did not find pigment granules within his cells. If due allowance is made for the fact that Langerhans worked with sections while the present investigation has largely been based upon the study of sheets of epithelium then it appears that the main points of difference have arisen through differences of technique and not because the elements investigated were intrinsically different. The findings of this investigation support the view that the 'cell of Langerhans' is the same as the (white) dendritic cell.

Weddell (1942) in a study of cutaneous nerve regeneration drew attention to the fact that the so-called Langerhans cells are abundant in the skin of the albino rabbit's ear and are revealed by methylene blue staining when the fine regenerating nerve terminals approach them. He suggests that these are probably nothing more than modified Schwann cells. The results obtained in the present study confirm the presence of elements similar to those described and illustrated by Weddell in the albino rabbit's ear (here described as white dendritic cells), but no indication has been obtained that they lie in series with, or are related to, the Schwann cell elements. In conclusion, if the identity of the 'cell of Langerhans' with the white dendritic cells is rejected and the nervous nature of the former is asserted then we are in fact asked to believe that within the epidermis two entirely (functionally) different categories of branching cells exist side by side with a remarkable similarity in form, incidence, location, etc.

SUMMARY

1. A reinvestigation of the anatomical basis of pigmentation of mammalian skin has been undertaken based mainly on the study of the pigmented epidermis of the black and white spotted guinea-pig.

2. It has been demonstrated that although pigment granules are found in most 'ordinary' epidermal cells of pigmented skin they are not of endogenous origin, but are derived from branched cellular elements which have been called pigmented dendritic cells.

3. These branching cells are located at the level of the basal-layer cells of the epidermis. From them branches are given off which travel along the intercellular spaces between the ordinary epidermal cells, dichotomizing frequently, and ultimately terminate in the form of 'caps' or 'end-buttons' closely applied to the boundaries of ordinary epidermal cells.

4. Evidence is presented which indicates that the pigment granules are elaborated within these dendritic cells and are passed on to the ordinary epidermal cells across the end-caps. This hypothesis accounts for the well-known initial polar-capping distribution of pigment within the epidermal cells.

5. Dendritic cells have a cell-lineage of their own and are not derived from ordinary epidermal cells of the basal layer as a functional modification.

6. Branched cellular elements which are similar in all respects to pigmented dendritic cells, save that they lack the melanogenic properties characteristic of the latter, have been demonstrated in the non-pigmented epidermis of man, the guinea-pig and the rabbit. These have been called white dendritic cells.

7. It is suggested that dendritic cells (both types) almost certainly fulfil some physiological function in the epidermis other than melanogenesis.

8. The relationship between the white dendritic cell and the 'cell of Langerhans' is discussed and it is concluded that they are identical. The theory that these cells have connexions with the nerves of the skin is not supported.

9. It is concluded that the mammalian epidermis is a compound tissue composed of at least two distinct cellular elements: the dendritic cells and the ordinary epidermal cells.

My grateful thanks are due to Prof. P. B. Medawar who supervised the work recorded in this paper and gave me great assistance at all stages, and to Dr O. L. Thomas who has given me invaluable technical advice and assistance.

The expenses involved in this research were defrayed by a grant from the Department of Plastic Surgery, Oxford University.

