Electron Microscope Studies of the Human Epidermis

The Cell Boundaries and Topography of the Stratum Malpighii*

By RICHARD G. HIBBS, Ph.D., and WALLACE H. CLARK, Jr.,‡ M.D.

With the Technical Assistance of MILDRED WATSON

(*From the Departments of Anatomy and Pathology, Tulane University School of Medicine, New Orleans*)

ABSTRACT

The thin skin of the left upper quadrant of the human abdomen has been studied by electron microscopy. Tissue removed with a high speed rotary punch was fixed in osmium tetroxide or potassium permanganate. The latter fixative in our preparations is superior to osmium for the demonstration of epidermal cell membranes and certain other membranous structures of the epidermis. The cytoplasmic membranes of basal cells and cells of the stratum granulosum have been found to be relatively straight, while those of most spinous cells are sharply scalloped. The deep cells of the stratum spinosum in the rete ridge area show cell membranes and cytoplasmic structure intermediate between true basal cells and most cells of the stratum spinosum. The extracellular material of the desmosome has been found to consist of alternate dark and light laminae similar to those described by Odland (13) and Horstmann and Knoop (7).

Many aspects of the structure of the epidermis have been clarified by recent electron microscope studies. A basement membrane has been clearly shown by Weiss and Ferris (21) and Selby (17). The coarse tonofibrils seen in light microscopic preparation have been shown to be composed of bundles of submicroscopic filaments (tonofilaments) by Porter (15, 16), Selby (17, 18), Charles and Smiddy (3), and Menefee (11). A dendritic cell, quite different structurally from the ordinary epidermal cell, has been described by Odland (13) and Clark and Hibbs (4). The structural characteristics of this cell indicate that it is the epidermal melanocyte and it has been shown to be "dopa-positive" in electron micrographs (5). Such cells were first demonstrated in electron micrographs by Birbeck, Mercer, and Barnicot (2) and Barnicot, Birbeck, and Cuckow (1) in studies of the hair bulb.

Electron micrographs have been quite valuable

in clarifying the structure of the "intercellular bridge." Porter (16) and Selby (17) have shown that cytoplasmic filaments and fibrils do not cross the cell membrane, but end in localized thickenings of the cell membrane. Horstmann and Knoop (7) and Odland (12, 13) have shown an intercellular striated or laminated material between these thickenings. Horstmann and Knoop (7), Odland (13), and Selby (17) have reviewed previous electron microscope studies and have carefully correlated these studies with the generally accepted views of epidermal structure as observed with the light microscope.

The present communication reports our observations made on human epidermis fixed in Luft's potassium permanganate (9) and Palade's osmium tetroxide (14). Potassium permanganate, in our hands, is usually superior to osmium tetroxide for the preservation of epidermal cell membranes. We have observed also a characteristic lamination of the intercellular portion of the desmosome, and our findings are compared with those of Horstmann and Knoop (7) and Odland (13). The

71

cell membranes of the epidermal cells are described and characteristic features are emphasized.

Materials and Methods

Tissue was selected from a series of forty-two biopsies of skin from the left upper quadrant of the abdomen. The biopsies were taken, without anesthesia, with a high speed rotary punch. The subjects, Negroes and Caucasians of both sexes, varied in age from 5 to 74 years. Fixation was by immersion in either Palade's buffered osmium tetroxide or Luft's buffered potassium permanganate. The electron micrographs were taken with an RCA-EMU3C microscope. The methods are described in greater detail in our previous publication (4).

OBSERVATIONS

Cellular Topography of the Epidermis:

In low power electron micrographs of epidermis, structures appear much the same as in light microscopic preparations. The various layers are easily discernible (Figs. 1, 2). In the rete ridges the basal layer is composed of cuboidal cells (Fig. 3), the cells becoming taller as the regions overlying the apices of the dermal papillae are approached (Fig. 2). In osmium-fixed tissue structural differences between cells of the basal layer and those of the stratum spinosum are not striking (Fig. 1). In permanganate-fixed tissue, the basal cells stand out in sharp contrast to the overlying cells (Fig. 2). Melanocytes, presenting a clear cytoplasm devoid of tonofilaments, are frequently encountered. Cells of this type are usually found between ordinary basal cells of the rete ridges, often with a part of their cytoplasm protruding into the corium but separated from the corium by the basement membrane. They are present in the stratum spinosum also, but in smaller numbers.

Tonofilaments are evident in the cells of all layers except the stratum corneum. In the basal layer, the tonofilaments are evenly distributed after permanganate fixation, giving the cytoplasm a "ground glass" appearance (Figs. 2, 3).

In the stratum spinosum, tonofilaments are present in large numbers, but are gathered in coarse bundles, which apparently pass, sometimes partially encircling the nucleus, from one side of the cell to another. In the cells of the deeper part of the rete ridge the tonofibrillar pattern resembles that of the ordinary basal cells more closely than that of other cells of the stratum spinosum.

In the cells of the stratum granulosum, tono-filaments are less numerous, but large masses of electron opaque substance, comparable in size, shape, and distribution to the coarse bundles of filaments in the spinous cells are present (Fig. 4). These masses are especially prominent in the region of the desmosomes and around the nuclei.

After osmium fixation tonofilaments appear much the same as after permanganate fixation, except in the basal cells where they are seen gathered in bundles almost as coarse as those of the spinous layer (Fig. 1).

