Electron Microscope Studies of the Human Epidermis

The Clear Cell of Masson (Dendritic Cell or Melanocyte)*

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ABSTRACT

The human epidermis has been studied by electron microscopy following osmium tetroxide and potassium permanganate fixation. An anatomically distinct cell in the human epidermis has been demonstrated with features similar to the melanocyte of the hair bulb described by Barnicot, Birbeck and Cuckow (3). It is dendritic in form and does not contain tonofilaments. "Intercellular bridges" are not formed. The mitochondria are larger and more numerous than those of other epidermal cells and the endoplasmic reticulum is more complex. Some of these cells contain melanin but others are melanin-free. The cell has been interpreted as being identical with the dopa-positive, clear cell of Masson (dendritic cell of Bloch or melanocyte). We have found that many membranous structures in the human epidermis are better preserved by permanganate fixation than by osmium tetroxide fixation.

The nature of the cell capable of synthesizing melanin in the epidermis of man and lower animals is in dispute. One group of workers believes that mammalian epidermis is composed of at least two distinct varieties of cells: a cell that is dendritic in form, and the ordinary epidermal cell (5). Proponents of this school distinguish the dendritic cell or melanocyte by its clear, faintly staining cytoplasm in hematoxylin and eosin preparations, by its long cytoplasmic processes demonstrable with ammoniacal silver nitrate, and its "positive" reaction on incubation with 3,4 dihydroxyphenylalanine (dopa) (4–6, 15, 16, 25). In addition, this "dualist" school adheres to the concept that the melanocyte is derived from the embryonic neural crest. The evidence, in mammals, supporting this view has been largely supplied by Rawles (20, 21).

The opposing school of workers believes that the basal cell may be transformed into a dopa-positive, dendritic cell (9, 17). Allen (1, 2) states that melanocytes have intercellular bridges and, quoting Ewing, says that they do not differ in any essential respect from the average epidermal cell. Allen further remarks that many epidermal cells with clear cytoplasm may be seen in dermatoses and in such cases dermatopathologists interpret the clear cytoplasm as evidence of intracellular edema. Further, he questions (1) the presence of "true" dendritic processes of the dopa-positive epidermal melanoblast.

This divergence of opinion hinges in part on the presence or absence of a dendritic cell which is consistently distinguishable from all other cells of the epidermis. The electron microscope studies of this report attempt to delineate clearly such a dendritic cell from the ordinary epidermal cells.

Materials and Methods

Biopsies of the skin of the left upper quadrant of the abdomen were obtained from forty-two white and Negro volunteers of both sexes. The ages varied from 5 to 74 years. Two mm. biopsies were taken without anesthesia using a high-speed rotary punch. By this method almost perfect cylinders of skin with a small amount of subcutis may be quickly obtained without subjecting the patient to objectionable pain. The

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method may be quite painful, however, if the tight skin over a bony prominence is biopsied. The abdominal skin was selected because the epidermis is so thin that its entire thickness may be included in a single section. The excised tissue was immersed, usually within 30 seconds after removal, in Palade's veronal-acetate buffered osmium tetroxide (18) and in some instances similarly buffered potassium permanganate (14). The corium was removed, except for that in the dermal papillae and a minute amount deep to the rete ridges, with a razor blade. The removal of the corium was found necessary in order to obtain relatively thin sections of good quality. The resulting blocks, which were less than 1.0 mm. in thickness, were fixed for 2 hours at about 4°C. and dehydrated in graded methyl alcohols. They were then embedded in partially polymerized methacrylate (1 part methyl to 12 parts *n*-butyl) and polymerization was completed at a temperature of 60°C. The blocks were oriented so that sections perpendicular to the surface of the skin could be obtained. Sections were cut with a Porter-Blum microtome and mounted on formvar-coated athene grids. The micrographs were taken with an RCA-EMU3C microscope.

RESULTS

These studies have established to our satisfaction that a morphologically distinct dendritic cell is constantly present in the abdominal epidermis of human beings. The dendritic cells are easily distinguished from the ordinary basal cells and also from another cellular element in the basal layer which we have tentatively designated as a "peg cell."

The general structural pattern of the ordinary epidermal cells is briefly presented in order to contrast it with the structure of the clear cell (dendritic cell or melanocyte). The details of the fine structure of the epidermis may also be found in the papers of Selby (22, 23), Porter (19), Charles and Smiddy (10), Weiss and Ferris (24), and Birbeck and Mercer (7). Additional papers relating to this problem are found in the bibliography of Selby (22).

