Electron Microscope Observations of the Melanocyte of the Human Epidermis

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PLATES 17 TO 21

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ABSTRACT

Using standard osmium fixation and methacrylate embedding techniques, a study has been made of the melanocyte of human biopsy skin removed under general and local anaesthesia. Melanogenesis was easily observable in the melanocytes, but immature pigment granules were rarely seen in the Malpighian cells. The passage of melanin from melanocyte to Malpighian cell—cytocrine secretion—is thought to have been observed. Phagocytes near the dermal-epidermal junction seem to have their pigment granules in vacuoles, rather than surrounded directly by the cytoplasmic matrix as in the melanocytes. This, together with the failure to observe "effete" melanocytes, prompts the suggestion that the phagocytes are melanocytes which have migrated from the epidermis into the dermis.

A melanin granule is shown with alternating dark and lighter transverse striations, concerning which structure little can at present be said.

The distinctive cells seen in the basal layer of the epidermis, which Masson (1, 2) called "clear cells" on account of their appearance in stained sections, have been the subject of much uncertainty. They have also been the subject of terminological profusion: the clear cell, the melanoblast, the melanocyte, the dendritic cell, and even the Langerhans cell, meaning the same thing. Clear cell is a good description of appearance in section, and melanocyte an accurate description of function, and it has been recommended (3) that the latter term be used to the exclusion of all others. The recommendation has met with wide, though not complete (4, p. 62), agreement, and is followed in this paper.

Pautrier *et al.* (5) early suggested that the melanocytes form a syncytial system to nourish the epidermis with nutrients obtained from the dermis. Billingham (6) considers that the cells, by means of the branching network formed by their dendrites, are ideally suited to spread virus-type infections through the epidermis. Later, with Medawar (7), he regards the cells as a definite system called the epidermal glial system. These authors also agree with Masson (1) that the Langerhans cell, considered by Langerhans (8) to be a distinct cell of neural function, is merely an effete melanocyte which has ascended the epidermis in company with the developing epidermal cells.

Melanocytes originate from the neural crest (9), but despite this neural relationship their only clearly demonstrated function is melanogenesis. In black skin of the guinea pig, or pigmented human skin, they are constantly laden with melanin (6), but in white guinea-pig skin and non-pigmented human skin they contain no pigment granules. However, by mildly stimulating the non-pigmented human skin (provided it is not vitiliginous) with, for example, ultraviolet light, the melanocytes are distinguishable after immersion in dopa (3,4-dihydroxyphenylalanine) by the heavy precipitate of melanin which forms within them.

Pease (10), using earlier, and consequently rather unsatisfactory, techniques, has shown the electron microscopic appearance of the melanocytes, with pigment granules and dendrites. Selby (11) examined human, rat, and mouse epidermis under the electron microscope but was unable to find any melanocytes. Very recently Barnicot and Birbeck (12) gave a good illustration of a melanocyte in the human epidermis.[1]

[1] Two papers have subsequently appeared in *J.*

The present paper will describe more fully the appearance of the melanocyte under the electron microscope, showing how completely it differs from the surrounding basal cells, and how certainly it is concerned with melanogenesis.

Material and Methods

For all but Figs. 2 and 7 *D* the human material used in this study was perianal skin obtained from a 19-year female under general anaesthesia. Fixation commenced about 15 minutes after removal. For Fig. 2 biopsy material was taken from the right flank of a 48-year female using 2 per cent procaine as local anaesthetic. This skin was regarded as normal although it came from close to the edge of a warty naevus which was being excised. Fixation commenced 10 minutes after injection. Material for Fig. 7 *D* was taken from the mons pubis of a 23-year female under general anaesthesia.

The techniques of fixation, embedding, and sectioning have been described in a previous publication (13), and accord with standard practices.

OBSERVATIONS

The general appearance of the melanocytes at the dermal-epidermal junction is shown in Fig. 1 *A*. The cytoplasm of these cells is so obviously different from that of the adjacent basal cells that it is impossible to confuse them. Principally the different appearance is due to the complete absence of tonofibrillar material in the melanocyte; indeed fibrillar material of any kind is generally absent, and at most has only been scanty. Consequently, the melanocyte has no tonofibrillar attachment points on its membrane, which is quite separate from that of adjacent basal cells, as shown in Fig. 1 *B*, which is enlarged from 1 *A*. Such attachment points are normally seen on the wall-membranes between basal cells as prickles or, where the wall-membrane forms part of the dermal-epidermal junction, as half-prickles (13).