Cell Boundaries:

Adjacent cytoplasmic membranes of any two basal cells may be traced from the dermo-epidermal junction to the first layer of the stratum spinosum as two roughly parallel, almost straight electron opaque lines. At intervals in the membranes are apposed, localized areas of increased density into which small bundles of tonofilaments insert (Figs. 6, 7). This complex structure, the desmosome, has been generally accepted as the electron microscopic counterpart of the intercellular bridge as observed with the light microscope. Fawcett and Selby (6) have noted similar structures at interfaces between cells of other tissues. Vogel (20) has described them in a variety of epithelial cells. The desmosomes between adjacent basal cells and those between the spinous cells that lie deep in the rete ridge are relatively small and less numerous than those in the remainder of the stratum spinosum. The desmosomal membranes (α-layer of Horstmann; attachment plaque of Odland) of these regions usually lie parallel to the nuclear membrane. The tufts of tonofilaments inserting into the membranes are short and relatively inconspicuous. Occasionally groups of small circular profiles (possibly non-myelinated nerve fibers) are present intercellularly between desmosomes (Fig. 6). Even in the best light microscopic preparations of normal epidermis, the desmosomes between basal cells are difficult to demonstrate.

At interfaces between basal and spinous cells over the dermal papillae, the cell membranes have a scalloped appearance in contrast to the relatively straight appearance of the cell membranes at the interface between two basal cells. Desmosomes are randomly distributed along the scalloped membranes so that many of them are not parallel to the nuclear membranes as are those between basal cells, but lie at various angles to it. The desmosomal areas of the cell membranes (at-
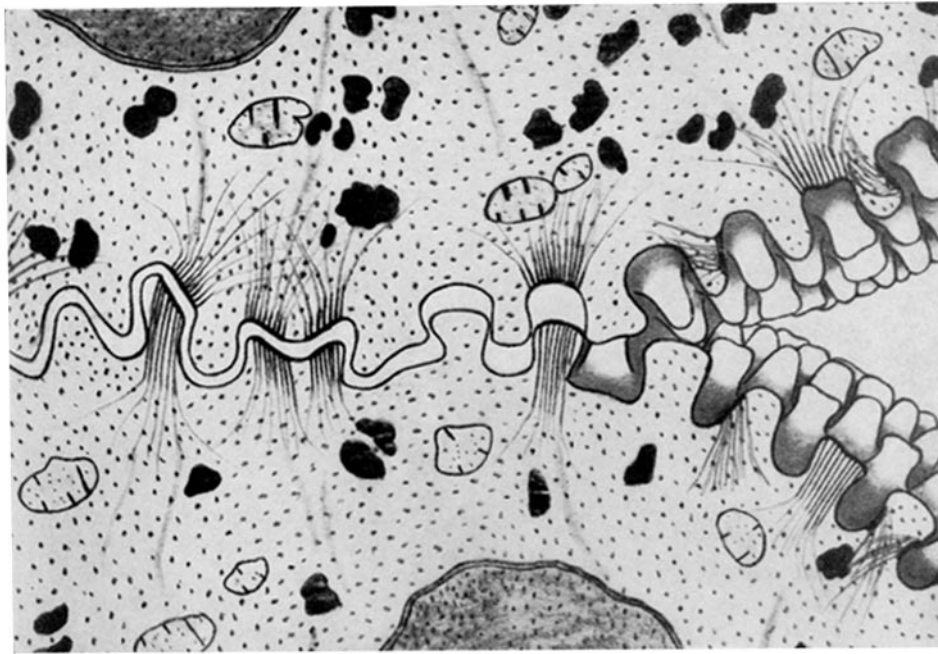
Text-Fig. 1. A diagrammatic drawing of the interface between two spinous cells. The two cells are pulled apart on the right to show the three-dimensional relationships of the interdigitating processes on the cell surfaces.

tachment plaques) are relatively straight, as though they were stiffened at these points, with most of the curving of the membranes occurring between desmosomes (Fig. 5). This irregular curving of the cell membranes and angulation of the desmosomes is even more pronounced where spinous cells (except those deep in the rete ridges) are in contact with each other (Figs. 8, 9).

This same membrane pattern is evident at junctions between spinous cells in sections cut parallel to the skin surface. This suggests that the interface is made up of blunt interdigitating processes protruding from the surfaces of both cells, with the desmosomes located at intervals on these processes, as illustrated in Text-fig. 1. This concept is verified by observations made on sections cut tangentially to the cell surfaces, in which these processes are seen in cross-section.

The cell boundaries in the stratum spinosum of the deeper half of the rete ridges deserve special mention. Those which lie perpendicular to the skin surface closely resemble the boundaries between basal cells (Fig. 3). There is little curving or interdigitation of membranes, and the desmosomes almost always lie on a plane parallel to

the nuclear membrane. The cell boundaries parallel to the skin surface do show some curving and interdigitation, which becomes more pronounced near the surface of the skin. The bundles of tono-filaments in these cells are much less prominent than in the rest of the stratum spinosum.

Contrary to the common belief that large inter-cellular spaces are present between desmosomes, we have found only a small amount of true inter-cellular space. Where such spaces are present, they often contain a finely granular material which is relatively electron opaque after permanga-nate fixation (Fig. 8). The nature and importance of this material is as yet unknown.

In the stratum granulosum and stratum corneum the cell membranes have lost their scalloped appearance and appear straight (Figs. 4, 4 a). This is particularly evident in the superficial layers of the stratum granulosum. In the stratum corneum one may see adjacent scales adherent at the region of the desmosomes, but slightly sepa-rated between (Fig. 14). This may produce a scalloped effect, but not the complex interdigi-tation observed in the spinous layers.
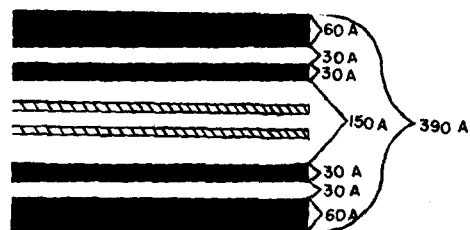
TEXT-FIG. 2. A diagrammatic representation of a section through a desmosome. The outer dark 60 A plates represent the desmosomal cell membranes, while the alternate dark and light lines between represent laminae of extracellular desmosomal material. The two cross-hatched lines within the central 150 A area represent two very thin laminae which have been seen in our own preparations inconstantly.

