

AN ELECTRON MICROSCOPE STUDY OF THE EPIDERMIS OF
MAMMALIAN SKIN IN THIN SECTIONS

I. DERMO-EPIDERMAL JUNCTION AND BASAL CELL LAYER*

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The structure of mammalian epidermis has been of considerable interest to investigators concerned with aspects of its synthesis of keratin, as well as with its neoplastic and other pathological changes. With the development of techniques for the examination of tissue sections with the electron microscope, several electron microscopic observations of skin have been reported (1-8), but no comprehensive studies of mammalian epidermis, employing the techniques currently regarded as optimum, have yet appeared. The present study was designed, therefore, to use recently established methods of thin sectioning and electron microscopy to extend the knowledge of certain features of the cytology of mammalian epidermis to the submicroscopic and macromolecular level.

Submicroscopic filaments in epidermal cells were described in the earliest electron microscopic studies (1-3). All authors related these filaments to the tonofibrils of light microscopic studies. Pease (2) considered both the submicroscopic and microscopic tonofibrils as artefacts of fixation of the cytoplasmic gel, while Adolph *et al.* (1) noted that the diameter of the submicroscopic filaments varied with fixation, being minimal after osmium fixation, and suggested that only the larger fibrils should be considered as artefacts. Pease also described a thin cell membrane separating the basal cell cytoplasm and filaments from the dermis, and cell membranes connecting the centers of intercellular bridges in "prickle" cells. He postulated functional relationships between pigment granules, granules in the knot of Ranvier, and mitochondria because they appeared to have similar densities. The value of the electron

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micrographs upon which these various observations were based was, however, severely limited by the thickness of sections, inadequacy of fixation, and removal of embedding matrix from the sections prior to examination.

More recent studies of skin have employed buffered osmium fixation (10), which preserves living cell structure better than any other known method, and the plastic embedding (9), in general use today, which is so much harder than the skin itself that ultrathin sections of this tissue may be cut as easily as of any other. Ottoson *et al.* (4) prepared frog skin with these improved methods of embedding and fixation and described a submicroscopic membrane 200 to 300 Å thick demarcating the dermis from the epidermis and separated by a small space from the cell membrane of the basal epithelial cells. Weiss and Ferris (5) observed the same membrane in the skin of *Amblystoma* larvae, as well as in frog skin, and described other details of the dermo-epidermal junction. On its epidermal side there is a single layer of dense granules and then a layer of unusual "bobbin-shaped" bodies spanning the cellular ectoplasm. In the endoplasm there is a continuous feltwork of fine filaments which are believed to be closely packed canals terminating at the layer of "bobbins." Porter (6) also studied *Amblystoma* larval skin and reports a similar structure at the dermo-epidermal junction and also at the intercellular bridges. Instead of "bobbins" he describes periodic thickenings of the cell membranes to which cytoplasmic filaments are connected. He makes the important point that the filaments terminate at cell membrane thickenings in the intercellular bridges, and so do not cross through the bridges from one cell to another. In both larval and adult amphibian skin, the collagen fibrils in the dermis, immediately below the epidermis, are arranged in sheets parallel to the epidermal surface, and blend into a layer of amorphous material directly below the submicroscopic membrane and the basal cells (6, 8).

In another electron microscope study (14), designed to examine cytoplasmic changes in normal, hyperplastic, and neoplastic prickle cells, only coarse tonofibrils were observed because of the thickness of sections and removal of the embedding matrix, but the point was made that the quantity of the fibrils was increased in hyperplasia and decreased in neoplasia and that the decreased quantity of tonofibrils was accompanied by a decreased quantity of cytoplasm.

These scattered studies leave unanswered many questions concerning the submicroscopic structure of the various layers of normal mammalian skin (which differs from amphibian skin in several ways, including the synthesis of keratin). The chief questions concern (1) the behavior of connective tissue fibers, cytoplasmic fibers, and cell membranes at the dermo-epidermal junction; (2) confirmation of the presence of a submicroscopic membrane limiting the connective tissue at this junction; (3) the fine structure of cytoplasmic tonofibrils, their behavior at cytoplasmic junctions, and their relation to epidermin and keratin; (4) other cytoplasmic components of epidermal cells; (5) comparison of cytoplasmic filaments, intercellular bridges, nuclei, and other characteristics of cells in the basal, prickle, granular, glassy, and cornified layers of the epidermis; (6) the morphology and localization of pigment.