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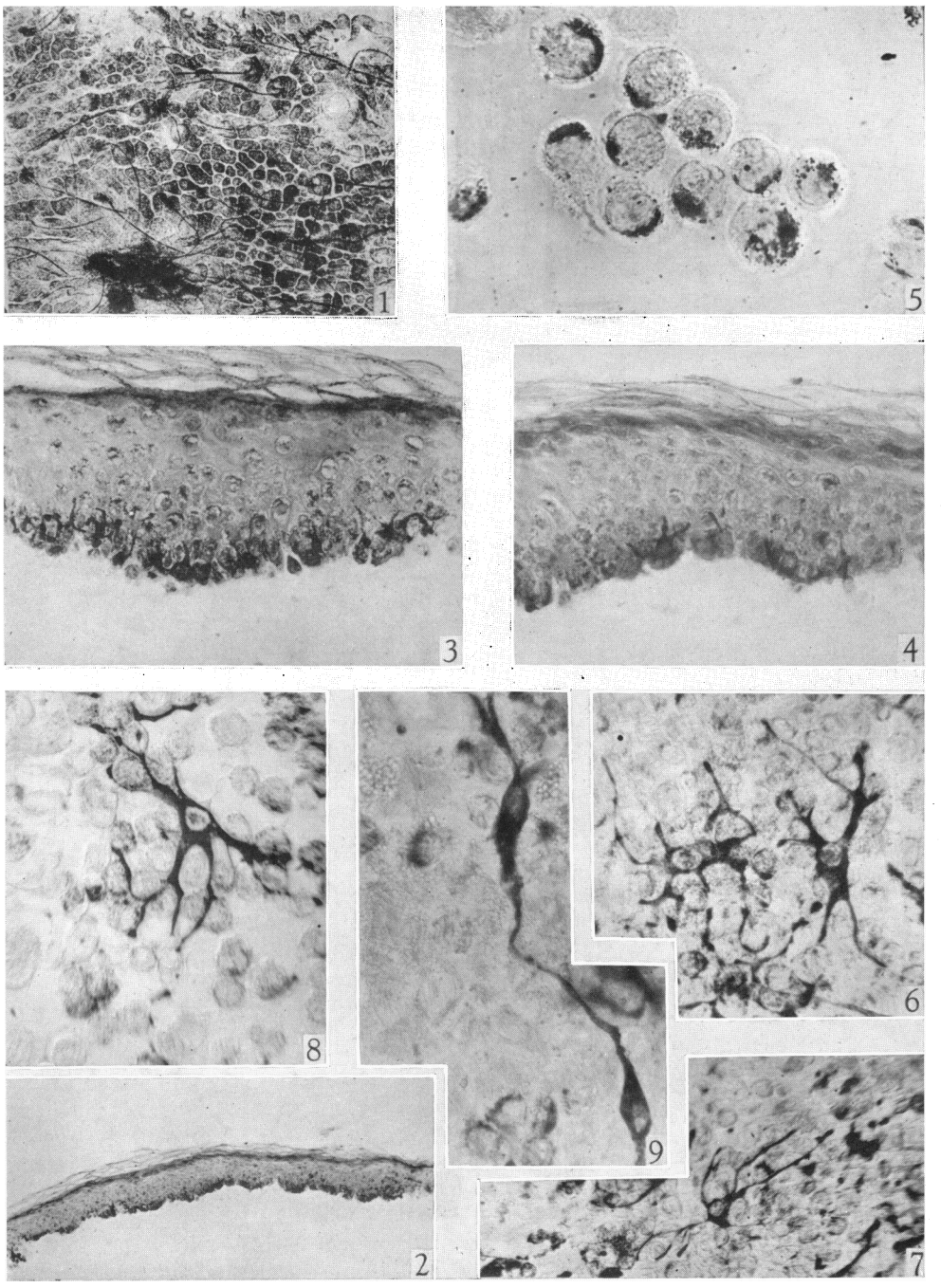
EXPLANATION OF PLATES

PLATE 1

Fig. 1. Whole mount preparation of pure pigmented epidermis from guinea-pig's ear, unstained and viewed from the underside to show the characteristic ridging pattern. The 'valleys' are normally occupied by the so-called 'dermal papillae'. (×20.)