Although the melanocytes here have the appearance of being rounded off, they must to some extent be branched because transverse sections of dendrites appear in the figure. A Golgi apparatus is often present.

Dense granules of melanin are seen in the melanocytes, in the dendrites, and in the basal cells, the granules in the last attaining a much larger size than any in the melanocytes or their branches. Melanin has never with any certainty been detected lying free in the intercellular spaces. Its formation is easily seen in Fig. 6, which shows transverse (*dt*) and longitudinal sections (*dl*) of stages in the formation of the granules. These stages are less robust in appearance than those of the hair (14; also unpublished observation).

Occasionally the dendrite is sectioned longitudinally as it branches from the cell, as shown in Fig. 2 where, however, the cell is devoid of pigment. The bulbous "body" of the cell is covered on its basal surface with a scanty reticulum of collagen, or reticular, fibrils indicating that it is in the epidermis. To the right of the melanocyte there is another cell with very similar cytoplasm whose shape and position are such that it is easy to imagine that it has been fixed in the act of penetrating the indented epidermis. This cell is not covered by reticular fibrils and is, therefore, in the dermis.

Part of a longitudinally sectioned dendrite is shown in Fig. 3 *A*, where the melanocyte contains abundant melanin. The majority of the granules are fully formed, and peripherally located, being especially numerous in the dendrite. Developing melanin also is detectable, and a vesiculate region, possibly related to a Golgi region, is noticeable in the centre of the cell. Figs. 3 *B* and 7 *A* are enlargements of regions indicated on the main figure, and show what is possibly a breakdown of membranes between the melanocyte and the basal cells in order presumably to allow passage of the melanin granules from one into the other—the cytocrine secretion of Masson (15). The evidence for this is especially strong in Fig. 7 *A* because here the easily observable separate membranes suddenly disappear on both sides of a short gap through which a melanin granule is passing.

Fig. 4 shows a rather unusual epidermal cell apparently situated in the layer above the basal layer but with a tongue projecting downwards almost to the junction, in the direction indicated by the arrow. The identity of this cell as an epidermal cell is made certain by the appearance of its cytoplasm, which contains tonofibrils, and the presence of prickles; the melanin it contains shows immature granules (enlarged in Fig. 7 *C*) only rarely seen in epidermal cells.

The phagocyte shown in Fig. 5 was one of many that could be seen in the dermis by means of the

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Biophysic. and Biochem. Cytol., 1958, **4**, by G. F. Odland, 529, and by W. H. Clark and R. G. Hibbs, 679, in which the melanocyte is more extensively studied. In general, our observations agree with those of Odland, and of Clark and Hibbs, both of whom illustrate more extensive dendritic branching than we do. It is interesting to read of their uncertainty in distinguishing electron microscopically between dendrites and non-myelinated nerve axons in the epidermis, a difficulty which we also have experienced.

phase contrast microscope. The cells were close to the dermal-epidermal junction and their cytoplasms were rich in melanin. As the figure shows, there is a striking difference between the melanin in this cell and that of the melanocyte, for here the granules are located collectively within vacuolar membranes (see enlargement in Fig. 7 *B*) whereas in the melanocyte they were singly located in the cytoplasm. Some of the granules in the phagocyte vacuoles are still intact, but others appear to have broken down into dense, finely particulate, material. The cytoplasm of these cells is otherwise little different from the melanocytes, the main difference perhaps being that the mitochondria are rather larger.

Fig. 7 *D* shows the banded appearance sometimes seen in melanin granules. Alternating dark bands are of slightly different densities.

DISCUSSION

Electron microscopy shows that melanocytes are devoid of tonofibrillar prickles, as noticed also by Billingham (6), and do not enter into the tonofibrillar system linking the cells of the epidermis. They are, therefore, quite distinct from epidermal cells, which is not surprising because Rawles' work has shown the different origins of these cells in mammalian tissue. The unspecialized, loose, manner in which the melanocyte enters into the composition of the epidermis must inevitably raise the question whether it migrates freely between dermis and epidermis. There can be no doubt that in, for example, transplanted embryonic material migration of prospective melanocytes can occur, and Rawles has shown that they are more strongly attracted to some sites, such as the developing hair, than to others. It is the mobility and regeneration of the melanocyte in the mature tissue that nowadays appears to be of greater interest.