The Fine Structure of the Desmosome:

The desmosome consists of two apposed plates which are specialized areas of the cytoplasmic membranes of the cells involved, separated by a series of alternate light and dark laminae. The specialized areas of the cell membrane have been called attachment plaques by Odland (13) and are the α-layer of Horstmann (7). In our preparations the attachment plaque averages 60 A in thickness. We have been able to demonstrate in all layers of the epidermis alternate light and dark laminae as shown in Text-fig. 2 (Figs. 11 to 14). Except for the central area (cross-hatched lines and intervening clear area of Text-fig. 2) these laminae correspond to those described by Odland. Horstmann and Knoop (7) did not describe material equivalent to Odland's intercellular contact layer, but this difference is quite likely due to species differences and Horstmann believes that desmosomes are formed differently in different places. The one illustration of Horstmann and Knoop that shows doubling of each dark layer of the desmosome could be a focusing error (8).

In our preparations the continuity of cell membranes through a series of desmosomes has been more consistent and easier to demonstrate after permanganate fixation than after fixation with osmium tetroxide (Figs. 9, 10) and the desmosomal lamination is more distinct. Odland (13), however, has shown good cell membrane continuity with osmium tetroxide. We have not seen the lamination of the attachment plaque or

the tonofilament striation described by Porter (16) and Odland (13).

DISCUSSION

The "intercellular bridge" observed with the light microscope consists not only of the desmosome, but also the apposing tufts of tonofilaments that insert into the desmosomal membrane (13). In electron micrographs the converging bundles of tonofilaments are seen to be entirely intracellular, while the "intercellular bridge" in most light microscopic preparations certainly appears to be intercellular. We attribute this apparent discrepancy to the action of the fixatives ordinarily used in tissue preparation for light microscopic studies, or to intercellular edema of the epidermis (spongiosis). Formalin fixation and paraffin embedding apparently result in cytoplasmic alteration so that the cell membranes are separated between desmosomes. Consequently, the cell membranes are pulled back to a point near the site of tonofibrillar convergence, leaving the convergent filaments and desmosomal membranes as a "spine" at the cell surface. The resolution of the light microscope does not permit one to determine that this spine of tonofilaments is entirely intracellular. When intercellular edema is present, "bridges" are especially prominent. Presumably the edema plus the fixation effect just described pushes the cell membranes even farther apart between desmosomes.

Adjacent membranes of basal cells, even when passing through a desmosome, tend to form straight lines. At the junction between basal and spinous cells, and between spinous cells, the cell membranes are sharply scalloped; but this pattern is again altered in the stratum granulosum and stratum corneum where the membranes, as between basal cells, are flat. This changing form of the cell boundaries in the various layers appears to be related to the structural arrangement of tonofilaments within the cells.

The cytoplasm of the basal cells following permanganate fixation contains numerous fine filaments in contrast to the coarse bundles seen in the cells of the stratum spinosum. Furthermore, the tonofibrillar bundles associated with the desmosomes of the basal cells and the cells deep in the rete ridge are much less prominent than those of an ordinary spinous cell. This difference is clearly shown at the junction

between a basal cell and a spinous cell in the region over a dermal papilla where the basal half of the desmosome has a short bundle of tonofilaments and the spinous half a long bundle (Figs. 5, 15). It appears that the delicate tonofilaments of the basal cells allow a relatively greater structural plasticity. The small tonofibrillar tufts apparently fail to anchor the desmosomes so that the desmosomal membranes respond to mechanical forces along with the rest of the cell membrane. This would leave the cell surfaces between basal cells relatively uniform. Furthermore, these cells are not firmly bound to each other since there are no interlocking processes and fewer desmosomes. There is also some evidence that those desmosomes present are not as strong as those of other regions, as we have seen occasional desmosomes pulled apart and sometimes the intercellular component of a desmosome is seen to be broken up or out of position (Fig. 11). Both conditions are probably artifacts, but even so they suggest an inherent weakness since this condition is rarely seen in the stratum spinosum.

Weakness of desmosomes also apparently exists in the upper layers of the stratum granulosum and in the stratum corneum. This weakness is revealed in the stratum granulosum by frequent separation of desmosomes, while in the outer stratum corneum only an occasional desmosome is intact.

It is hard to conceive that firmly anchored spinous cells can slide over each other or separate during or following mitosis. Thuringer (19) has shown that mitotic activity occurs both in the basal layer and stratum spinosum. The cells in the stratum spinosum that divide are probably those we have observed in the rete ridge area that more closely resemble basal cells than they do the usual cells of the stratum spinosum. It is therefore possible that the cells with the "ground glass" cytoplasm and delicate desmosomes, whether in the basal layer or stratum spinosum, are those capable of mitosis.

The desmosome is reactive with the periodic acid-Schiff technique and this reaction persists after hydrolysis with saliva and diastase. The desmosome is also faintly sudanophilic (10). These observations suggest that some part of the complex structure of the desmosome is a polysaccharide, possibly associated with a lipide or lipo-protein.