In our electron microscopic study, the fine structure of human skin has

been the prime concern because of considerable interest in pathological changes in this tissue. Rodent skin has been included since it was anticipated that this skin treated in certain experimental procedures might subsequently be used in the present study. Because of the mass of illustrative material involved, this report is concerned exclusively with the basal cell layer (stratum germinativum) of normal skin and its junction with the dermis while a following report will deal in a similar way with the outer cell layers of the epidermis

Methods and Materials

Specimens of skin from the following sources have been studied: rat footpad, mouse back, human footpad, white human breast and nipple, Negro breast, human leg, and abdominal skin from a 12 week human fetus. Strips of skin (as thin and narrow as is practicable in dissection) are excised from the anesthetized host and immediately immersed in a large drop of osmium tetroxide (buffered at pH 7.4) (10) on a dissecting board. After further subdivision into 1 mm. wide strips the specimens are immersed in at least twenty volumes of the 1 per cent buffered osmium fixative for the 4 hour period advised by Palade (10). Great care is taken not to injure the epidermal surface in any way during dissection. Since a transverse section of the epidermis was desirable to orient the observer with respect to the various layers of the epidermis, the strips of skin were embedded in capsules so that a transverse surface would present itself to the knife. It was found that they could be embedded with their long axis parallel to the long axis of the capsule by keeping the capsules in the horizontal position during polymerization of the plastic provided the capsules were first sealed with duco cement and the embedding material and specimen introduced through a small hole at the side, which was subsequently sealed over.

When it became evident that so much of the structure of skin concerned membranes and filaments, attempts were made to "electron-stain" with phosphotungstic acid (PTA) according to the general procedure employed in studies of sectioned muscle (15). Following the 4 hour fixation in buffered osmium tetroxide, the pieces to be electron-stained were transferred to 1 per cent phosphotungstic acid in phthalate buffer at pH 5.4 overnight. In this way the density of the finest filaments was enhanced and the contrast of the resulting electron micrographs increased. After staining, the specimens were dehydrated in the usual way but with phosphotungstic acid solution added to the alcohols, since in Hanson and Huxley's experience¹ considerable stain washed out in the alcohols unless it was replaced there. Control specimens left in distilled water overnight after osmium fixation were also prepared and studied.

The tissue blocks were sectioned in a mechanically advancing microtome built in our Institute (16) which permits sectioning for light and electron microscopy with the same setting of the block and knife. One micron thick sections were prepared for phase or ordinary light microscopy by removing the embedding matrix with xylol, mounting in diaphane on glass slides and covering with a No. 1 coverslip (17). In the study of skin, in which the positioning of cells in the various layers is so important, it was very useful to determine the orientation of ultra-thin sections by light microscopic examination of serial thick sections. The sections for electron microscopy were picked up on collodion-coated copper grids and, without removal of the embedding matrix, examined in the electron microscope (RCA model EMU-2 b) equipped with intermediate lens and 20 to 25 μ objective aperture.

¹ Hanson, J., private communication.

OBSERVATIONS

Embryonic Human Skin.—The two-cell layer of primitive epidermis and scattered mesenchymal cells in the dermis, as found in fetal human skin, are illustrated in the light micrograph of Fig. 5. Cells of the basal layers of cells or stratum germinativum are those which divide and subsequently move outwards to form the second layer or periderm. At this stage in fetal growth extensive keratinization is not yet evident and the cells have not yet differentiated into the mature epidermal cell layers.

According to electron micrographs (see Figs. 6 to 9), the junction between epidermis and dermis is formed simply by the basal cell membrane on one side and another membrane on the dermal side which is separated from the cell membrane by an approximately 300 Å space. Two membranes can be seen separating the two adjacent basal cells in Fig. 8. These part from each other to follow each cell along the dermo-epidermal junction. The membrane on the dermal side is continuous across the space between the two adjacent cells and extends without interruption along the total length of the epidermis. It has not been observed with any particular connection to mesenchymal cells. The low power electron micrographs show that there is a dense accumulation of filaments and perhaps amorphous substance that forms a band extending from this interface for about $\frac{1}{2}$ micron into the dermis. These filaments are about 150 to 250 Å in diameter but have not been examined at higher resolutions or after PTA staining so that their fine structure, exact diameter, and presence or absence of striation have not been determined.