The cytoplasm of the ordinary cells that make up the basal layer, the stratum spinosum, and even the stratum granulosum, contains numerous delicate filaments (the tonofilaments of Selby), which converge on localized areas of increased density of the cell membrane. These bundles of convergent tonofilaments apparently represent the same structures described by light microscopists as tonofibrils. Every point of tonofibrillar convergence on a cell membrane is apposed on the membrane of the adjacent cell by a similar convergence of filaments. The two groups of tonofilaments and the associated areas of cell membranes with increased density make up a complex structure which apparently is the ultrastructural counterpart of the intercellular bridge as seen in light microscope preparations (Figs. 4 and 7). It should be made clear that the use of the term "intercellular bridge" does not imply conformity with the light microscopist's concept that it is a tonofibril coursing from one cell into another. The studies of Porter (19), Selby (22, 23), and Charles and Smiddy (10) have shown that the apposing bundles of tonofilaments stop at the cell membrane. The apposing, dense regions of the cell membranes into which these bundles of tonofilaments insert correspond to the node of Bizzozero (Fig. 4). Fawcett and Selby (11) have used the more general term of desmosome for these "adhesion plates" at the cell surfaces.

The human epidermal cell contains a moderate number of rather small, round to ovoid mitochondria. The mitochondria are not so large, nor are the cristae so prominent as those seen in most other types of cells. Furthermore, the number and size of the mitochondria decrease rapidly as the stratum granulosum is approached. The endoplasmic reticulum is scanty and a well developed Golgi area is seldom seen. Cells of all layers of the epidermis may contain dense melanin granules. These are even noted in the stratum corneum, particularly in deeply pigmented skin. These granules vary in number and size, being fewer and smaller in Caucasians and more numerous and larger in Negroes (22). Finally, the cell membranes of adjacent epithelial cells are seen to interdigitate in a deeply scalloped or serrated fashion.

The basal cells present certain additional characteristic features. In osmium-fixed tissue, tonofilaments can be seen to converge on local regions of the cell membrane facing the dermis. At these sites the cell membrane is increased in density in a fashion similar to that observed at the desmosome. However, in the absence of an apposing cell, the converging tonofilaments are seen at the dermoepidermal junction as a row of tuft-like structures on the cytoplasmic surface of the cell membrane. The general appearance of the cytoplasm of the basal cells also differs from that of other epidermal cells. In osmium-fixed material the tonofilaments appear to be in smaller bundles and are less numerous than in cells of the stratum spinosum. Permanganate fixation results in an even clearer delineation of the basal layer. In such preparations

the cytoplasm is seen to contain more numerous and finer filaments less than 100 A in diameter. This fine filamentous cytoplasm is in sharp contrast to the coarser fibrillary pattern of cells of the stratum spinosum (Fig. 4). In general, permanganate is superior to osmium tetroxide in demonstrating the cell membranes of the epidermal cells and with this fixative mitochondria are also more easily identified.

The "peg cell" presents certain features in contrast to the structural pattern of the cells of the basal layer and stratum spinosum (Fig. 2). It is usually located in the basal layer, is much smaller than the average basal cell, and is roughly pyramidal in shape. The cytoplasm is quite dense, apparently because of the numerous densely packed tonofilaments. Melanin granules are usually abundant. The "peg cell" forms "intercellular bridges" with adjacent cells and exhibits "tonofibrillar tufts" at the dermo-epidermal junction, as do ordinary basal cells. It may possess short cytoplasmic projections indenting the cytoplasm of adjacent cells. The "peg cells" have many features in common with basal cells and may only be a variant of these cells. This brief description is not intended to report a new variety of cell, but only to draw attention to these cells, which may be quite prominent in certain low-power electron micrographs.