BIBLIOGRAPHY

1. Barnicot, N. A., Birbeck, M. S. C. and Cuckow, F. W., The electron microscopy of human hair pigments, *Ann. Human Genetics*, 1955, **19**, 231.
2. Birbeck, M. S. C., Mercer, E. H. and Barnicot, N. A., The structure and formation of pigment granules in human hair, *Exp. Cell Research*, 1956, **10**, 505.
3. Charles, A., and Smiddy, J. G., The tonofibrils of the human epidermis, *J. Inv. Dermat.*, 1957, **29**, 327.
4. Clark, Wallace H., Jr., and Hibbs, Richard G., Electron microscope studies of the human epidermis. The clear cell of Masson (dendritic cell or melanocyte), *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 679.
5. Clark, Wallace H., Jr., Watson, B. E. M., and Watson, M. C., Electron microscope observations of the epidermal melanocyte following a modified "DOPA" reaction, *Am. J. Path. Abstr.*, 1959, **35**, 686.
6. Fawcett, Don W., and Selby, Cecily C., Observations on the fine structure of the turtle atrium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 63.
7. Horstmann, E., and Knoop, A., Electronenmicroskopische Studien an der Epidermis. I. Rattenpfote, *Z. Zellforsch.*, 1958, **47**, 348.
8. Horstmann, E., personal communication, November 17, 1958.
9. Luft, John H., Permanganate—a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
10. Montagna, W., The Structure and Function of the Skin, New York, Academic Press, Inc., 1956, 55 and 208.
11. Menefee, M. G., Some fine structure changes occurring in the epidermis of embryo mice during differentiation, *J. Ultrastruct. Research*, 1957, **1**, 49.
12. Odland, George F., The fine structure of the attachment between cells of the human epidermis, *Anat. Rec.*, 1958, **130**, 349.
13. Odland, George F., The fine structure of the interrelationship of cells in the human epidermis, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 529.