The epidermal cell cytoplasm contains mitochondria, submicroscopic particles, and very occasional filaments and vesicles. The mitochondria show evidence of internal membranes or septa. Scattered vesicles which may be sections of the endoplasmic reticulum (18) are occasionally evident. The submicroscopic particles are shown in Fig. 9 and have the same density and dimensions (80 to 180 Å) as those described by Palade (19) in the cytoplasm of many cell types. Where the filaments do appear, as in Fig. 6, (f) they have diameters less than those of the particulates (*i.e.* <100 Å), are in the basal portion of the cells, and appear to lead directly to the cell membrane. Adjacent epidermal cells are separated simply by the single cell membranes described above, no special intercellular bridges having been observed in the 12 week old fetal specimen examined.

Adult Rat Skin.—Fig. 10 shows a low power electron micrograph of a thick section of rat skin from which the embedding matrix was removed with xylol. The great density of the connective tissue, covering the lower right hand corner of the micrograph, can be resolved into transverse or longitudinal sections of fibers 400 to 500 Å wide. These are evidently wider and packed more densely than the filaments in the epidermal cells. The great density of portions of the connective tissue in comparison with that of the epidermal

cells and the resultant failure of the methacrylate to penetrate adequately through the dermis or across the dermo-epidermal junction frequently cause tearing and rupture of the sections at the junction. Fig. 11 illustrates a section, much thinner than that of Fig. 10, from which the embedding matrix has not been removed and which is taken of a specimen that had also been stained with PTA. The filamentous structures are clear and a fairly regular distribution of density inside the cells along the dermo-epidermal junction is evident. In the enlarged portion of Fig. 11 which is shown in Fig. 22 these densities appear to be discrete bodies or granules with the cytoplasmic filaments directed towards them. Here the densities have a triangular shape in section. Neither cytoplasmic nor connective tissue filaments appear to cross over the boundary between the cell and connective tissue.

The basal cell cytoplasm contains mitochondria, filaments, and submicroscopic particles as illustrated in Fig. 22. Submicroscopic particles occur primarily in clusters with only occasional association with vesicles, and have diameters larger than those of the filaments. In these rodent specimens no intercellular bridges have been seen and filaments appear directed toward the dermis rather than to intercellular junctions. Single cell membranes have not been delineated, presumably due to confusion with cytoplasmic filaments. These specimens are further illustrated in the light micrographs of Figs. 2 and 3.

Adult Human Skin.—The light micrographs of Figs. 1 and 4 illustrate sections of human breast skin comparable to that in the electron micrograph of Fig. 12 which includes an approximately perpendicular cut across the dermo-epidermal junction. Pigment granules (*P*) are evident in the basal cells with much fewer granules appearing in cells of the next layer. Collagen fibers run approximately parallel to the dermo-epidermal junction. The filaments in basal cells run predominantly from the nucleus to the cell borders and appear to be narrower and less densely packed than collagen fibrils and separated from them by the junction.

Details of the junction are better seen in higher magnification views such as those of Figs. 15 and 17 to 20. A submicroscopic membrane separating the dermis from the epidermal cells is evident in all these sections. It is cut transversely in some sections and tangentially in others and is separated from the epidermal cytoplasm by another much narrower membrane which is only occasionally distinguishable (see arrows in Fig. 15 and (*c*) in Figs. 19 and 20). In the specimens stained with PTA (Figs. 17, 18, and 20) the striation of the collagen fibrils in the dermis is brought out and these are readily distinguished from the cross-section of the membrane separating them from the basal cells. They are also distinguishable from the membrane by the fact that their diameter is smaller. In Fig. 15, the densities spaced along the basal cell membrane are resolved into three groups of two rows of approximately

spherical densities; these densities appear to represent discrete granules. Cytoplasmic filaments are continuous with these granules and may not be seen in the space between the granules and the dermal membrane. These granules may naturally be sectioned in a variety of ways, giving rise to various profiles: circular in Fig. 15 and narrower plates of various lengths in Figs. 17 to 20. In Figs. 18 and 20 circular profiles of less density are found between these granules and the cytoplasm, which are only occasionally seen and always in circular profile, so that they apparently represent spherical granules similar to those described by Weiss and Ferris (5) in *Amblystoma* as present between the ectoplasm and "bobbins." In human skin they occur in a particularly clear zone of the ectoplasm and are not attached to cytoplasmic filaments.