In the present paper questions concerning the mobility of the melanocyte, and the interaction between it and the epidermis, arise for a variety of reasons. In Fig. 2 a cell was seen in the dermis, near the junction, which had the appearance of a melanocyte in position to enter the epidermis. In another instance, not illustrated, a melanocyte was seen which, because it was above the reticular fibrils which help to delineate the dermal-epidermal junction, was undeniably in the epidermis, yet nine-tenths of its volume projected into the dermis; this may indicate either that it had just entered the epidermis, or that it was about to leave. In this cell, as in all melanocytes, the melanin granules were located directly in the cytoplasm, whereas in the numerous phagocytes seen near the epidermis in this material, the pigment is located within vacuoles. It is, therefore, difficult to understand why, since the pigment must come from the epidermis (15), phagocytes (distinguished by their vacuolated pigment) are not seen with pseudopodia reaching into the epidermis, or at least making contact with the basal cells or the melanocytes. Possibly this may be due to insufficient observations, but it may also be due to the migration of the melanocyte, still containing some unextruded pigment, into the dermis. This would imply that after the melanogenic life of the melanocyte is over it enters the dermis to undertake phagocytic functions, or perhaps only to die. It would imply furthermore, that effete melanocytes, said to occur in the suprabasal cell layers, would not normally be seen in the epidermis: with this our observations concur, because although non-epidermal cells are readily recognizable in the epidermis by their absence of prickles, they are seen only rarely, in pathological conditions, and appear absent even when melanocytes are abundant. Neither have effete melanocytes been seen in the developing hair cortex (unpublished observation), where, if they occurred, it seems highly unlikely that they would not have been noticed. The different cytoplasmic state of the melanin, whether it is found in vacuoles or directly in the cytoplasm, may be a result of the changed environment of the melanocyte, or of its physiological deterioration. In the epidermal cells also, while the pigment granules may frequently occur in vacuoles, they most often do not.

If the presence of effete melanocytes in the epidermis is denied, the nature of the Langerhans cell again arises. The recent papers by Fan and Hunter (16) showing the consistent appearance of the Langerhans cell in the epidermis, and the work of Weddell (see 17) and of Miller *et al.* (18), which proves that the epidermis is innervated, make it difficult to believe that Langerhans was entirely wrong in ascribing a neural function to the "cell" which bears his name. Though perhaps not exactly as he depicted it, there possibly occurs in the epidermis a structure to which the name Langerhans cell may properly be given.

The evidence given for melanogenesis in the melanocyte is not difficult to obtain (Fig. 6); rarely immature pigment granules pass from melanocytes into the epidermal cells, but it is unlikely that the latter are melanogenic because it is only when melanocytes are present that pigmentation takes place (9).

The fact that melanogenesis occurs in the

melanocyte, and mature melanin is found in the epidermal cells, renders cytocrine secretion from one into the other a necessity. The strong evidence given for such secretion in Fig. 7 *A* is not conclusive. A sharp breakdown of part of the closely adpressed membranes between the melanocyte and adjacent basal cell with a pigment granule passing through the gap is evidence that is perhaps not easy to reject. But thin membranes are very prone to disappear in electron micrographs for no other reason than that they bend a little and so become obliquely sectioned. With dense, and presumably quite hard, material like melanin coming so close to such a membrane the chance of membrane "breakdown" being an artifact may be great. However, should the observations prove not to be artifacts, the mechanism of melanin transfer, once the membrances between dendrite and epidermal cell had "fused," would appear to be by simple mechanical pressure. The long thin dendrite packed with pigment granules, though not invariably the instrument of transfer, is an especially suitable one, because it would be very sensitive to squeezing pressures either from environmental factors at the skin surface, or by pseudopodic retraction of the dendritic cytoplasm. The converse of this, the passing of melanin from the bulkier epidermal cells into supposed phagocytic pseudopods, is not so easy to visualize.

Masson (15) and Pinkus (19) have published figures showing mitotic division of the epidermal melanocyte. Observations of this kind are rare, and it is not surprising that we have seen none.

The periodic structure shown by the melanin granule in Fig. 7 *D* is rarely seen, and observations of the striations may depend on the correct orientation of the sectioning. At present it is difficult to suggest a molecular basis for the striations.