14. Palade, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.
15. Porter, K. R., Observations on the sub-microscopic structure of animal epidermis, *Anat. Rec.*, 1954, **118**, 453.
16. Porter, Keith R., Observations on the fine structure of animal epidermis, *Proc. Internat. Conf. Electron Micr.*, London, 1954, 539–547.
17. Selby, Cecily C., An electron microscope study of the epidermis of mammalian skin in thin sections.

I. Dermoepidermal junction and basal layer, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 429.

18. Selby, Cecily C., An electron microscope study of thin sections of human skin. II. Superficial cell layers of footpad epidermis, *J. Inv. Dermat.*, 1957, **29**, 131.

19. Thuringer, Joseph M., Studies on cell division in the human epidermis. II. A. Rate of cell division in the prepuce. B. Influence of various factors on cell division, *Anat. Rec.*, 1928, **40**, 1.

20. Vogel, A., Zelloberflache und Zellverbindungen im elektronenmikroskopischen Bild, *Verhandl. deutsch. Ges. Path.*, 1958, **41**, 285.

21. Weiss, P., and Ferris, W., Electron micrograms ot larval amphibian epidermis, *Exp. Cell Research*, 1954, **6**, 546.

EXPLANATION OF PLATES

PLATE 41

FIG. 1. Electron micrograph of osmium-fixed skin. Note the discontinuity of the cell membranes characteristic of osmium fixation. The dermo-epidermal junction is indicated by arrows.

BC, Basal cell. × 4,000.

FIG. 2. Electron micrograph of permanganate-fixed skin over dermal papilla. Note continuity of cell membranes. Dermo-epidermal junction is indicated by arrows.

BC, Basal cell. *PC*, Process of basal cell. × 4,000.
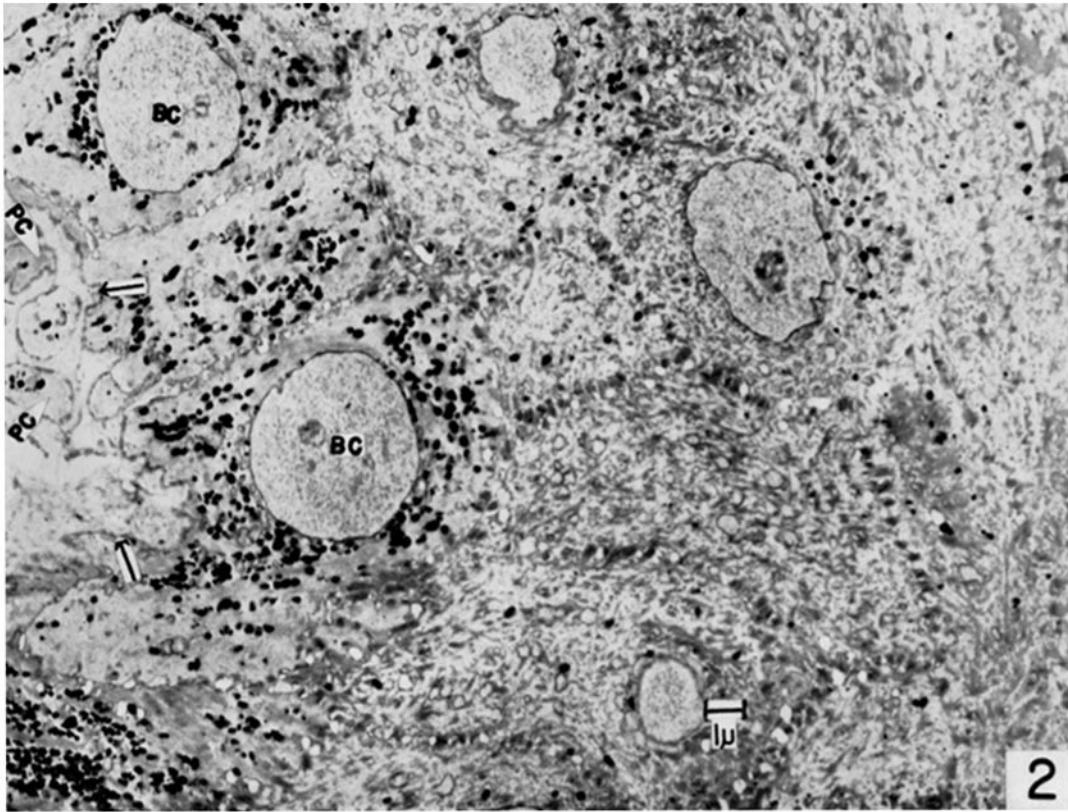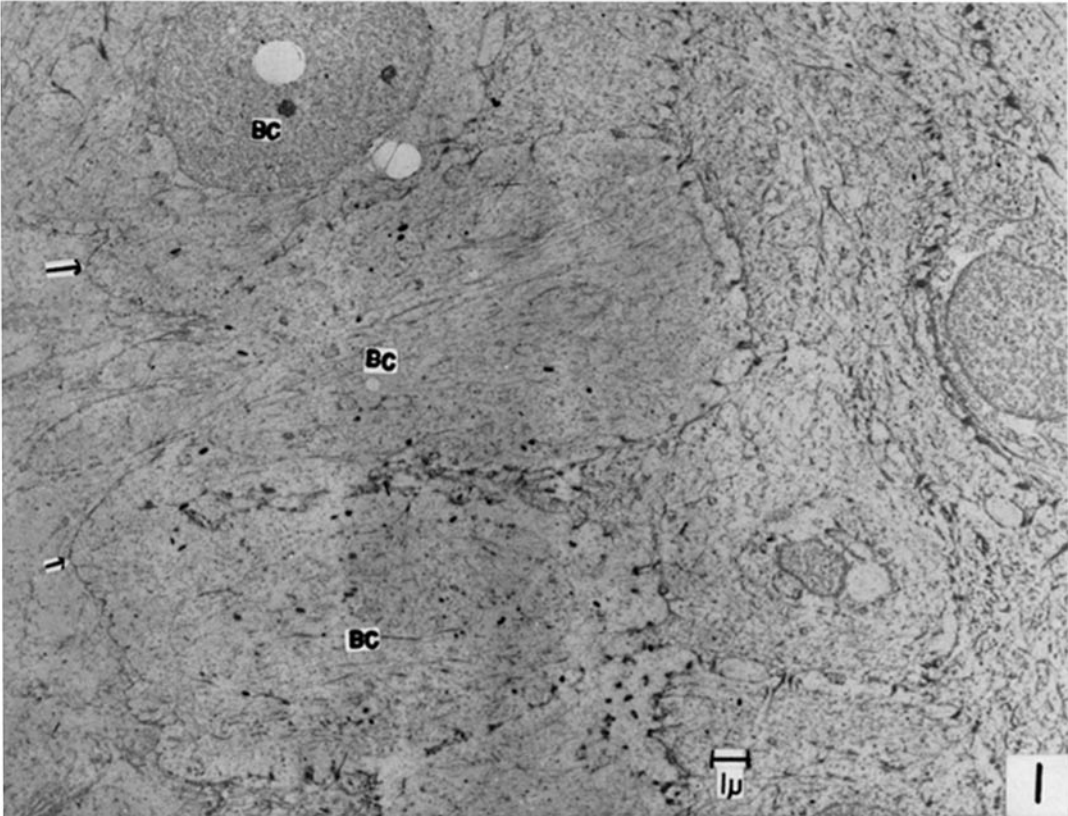
(Hibbs and Clark: Human epidermis)