The basal cell cytoplasm is characterized by pigment granules and specialized intercellular bridges, in addition to the mitochondria, filaments, and submicroscopic particulates found in rodent skin (see Fig. 21). Cells were extremely uniform in both shape and cellular contents (after allowing for different angles of sectioning). Sections were conscientiously scanned for any images representing the typical "clear" cell discussed in the pathological literature (13). Occasional basal cells were found possessing a large perinuclear region completely devoid of cytoplasmic filaments, similar to that in the prickle cells of Fig. 12. Mitochondria and pigment granules could be found within this space while the nucleus was sometimes, although not necessarily, denser than other nuclei. Thus, although a different fixation was involved, it was not felt that these cells followed the classical histological description of clear cells, and no other images more closely resembling this description could be found in well fixed specimens.

Pigment granules are illustrated in Figs. 12 to 23 as oblong bodies with irregular outlines. Granule lengths, in section, varied from 0.14 to 0.45 μ , so that the granules must usually be longer than the thickness of section and hence only partially included in each section. Because of random variation in the angle at which each granule is cut, the distribution of sizes of whole granules could not be readily estimated. The smallest dimension of a section through each granule is, however, equal to the smallest dimension of the whole granule. Measurements were carried out on the smallest dimension of the sectioned granules. These are as follows:

White skin: smallest diameter $d_1 = 0.130 \mu$	s.d. = 0.036 μ
Negro skin: smallest diameter $d_2 = 0.185 \mu$	s.d. = 0.047 μ

Employing the t test of Fisher for $P \leq .01$ d_1 , was found to be significantly smaller than d_2 . Although the granule lengths could not be statistically compared, because of random sectioning, they appeared generally greater in Negro than in the white skin.

Even in the thinnest section examined in this study, pigment was too dense

to reveal internal structure. The granules do not possess true crystal faces, as do salt crystals, but have variable angular outline. They are distributed at random throughout the cytoplasm without any noticeable relation to other cell components. Occasionally indentations in the nucleus cause a small island of cytoplasm to be sectioned in the same plane as the nucleus so that pigment granules in this portion of cytoplasm may falsely appear to be within the nucleus. Apart from the occasions when this occurs, no pigment is observed in the nucleus.

DISCUSSION

The specimens upon which these observations were carried out appear to be moderately well fixed (10): nucleoplasm is homogeneous, nuclear and cell membranes are finely delineated without associated precipitate, non-specific cytoplasmic vacuoles are absent, the small particulate component has the same dimensions reported by others (19), cytoplasmic filaments appear singly without clumping, and nucleoli have their usual coiled appearance (22, 23). Although the mitochondria always have a less dense interior which often shows traces of internal membranes, they do not have as clear-cut an internal structure as in other cells studied in this laboratory or described by other authors (20, 21). The reason probably is that fixation adequate for the dermis is not adequate for the epidermis, since characteristic internal and external membranes are seen in the mitochondria of fibroblasts close to the epidermis. The fixative may not penetrate as readily through the cornified and densely fibrous cells of the epidermis as through the connective tissue and/or a pH of 7.4 and osmotic solution used may not be optimal for epidermal cells. The alternative possibility, that mitochondria do not have such an internal structure in epidermal cells is quite unlikely, apart from the consideration that they may well have fewer internal membranes than in cells with greater metabolic activity. However, the membranes and filaments which make up the unique structure of the epidermis and with which this report is primarily concerned have apparently been adequately preserved in the specimens studied. Their dimensions correspond to those of fine membranes and filaments described in other studies of these (4-6) and other cell types (24) and they appear continuous in sections. Membrane and filamentous structures have also retained their relative positions and dimensions in the specimens stained with PTA as well as having their electron-density increased (compare Figs. 18 and 19). In Fig. 19, amorphous material has been removed by the overnight washing but filaments remain continuous with granules at the junction and the dermal membrane still follows the contours of the basal cell.