As has been mentioned, our studies have demonstrated the regular presence of an additional type of cell which is characteristically located in the basal region. It has a relatively "clear" cytoplasm which may extend in long dendritic processes between other epithelial cells. It usually appears along either side of or at the apex of a rete ridge and is rarely seen overlying a dermal papilla. It may be situated between basal cells, but more frequently appears "tacked on" to the basal layer with about one half of its cytoplasm protruding between basal cells and the remainder projecting into the corium (Fig. 1). In contrast to the extremely convoluted cell membrane of the typical epidermal cell (Figs. 2 and 4), its cell membrane is uniformly curved or gently undulating (Figs. 1, 5 to 7). The cytoplasm of the clear cell has a characteristic appearance. In the clear cell no tonofilaments have been identified and consequently no points of tonofibrillar convergence or localized densities of the cell membrane are seen. It follows that no "intercellular bridges" are formed. The cytoplasm contains numerous mitochondria (Fig. 6) which are rather large in comparison with those of a basal cell. In most clear cells a well developed endoplasmic reticulum is evident (Figs. 6 and 7). This reticulum is not so complex as that in many glandular cells, but is more abundant than in any other cell of the epidermis. The endoplasmic reticulum is better demonstrated in permanganate than in OsO_4-fixed tissues. OsO_4 fixation appears to have a destructive effect on some membrane-limited structures of the epidermis, even with relatively short periods of fixation. Permanganate preserves and "stains" all membranes well, though it does not bring out the granular component of the endoplasmic reticulum. Some vesicular components of the cytoplasm cannot be definitely identified as endoplasmic reticulum and these could be comparable to the Golgi region mentioned by Birbeck, Mercer, and Barnicot (8). The clear cells usually but not invariably contain a moderate number of small, uniform melanin granules. The cytoplasm may contain also a number of dense, spherical or ovoid bodies (designated as opaque bodies in the illustrations) that are less than 1 micron in diameter and are neither melanin granules nor mitochondria (Figs. 3 and 6). Their nature is unknown. Continuous segments of cytoplasmic processes of these cells are not usually seen, particularly in thin sections. Occasionally, however, one is fortunate enough to demonstrate a dendritic process continuous with the perikaryon, and one may even see dichotomous division of such a process (Fig. 7). The cell membrane along the dendritic process may appear to be interrupted, forming small pores (Fig. 7). This could be an artifact. More often one sees the dendrites cut in cross- or oblique section as isolated rounded masses of cytoplasm interposed between other cells. The relatively abundant endoplasmic reticulum, larger mitochondria, opaque bodies, and absence of tonofilaments seen in these masses of cytoplasm are features peculiar to the cytoplasm of the clear cells (Figs. 4 to 7). One cannot be certain that all structural profiles between epidermal cells are the dendritic processes of clear cells. Some of the smaller structures could be non-myelinated nerve fibers, but we have not been able to identify with certainty any nerve endings.

DISCUSSION

Barnicot, Birbeck, and Cuckow (3) and Birbeck, Mercer, and Barnicot (8) have previously described in electron micrographs the melanocyte of the hair bulb. These authors state that the melanocyte, in this location, has a cluster of "ergastoplasmic

membranes," many mitochondria, and an area adjacent to the nucleus containing many vesicular structures which is comparable in a geometric sense to the Golgi regions of other cells. On the other hand, Selby (22), in studying the epidermis, states that no cell corresponding to the clear cell (of Masson) was seen in her material.

The cell we have described does not differ in any essential respect from the descriptions of Barnicot, Birbeck, and Cuckow (3), and Birbeck, Mercer, and Barnicot (8); however, the melanocyte of the general body epidermis as observed in thin sections does not invariably contain melanin in contrast to the melanocyte of the hair bulb. One might infer that in the lightly pigmented skin, the melanocyte is not continuously synthesizing melanin. Perhaps as a reflection of the decreased rate of melanin synthesis, the endoplasmic reticulum and Golgi region of the epidermal melanocyte are not so well organized as in the melanocytes of the hair bulb. Our material does not show structures that may be definitely identified as a Golgi area, but the area just above the nucleus of the dendritic cell in Fig. 7 could be compared to "Golgi region" illustrated by Birbeck, Mercer, and Barnicot (8). Different fixation procedures make detailed comparison unreliable.

Tonofilaments and tonofibrils (bundles of tonofilaments) and the formation of "intercellular bridges" (convergent, apposing tufts of tonofilaments plus the desmosome) are characteristic of the epidermal cell. These filaments are consistently present in the Malpighian layers, and Giroud and Leblond (12) have shown, by x-ray diffraction, that the Malpighian layers exhibit a periodicity of 5.1 A identical to the α-pattern of the unstretched human hair. They conclude that a protein with the keratin pattern exists in the Malpighian zone and it is actually the precursor of the true keratin. From this it seems plausible to regard tonofilaments as the morphologic evidence of keratin synthesis. The apparent absence of tonofilaments and tonofibrils from the dendritic cell we have described suggests that the dendritic cell of the human epidermis does not synthesize keratin. The extensive studies (5, 6, 13, 15, 16) that have been made with the dopa reaction indicate that the dendritic cell observed with the light microscope can synthesize melanin, and we believe the dendritic cell of this study is identical with the dendritic cell described by the light microscopists. The available evidence, therefore, suggests that the dendritic cell can synthesize melanin and cannot synthesize keratin. We conclude, as Billingham (5) did, "that the mammalian epidermis is a compound tissue composed of at least two distinct cellular elements: the dendritic cells and the ordinary epidermal cells."