FIG. 3. Permanganate-fixed skin from the apex of a rete ridge. Note the absence of basal cell processes in the dermis. Arrows indicate the dermo epidermal junction.

M, Melanocyte. × 9,500.

FIG. 4. Electron micrograph of stratum granulosum and stratum corneum. Note the absence of interdigitating processes. Permanganate-fixed.

GC, Cell of stratum granulosum. *CO*, Stratum corneum. *SC*, Spinous cell. × 9,500.

FIG. 4 *a*. Higher magnification of area outlined. Permanganate-fixed.

DE, Desmosome. × 18,000.

(Hibbs and Clark: Human epidermis)
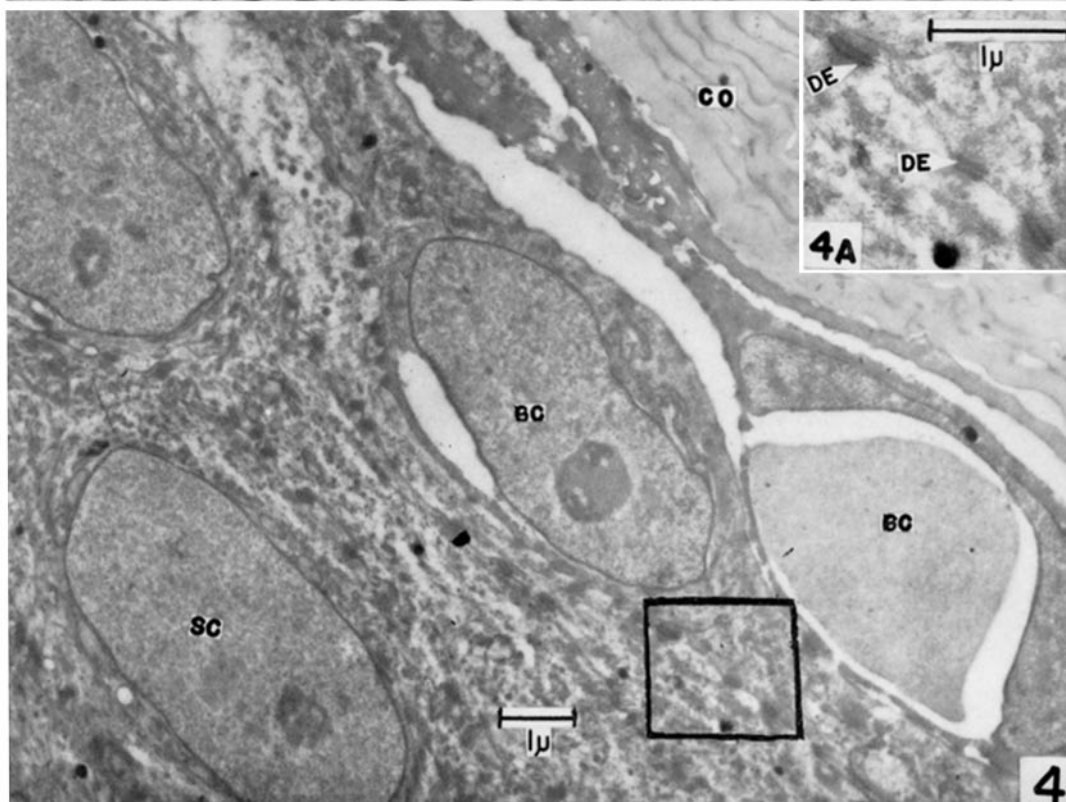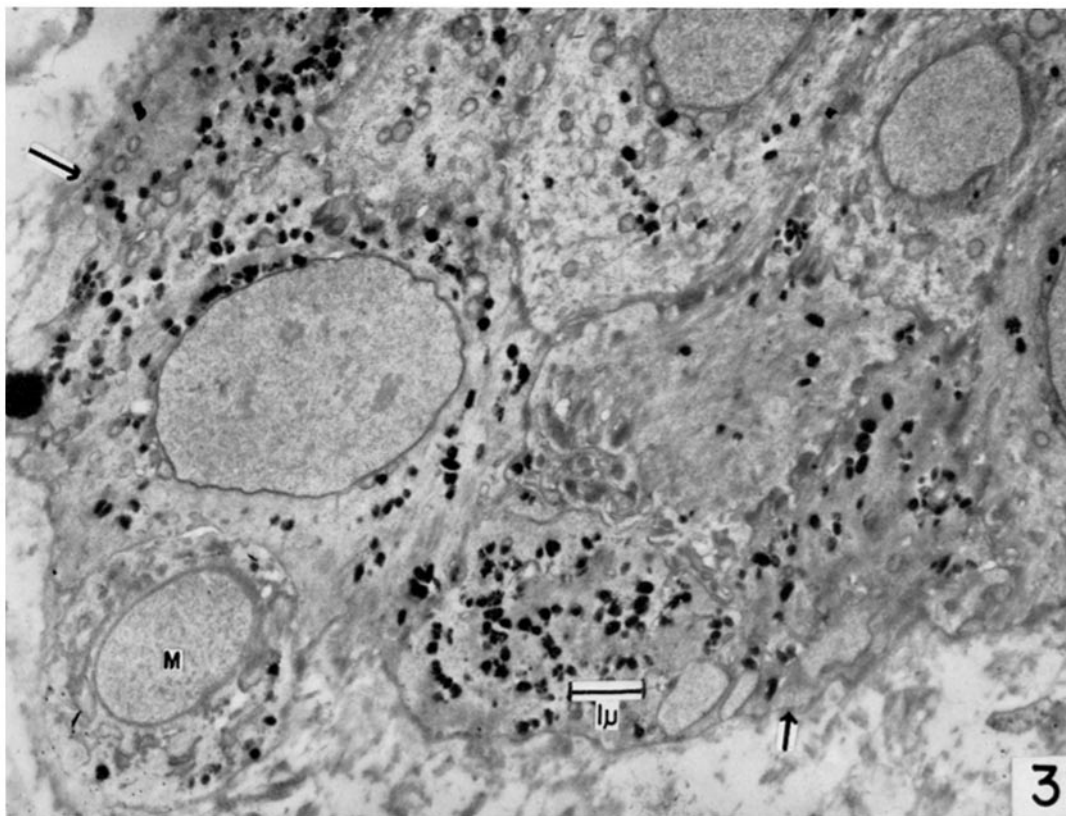
PLATE 43

FIG. 5. Cell boundaries between a basal cell and a spinous cell. Note the greater length of the tufts of tonofilaments (arrows) on spinous cell components of desmosomes. Permanganate-fixed. × 50,000.

FIG. 6. Junction between two basal cells. The straight cell membranes are demonstrated. Groups of small circular profiles (arrows) can be seen in the intercellular space between desmosomes. Permanganate-fixed.

DE, Desmosome. × 22,000.