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Explanation of Plates

Abbreviations Used

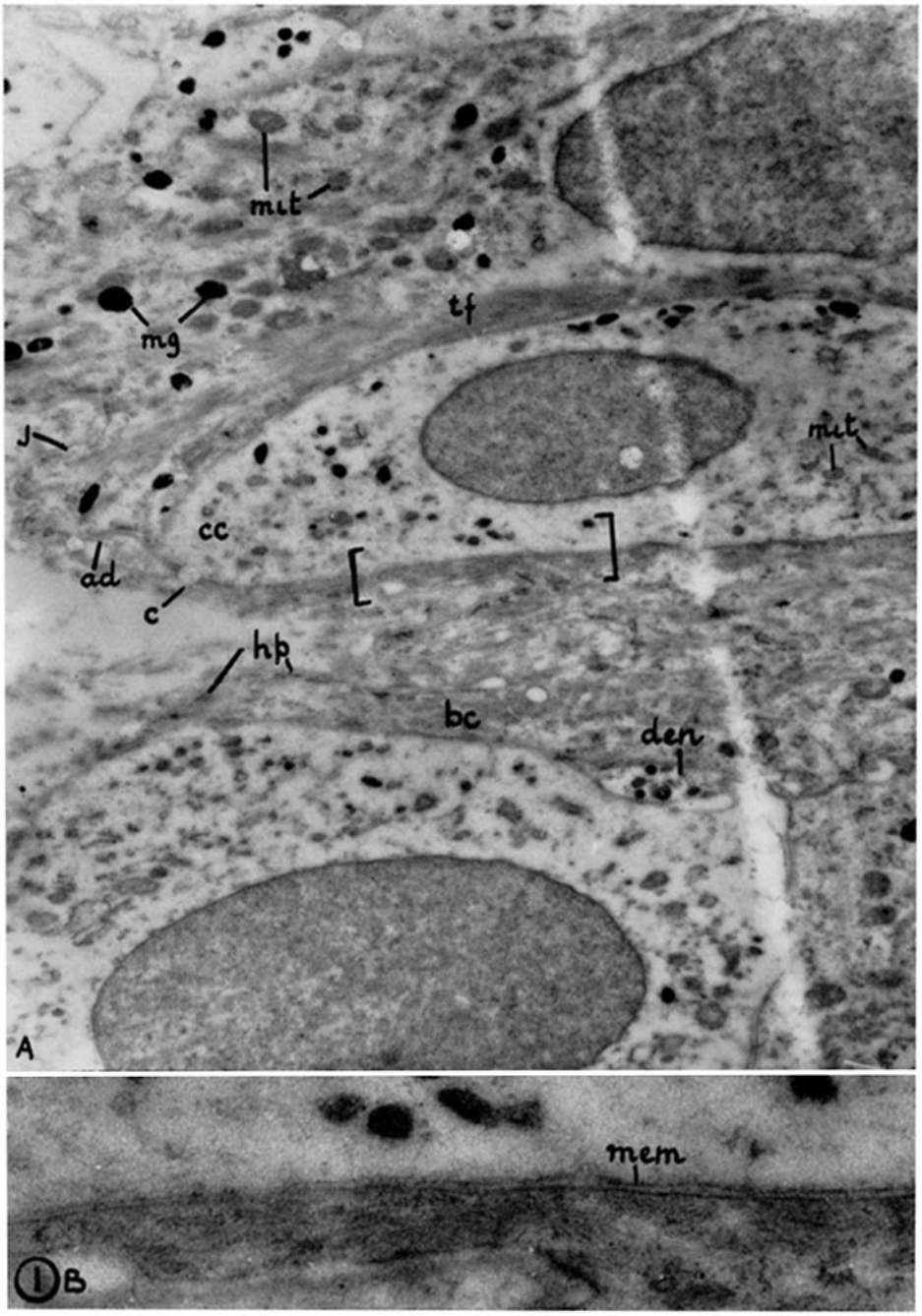
bc, basal cell; *c*, collagen fibrils of the reticulum; *cc*, melanocyte; *den*, dendrite; *er*, endoplasmic reticulum; *g*, Golgi apparatus; *hp*, half-prickle; *j*, dermal-epidermal junction; *mg*, melanin granule; *mit*, mitochondrium; *p*, prickle; *tf*, tonofibrils.

Plate 17

FIG. 1. *A* shows the dermal-epidermal junction with melanocytes sharply differentiated from the basal cells by the different appearance of the cytoplasm. The actual line of the junction is not easy to follow because it is confused by the adpressed reticulum of collagen fibrils, but it is possible to distinguish it, and to see the half-prickles as dark dots. Immediately on the dermal side of these half-prickles is a weaker osmiophilic line (*ad*) which is presumably the surface of adhesion between dermis and epidermis. The tonofibrils are tightly compacted and adpressed to the central melanocyte. Mitochondria and dense melanin granules are abundantly evident and dendrites are seen in transverse section.

B is an enlargement of the area bracketed in *A*, so as to show more clearly the separate nature of the melanocyte and basal cell membranes (*mem*).