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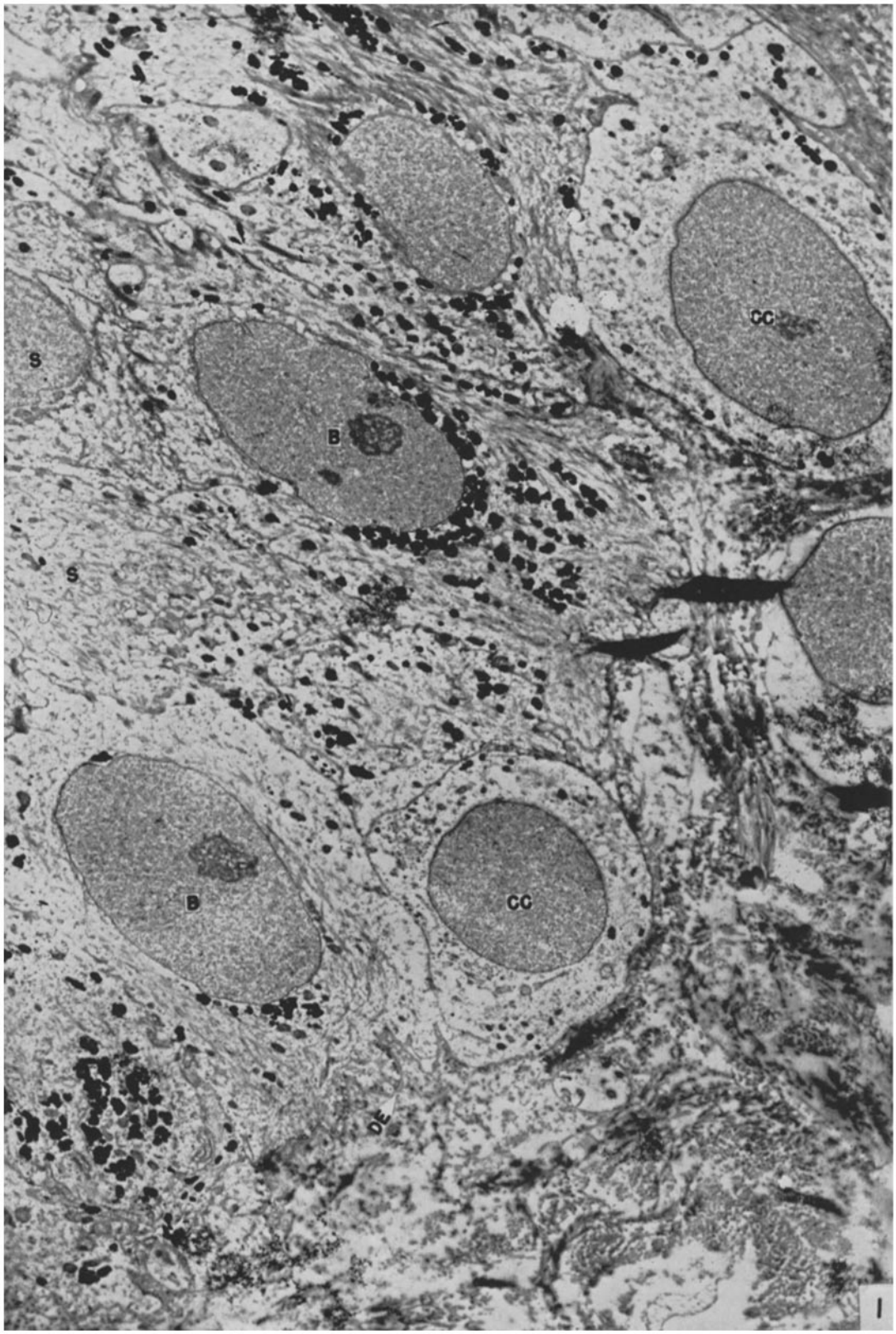
Explanation of Plates