FIG. 7. Slightly higher magnification of junction between two basal cells. The cell membranes are closely approximated. No vesicles or intercellular space is present here. Permanganate-fixed.
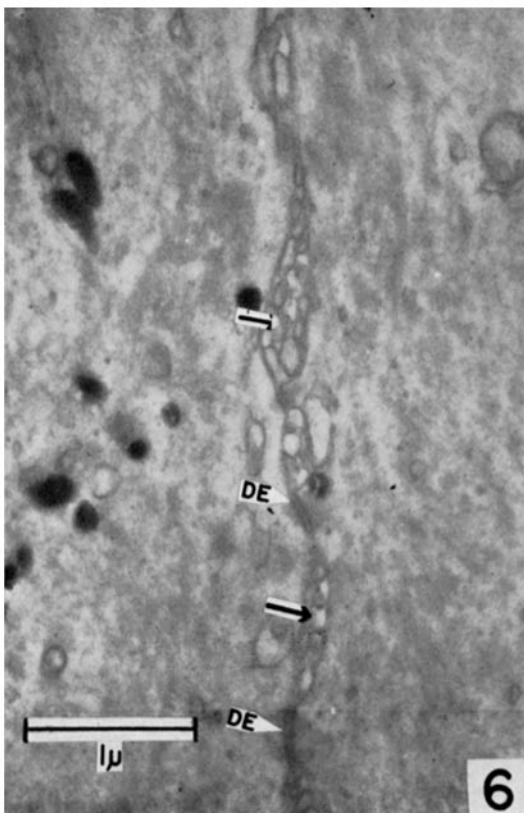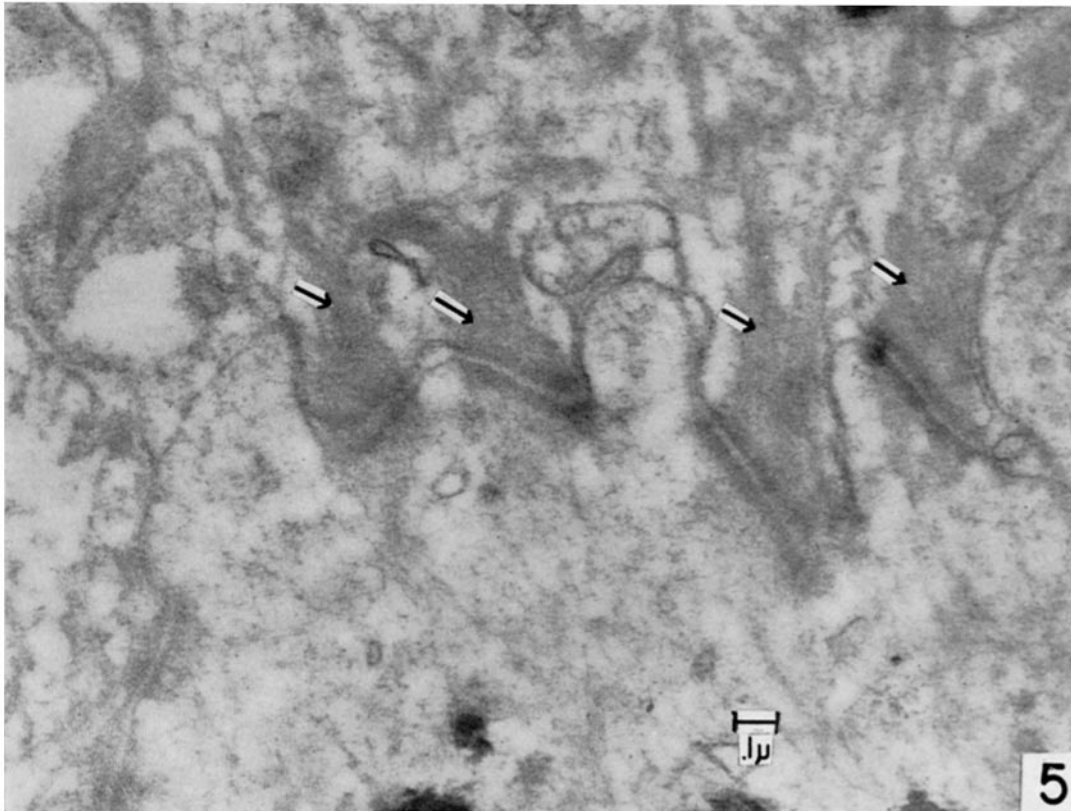
DE, Desmosome. × 32,000.
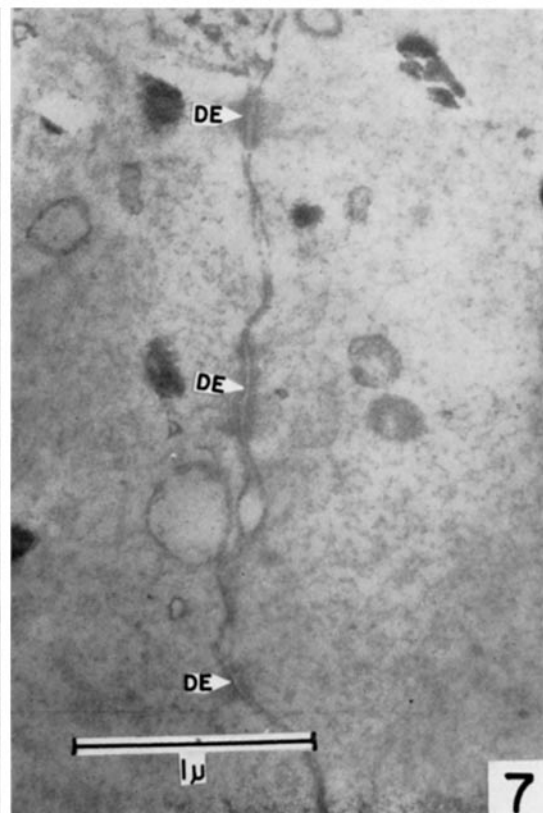
PLATE 44

FIG. 8. Small area of stratum spinosum showing the interdigitation of cell processes. Masses of dense granular substance (arrows) can occasionally be seen in the intercellular spaces. Permanganate-fixed. × 30,000

FIG. 9. The interface between two spinous cells showing details of cell boundaries. Permanganate-fixed.
DE, Desmosome. *TF*, Tufts of tonofilaments. × 45,000.