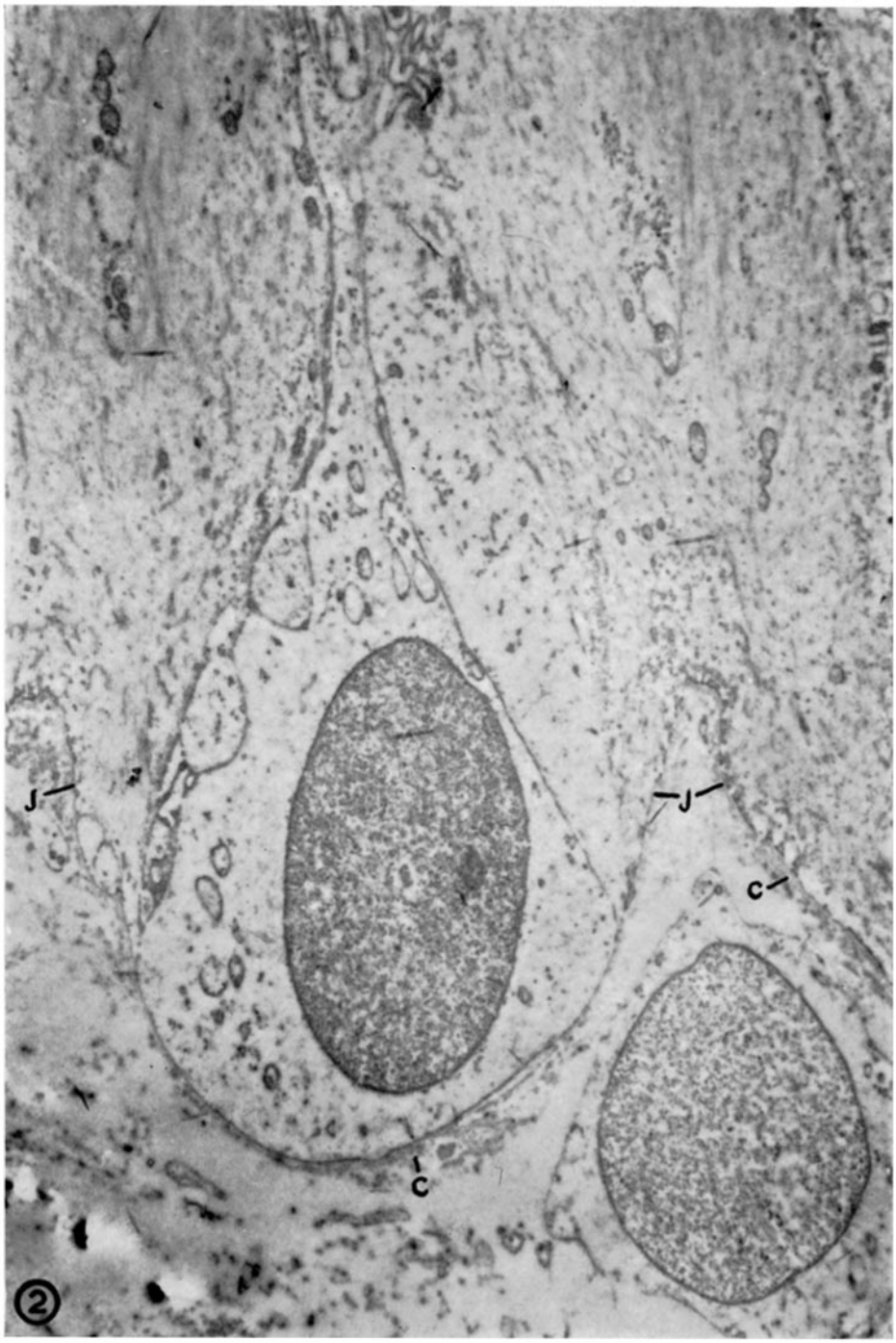
A, × 15,000; *B*, × 54,000.



(Charles and Ingram: Melanocyte of human epidermis)

PLATE 18

FIG. 2. Showing the dendrite of a non-pigmented melanocyte inserted between cells of the basal layers. The reticular, or collagen, fibrils enmesh the melanocyte, but not the cell to the right of it in the dermis. × 10,000.