Plate 335

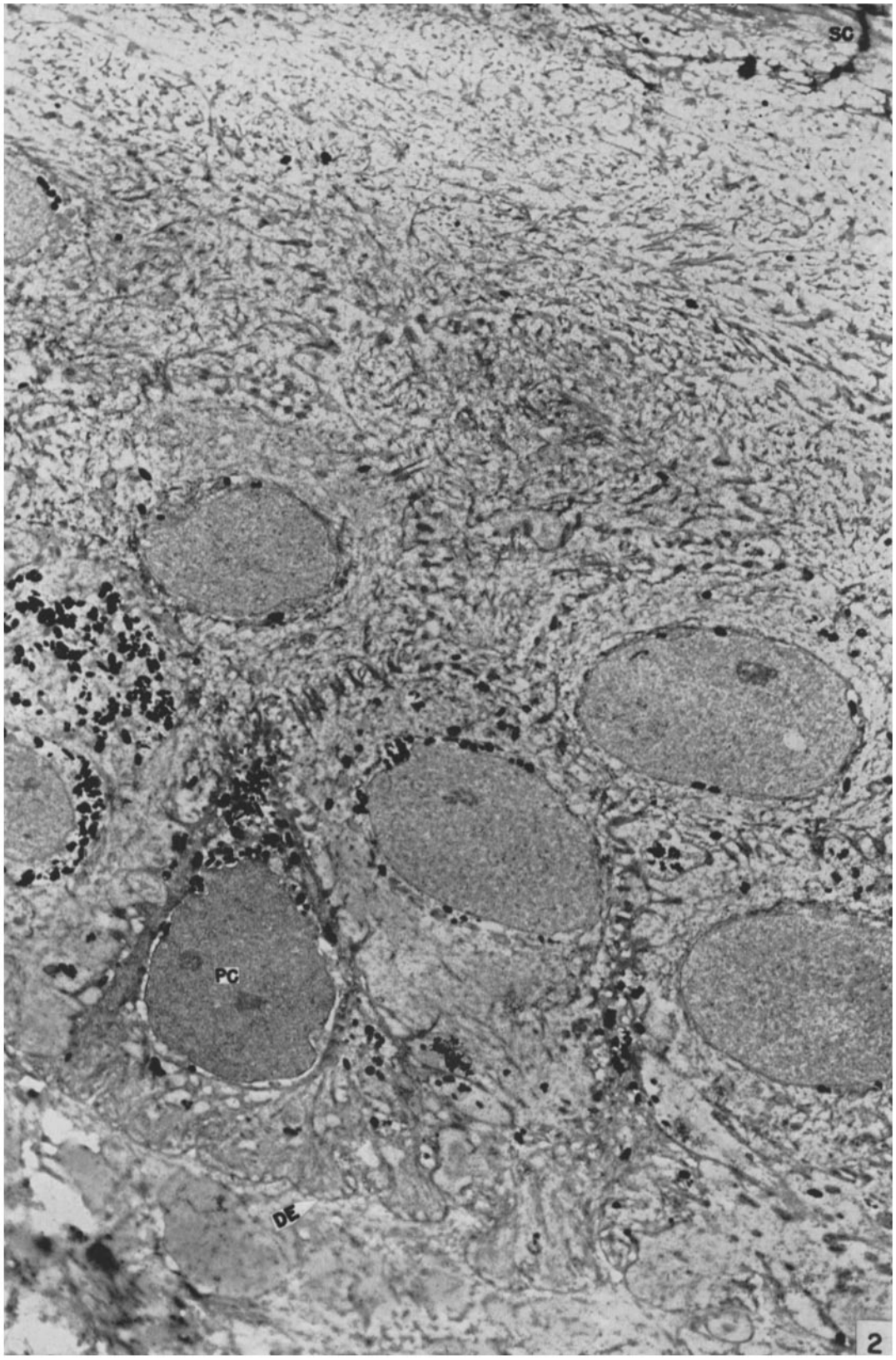
Fig. 1. A section perpendicular to the skin surface showing the dermo-epidermal junction (*DE*), basal cells (*B*), and portions of a few cells of the stratum spinosum (*S*). A clear cell (*CC*) is shown. Note its uniformly curved cell membrane, the small melanin granules, the absence of tonofilaments, and tonofibrillary tufts. These tufts are seen at the dermo-epidermal junction of the adjacent basal cell (*DE*). OsO_4 fixation. × 5,000.



(Clark and Hibbs: Human epidermis: melanocyte)

PLATE 336

FIG. 2. A "peg cell" (*PC*) is seen in the basal layer. Note the triangular outline and the extremely dense cytoplasm. The dermo-epidermal junction (*DE*) is indicated. OsO_4 fixation. × 5,500.