Figs. 2–4. Vertical sections of split black ear skin. The pure epidermis has been separated from the dermis by tryptic digestion and the sections prepared from it lightly stained with Ehrlich's haematoxylin and eosin. Dendritic cells are more prominent than in sections of full-thickness skin. (Fig. 2, ×75; Figs. 3 and 4, ×335.)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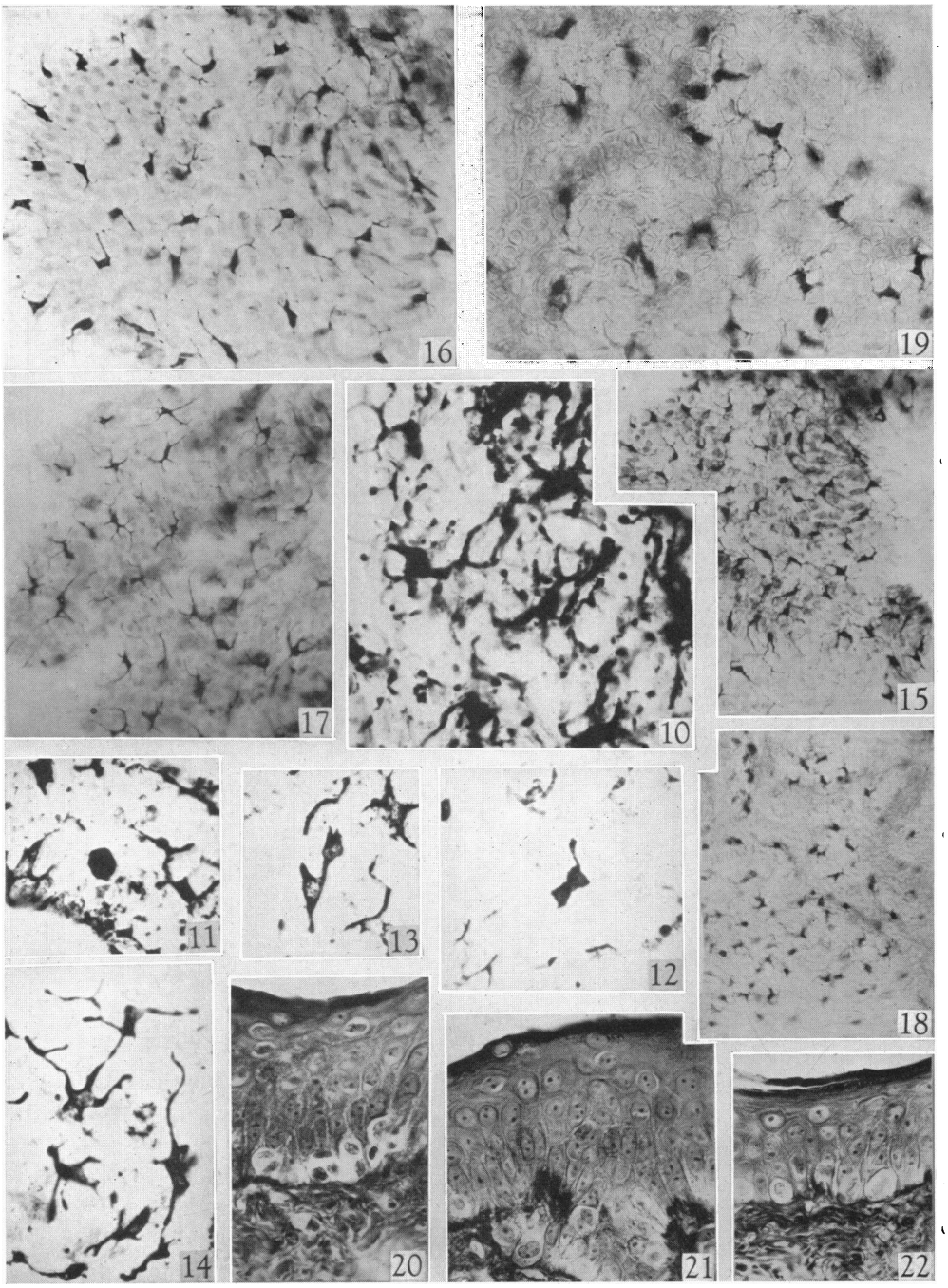

BILLINGHAM—DENDRITIC CELLS

Fig. 5. Isolated ordinary basal-layer epidermal cells from pigmented ear skin about 1 hr. after teasing out in Ringer's solution. Melanin granules are present *within* the cells as characteristic 'polar caps' located between the nuclear and cellular boundaries. It is evident that cytolysis has commenced. (×735.)