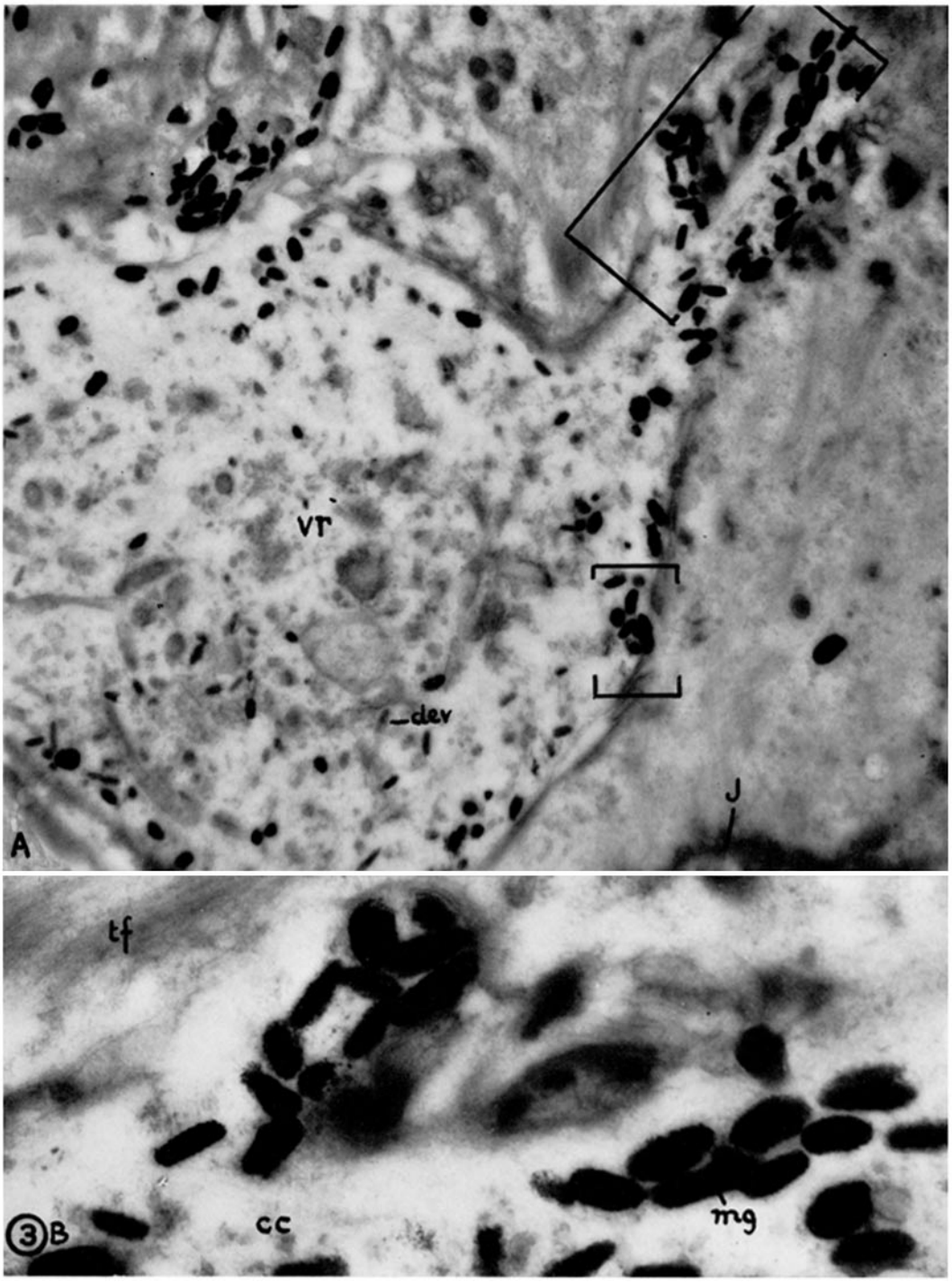


(Charles and Ingram: Melanocyte of human epidermis)

PLATE 19

FIG. 3. *A*, a pigment-containing melanocyte with dendrite. Developing granules are shown (*dev*), and the bracketed areas, enlarged in *B*, and Fig. 7 *A*, show possible breakdown of the membranes of the melanocyte and the basal cell in order to allow the melanin to pass from the one into the other. The vesiculated region (*vr*) is possibly a tangentially sectioned Golgi region.

A, × 15,000; *B*, × 42,000.