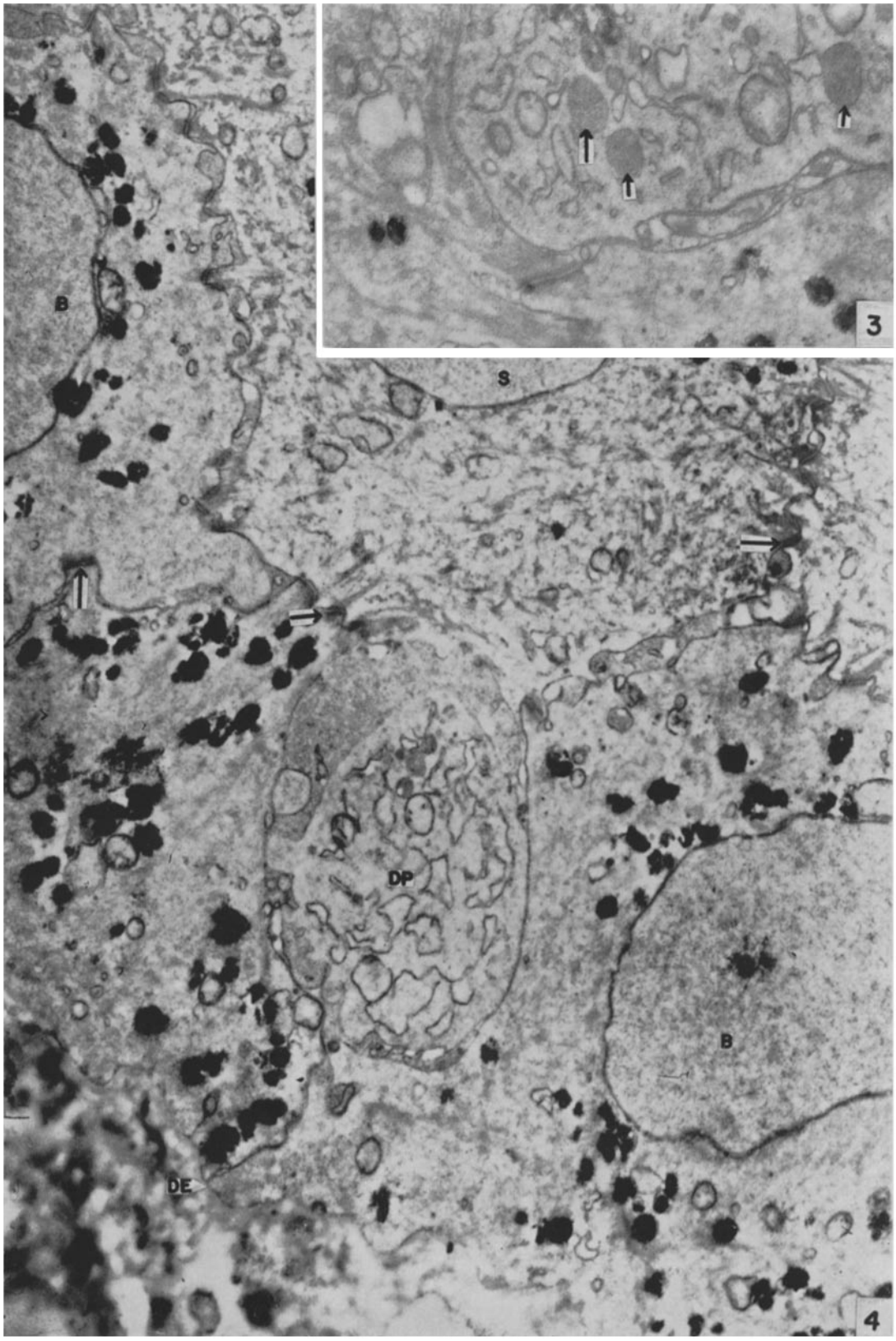


(Clark and Hibbs: Human epidermis: melanocyte)

PLATE 337

FIG. 3. A part of a dendritic process is shown at high magnification. Three opaque bodies (arrows) are shown. $KmnO_4$ fixation. × 55,000.

FIG. 4. An isolated dendritic process of a clear cell (*DP*) is shown in cross-section. The cytoplasmic characteristics are virtually identical to those of the clear cell illustrated in Fig. 7. In neighboring cells the converging tonofilaments and the associated areas of increased cell membrane density (desmosomes) are clearly shown (arrows). The differences in the cytoplasm of the basal cells (*B*) and the cell of the stratum spinosum (*S*) are brought out by permanganate fixation. A part of the dermo-epidermal junction (*DE*) is indicated. $KmnO_4$ fixation. × 14,000