FIG. 10. Details of cell boundaries from area comparable to that shown in Fig. 9, but fixed in osmium. Note discontinuity of cell membranes between desmosomes. Compare to Fig. 9.
DE, Desmosome. × 45,000.
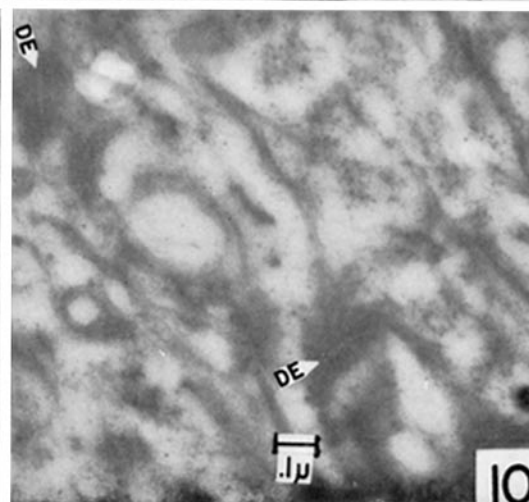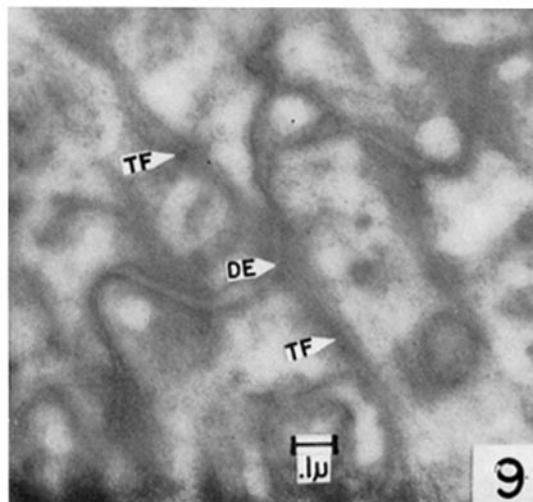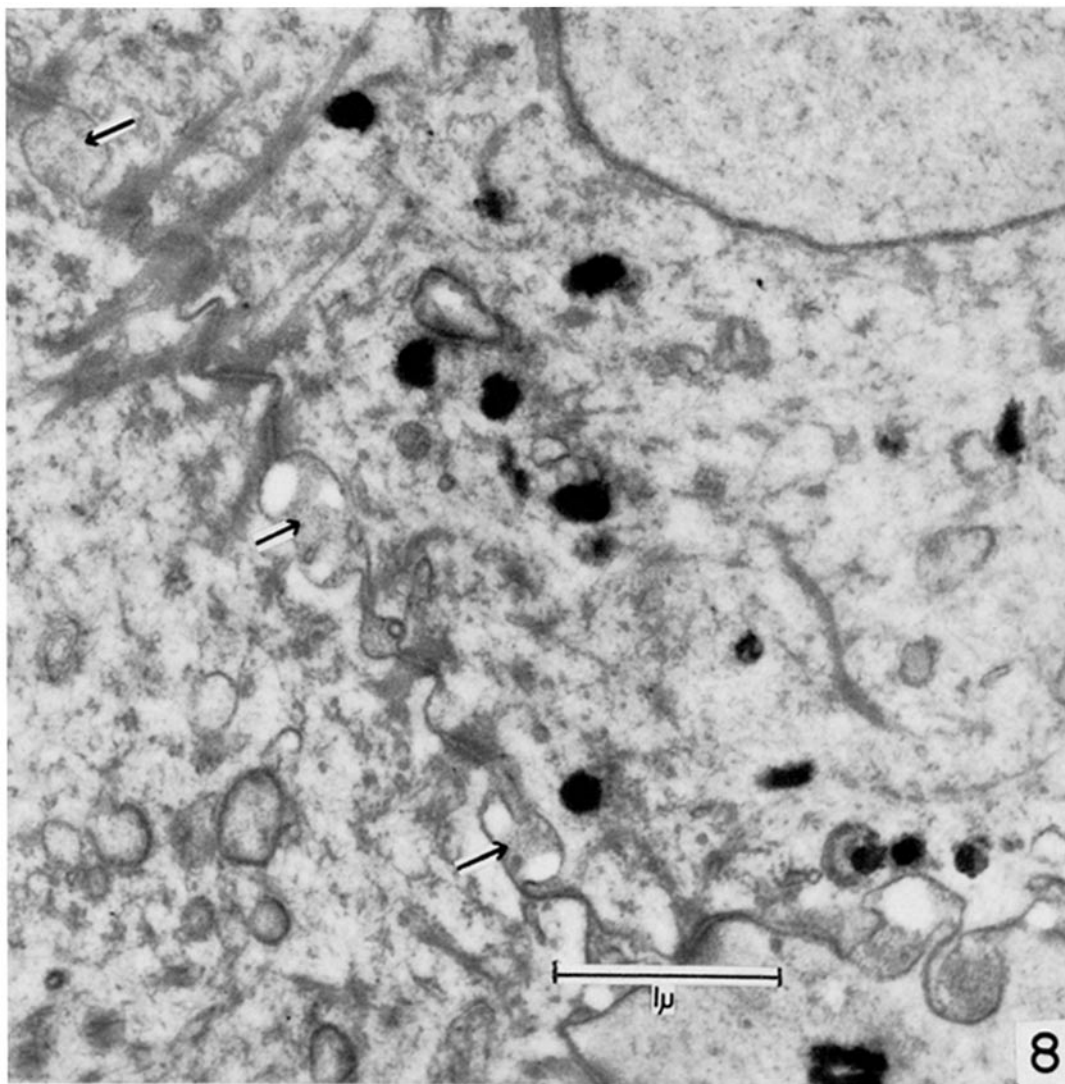
(Hibbs and Clark: Human epidermis)

PLATE 45

FIG. 11. High magnification of desmosomes between two basal cells. The intercellular desmosomal material in the lower part of the micrograph appears to be separated and pushed out of position. Permanganate-fixed.

DM, Desmosomal membrane. *ED*, Extracellular desmosomal material. × 80,000.

FIG. 12. High magnification of desmosome between basal cell and spinous cell. Note lamination. Permanganate-fixed.

ED, Extracellular desmosomal material. *DM*, Desmosomal membrane. × 80,000.

FIG. 13. High magnification of desmosome between cells of the stratum granulosum. Note prominent lamination. The nature of the dark spherical granules is unknown. Permanganate-fixed.

DM, Desmosomal membrane. *ED*, Extracellular desmosomal material. × 150,000.

FIG. 14. Desmosome of stratum corneum. Note the degenerate appearance of the intercellular component of the desmosome. Many small dense particles believed to be melanin can be seen. Permanganate-fixed.

DM, Desmosomal membrane. *ED*, Extracellular desmosomal component. × 80,000.

FIG. 15. The interface between a basal cell and a spinous cell. Note the long tufts of tonofilaments in the spinous cell (arrows) as opposed to the short tufts in basal cell. Permanganate-fixed.

DE, Desmosome. × 35,000.

(Hibbs and Clark: Human epidermis)