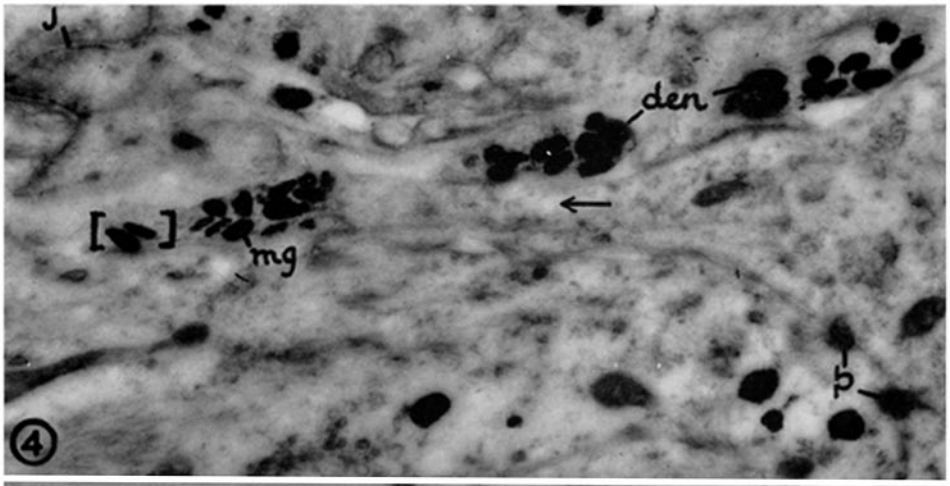


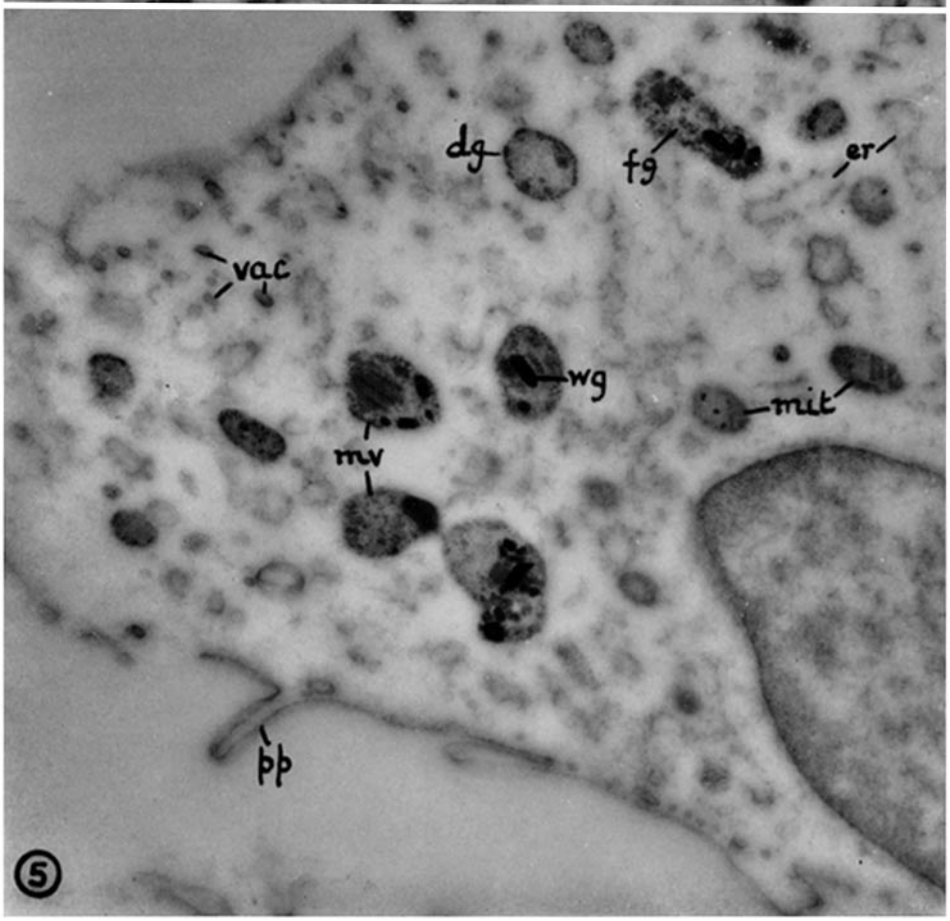
(Charles and Ingram: Melanocyte of human epidermis)

PLATE 20

FIG. 4. Part of a Malpighian cell projecting downwards (in the direction of the arrow) almost to the dermal-epidermal junction. The melanin granules in the bracket are enlarged in Fig. 7 *C* and an immature grain is seen to be present. Groups of melanin granules are enclosed in the cytoplasm of what are possibly dendrites in transverse section. × 15,500.

FIG. 5. Melanin-ingesting phagocyte located near the dermal-epidermal junction. The pigment appears to be in the process of digestion in vacuoles (*mv*) with limiting membranes (*vm*) seen in Fig. 7 *B*, which is an enlargement of the vacuole labelled (*dg*). Intact grains (*wg*) and broken down grains (*dg*) can be observed, in addition to much finely dispersed dense grains (*fg*). The cytoplasm shows pseudopodial projections (*pp*), possibly elements of an endoplasmic reticulum (*er*), and smaller vacuoles (*vac*). × 25,000.