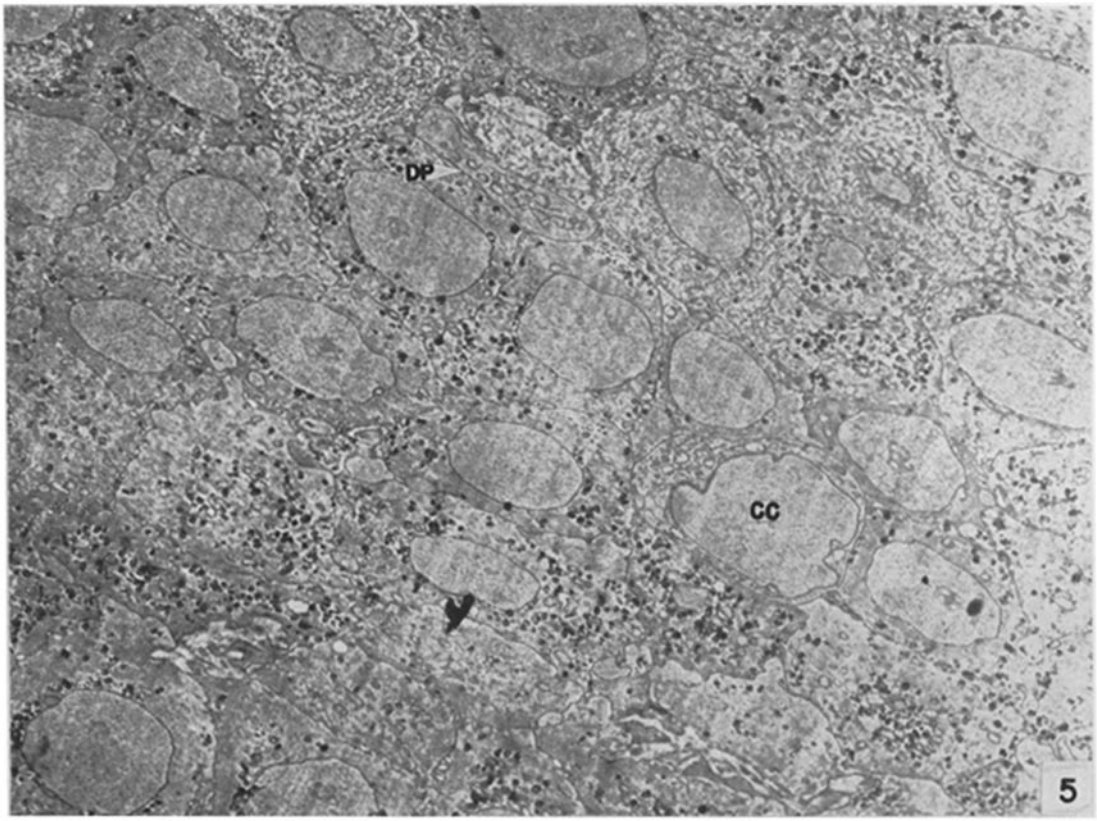


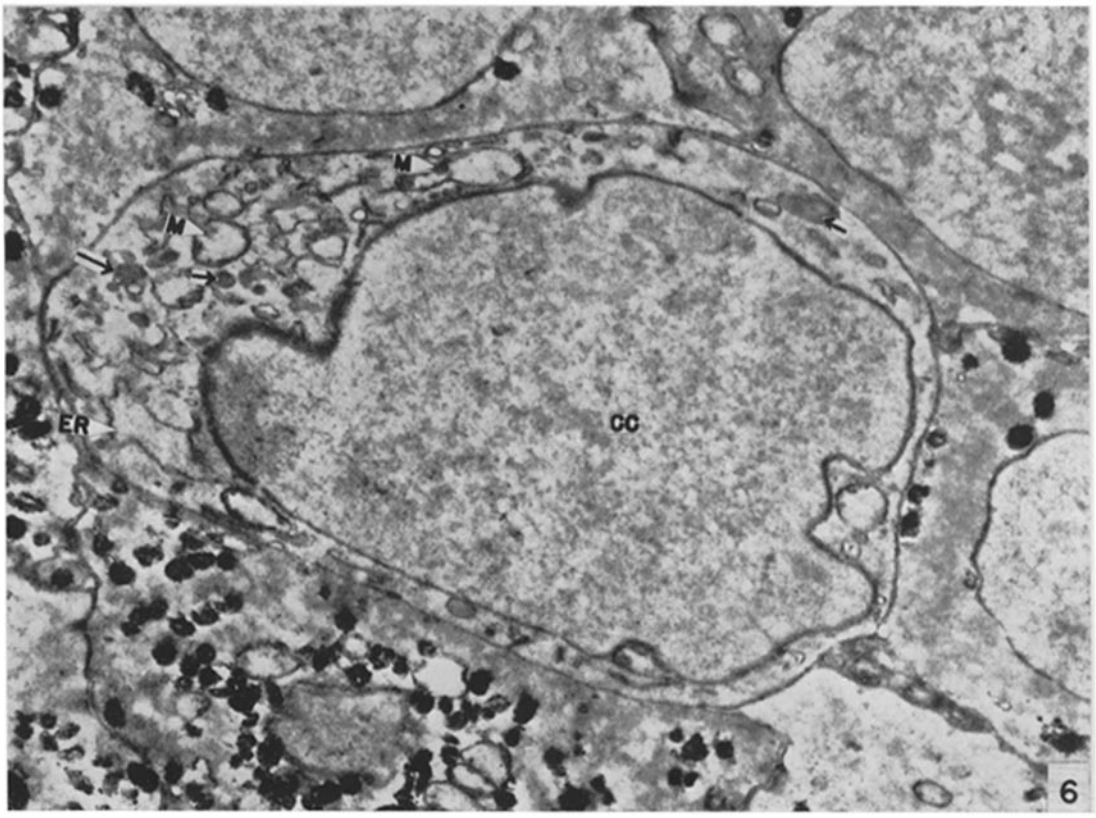
(Clark and Hibbs: Human epidermis: melanocyte)

PLATE 338

FIG. 5. An oblique section through the epidermis. A clear cell (*CC*) and an isolated dendritic process of a clear cell (*DP*) are indicated. The characteristic appearance of the clear cell and a clear cell dendritic process is apparent at low magnification. $KmnO_4$ fixation. × 3,000.