Figs. 6–9. Pigmented dendritic cells from surviving preparations of split black ear skin the undersides of which have been lightly scraped and the resultant clumps of cellular material lightly squashed in Ringer's solution. The terminal buttons or end-caps of dendritic cell processes can clearly be seen applied to the boundaries of ordinary basal-layer cells in Fig. 8. Note that melanin granules are present in nearly all ordinary epidermal cells. Fig. 9 shows two dendritic cells united by a common process. They may represent the division-products of a single dendritic cell which has recently divided, since they possess very few processes. (Fig. 6, ×310; Fig. 7, ×220; Figs. 8 and 9, ×390.)

PLATE 2

Fig. 10. Pigmented dendritic cells. This is a high-power photograph of a preparation exactly similar to that illustrated by Fig. 1 after subjection to Dopa treatment for 1 hr. The preparation is unsquashed and the distribution and optical depth of the dendritic cells of ear skin are normal. Ordinary epidermal cells are invisible, but numerous end-caps of dendritic cell processes, which are strongly Dopa-positive, can be seen. (×400.)

Figs. 11–14. Division stages of pigmented dendritic cells. The preparation is unstained and is of pure pigmented epidermis of the ear which had previously been rendered hyperplastic by transplanting it to a recipient area of the animal's chest for 12 days, viewed from the underside. Fig. 11 shows a dendritic cell which has retracted its processes and rounded off prior to division. Figs. 12 and 13 show later division stages in which daughter nuclei can be distinguished. In Fig. 14 two daughter dendritic cells are seen which are still united by a common process although each possesses its own fairly complex system of branches. (×270.)

Figs. 15–17. White dendritic cells as revealed by a variant of Gairns's gold impregnation technique. Figs. 15 and 16 are of the white ear epidermis of the spotted guinea-pig; Fig. 17 is of the white epidermis of the human forearm. Note that the distribution, number, size and mode of branching is just the same as with pigmented dendritic cells. Fixation with formic acid and lemon juice has, however, caused these cells and their processes to appear more compact and wiry. The nuclei of the ordinary epidermal cells of the basal layer are easily visible. There is absolutely no morphological difference between the white dendritic cells of human epidermis and those of the guinea-pig. (Fig. 15, ×100; Fig. 16, ×180; Fig. 17, ×135.)

Figs. 18 and 19. White dendritic cells of human skin. The preparation is of a Thiersch graft cut from the forearm and stained supravitally with methylene blue solution in Ringer. The nuclei, cell boundaries and cytoplasmic bridges of the ordinary epidermal cells can be clearly seen in Fig. 19. The processes of the dendritic cells have been rendered visible by the presence of deep-blue-staining granules within their cytoplasm. Compare these white dendritic cells, as revealed by methylene blue staining with those demonstrated by gold impregnation (see Figs. 15–17). (Fig. 18, ×95; Fig. 19, ×270.)

Figs. 20–22. White dendritic cells of human skin as revealed by Heidenhain's Azan method. The material was taken from the abdominal region, fixed in formol-mercuric chloride and paraffin sections cut at 15 μ. These cells are slightly larger than their ordinary basal-layer cell neighbours and they have a relatively thicker layer of cytoplasm around their nuclei. 'Prickles' can be seen bridging the spaces between the ordinary epidermal cells, but in no case can prickles be seen around the boundaries of the dendritic cells—a diagnostic character of these cells. Processes which arise from the cytoplasm of these white dendritic cells and disappear between the neighbouring cells can be seen in Figs. 20 and 21. (×400.)