(Charles and Ingram: Melanocyte of human epidermis)

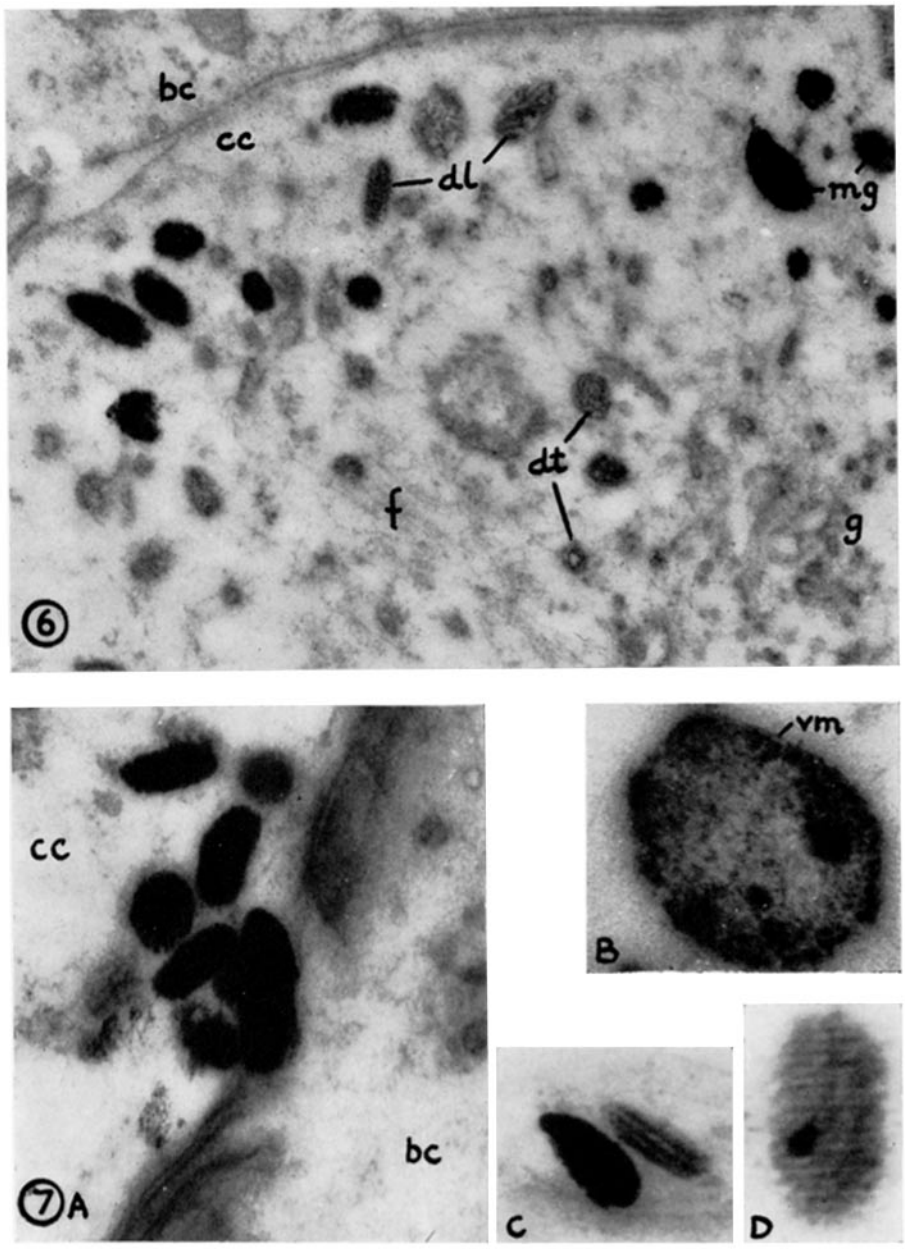
PLATE 21

FIG. 6. Part of a melanocyte enlarged to show stages in the formation of the melanin granules. Developing granules are seen in longitudinal (*dl*) and transverse (*dt*) section. A little fibrillar material (*f*) is present. × 42,000.

FIG. 7. *A*, *B*, and *C* are enlargements from Figs. 3, 5, and 4 respectively, and are described in the legends to those figures.

D is a melanin granule showing alternating dense and less-dense transverse striations.

A and *C*, × 54,000; *B*, × 85,500; *D*, × 190,000.



(Charles and Ingram: Melanocyte of human epidermis)