FIG. 6. The cytoplasm of the clear cell shown in Fig. 5 at higher magnification. The comparatively large and relatively numerous mitochondria (*M*), the endoplasmic reticulum (*ER*), and opaque bodies (arrows) are shown. Compare with the cytoplasm of adjacent basal cell. $KmnO_4$ fixation. × 15,000.

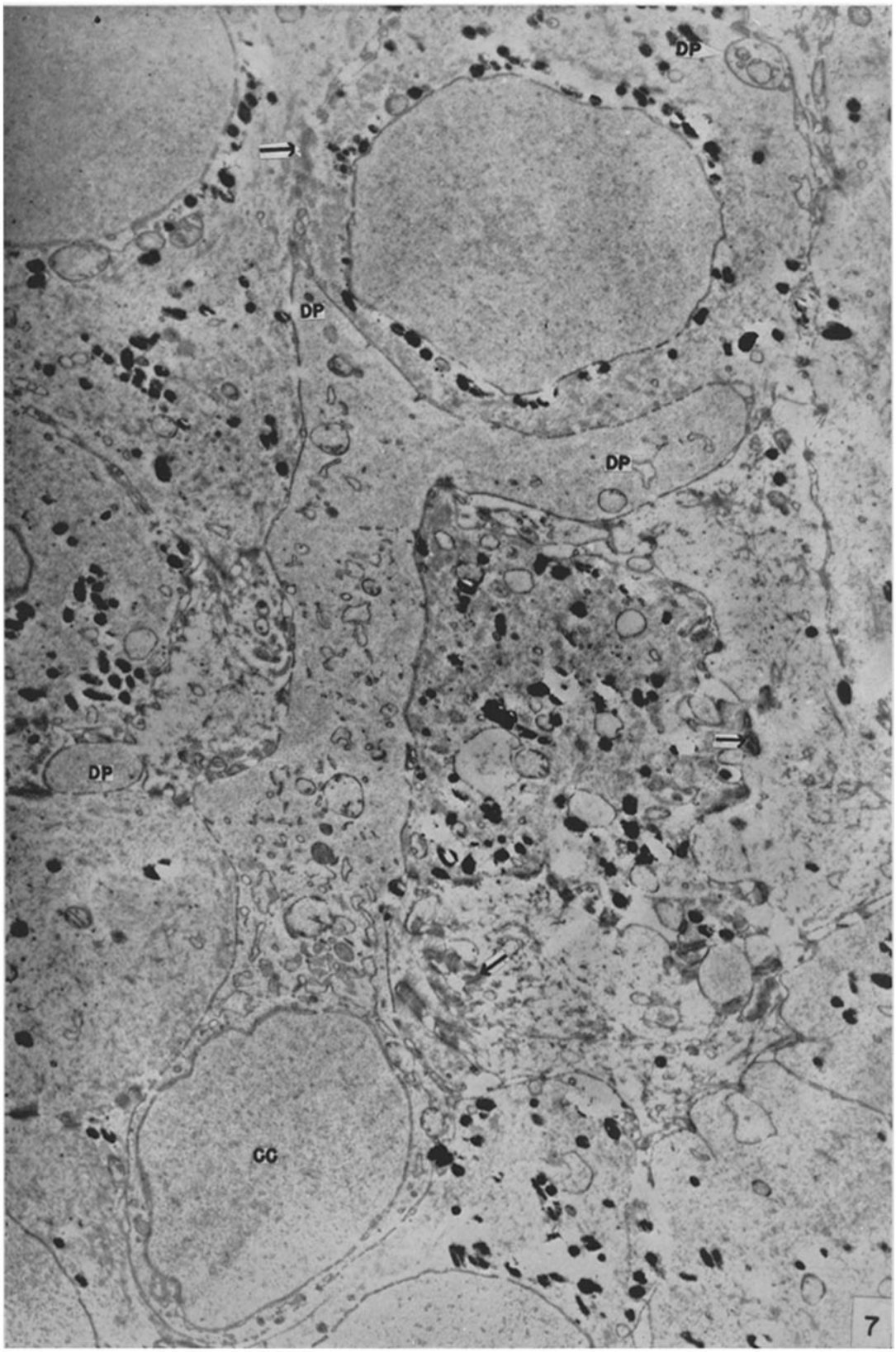




(Clark and Hibbs: Human epidermis: melanocyte)

PLATE 339

FIG. 7. A clear cell (*CC*) with its nucleus situated between the upper part of two basal cells is seen at the lower left. The branching processes (*DP*) extend upward between the epidermal cells. No "intercellular bridges" such as those between other epidermal cells (arrows) are present on the clear cell membrane. $KmnO_4$ fixation. × 12,000.



(Clark and Hibbs: Human epidermis: melanocyte)