Dermo-Epidermal Junction.—In the adult human, embryonic human, and rodent skins examined in this study, in adult frog skin (4) and in larval frog and *Amblystoma* skin (5, 6) a submicroscopic membrane is observed limiting

the connective tissue from the epidermal cells. In all cases this membrane is 300 to 400 A thick and is separated from the epidermis by an ~ 300 A space. These studies have demonstrated that this membrane closely follows the contour of basal cells even in dermal papillae and is distinguishable from connective tissue filaments by its diameter, lack of striation, and sheet-like structure. The important point raised by Ottoson *et al.* (4) should be reiterated here, that this is a "submicroscopic" membrane only indirectly visible in the light microscope and should not be considered as equivalent to the "basement membrane" of the histological literature. What the classical histologists mean by a basement membrane is a broad band of connective tissue material adjacent to the basal cell membrane. Electron microscopic demonstration of only a submicroscopic basement membrane is in agreement both with those who have argued that there is no typical basement membrane in skin (see Allen (13) and the reviews of Medawar (12) and Montagna (11)) and with those who argue that there is. The electron microscope shows that, as those denying the basement membrane believed, the connective tissue does not change radically in the immediate neighborhood of the dermo-epidermal junction and that there is no definite band of amorphous and reticular substance associated with it as there is, for instance, in the renal tubules (24), where a true basement membrane occurs. To distinguish this membrane from the thicker, typical basement membranes found in other tissues and from the epidermal cell membrane, it is proposed to call it the *dermal membrane*. The presence of this membrane demonstrates that there is no continuity of basal cell cytoplasm with connective tissue (as advocates of the basement membrane believed), explains the ease with which epidermis can be detached intact from the dermis and how basal epidermal cells may divide without disrupting the whole structure. The fine processes of these cells in thick skin, such as that of the footpad, certainly afford a good deal of intimate interdigitation of cytoplasm with connective tissue without actual continuity of these two (see Figs. 16, 18, 19, and 23).

The connective tissue filaments observed immediately adjacent to the dermo-epidermal junction must correspond to the argyrophilic fibers or the argyrophilic reticulum described by histologists in this region. Histological analysis has made it clear that all connective fibers of the upper reaches of the dermis, and particularly of the dermal papillae, are fewer, more openly packed, and "younger" than the stout densely packed mature fibers of the deeper connective tissue. Bear (25) reviews the available data concerning the histochemical and chemical properties of the finer argyrophilic fibers (commonly called reticulin) and concludes that they are basically collagen but have a particular affinity for polysaccharide, either adsorbed on or occluded within them. Since no particular band of amorphous material has been observed by the electron microscope associated with these filaments or the dermal membrane, the glycoprotein which can be demonstrated by histochemical

techniques (11-13, 26) between the connective tissue and basal cells may well be simply a result of the concentration of reticular fibers in this region. The alternative localization for the polysaccharide would be in the dermal membrane itself or between it and the basal cell membrane (13).

Bear's (25) and Gross's (27) contention that reticulin is basically collagen, is supported by the observation that the narrow filaments observed near the junction have the same periodicity (350 to 500 A) as the 800 A wide filaments of the deeper connective tissue. The widths of the latter filaments correspond to those classed as typical human collagen (27), while their period is considerably smaller than that (640 A) of PTA-stained extracted collagen, but the same as that of comparable filaments in frog skin (6). It is more difficult to blame this discrepancy in period upon damage to the collagen in fragmentation or extraction, since x-ray diffraction studies of unextracted collagen also indicate a 640 A period, than to shrinkage of the filaments during fixation and embedding. The discrepancy is increased by the fact that oblique, rather than exactly longitudinal sectioning of collagen would lead to the appearance of a larger rather than a smaller striation (and hence the variation in the period measured in sections). Fibers in the upper reaches of human dermis range from 300 A in diameter while they were even narrower (150 to 250 A) in the only early example of embryonic skin that was studied. Thus the view that connective tissue fibers "age" by grouping themselves into wider bundles (25, 27) is supported. The narrowness, and collagen-like striation of these fibers are consistent with the view (25, 27) that they are equivalent to an immature collagen, which, incidentally, may be found in other than mammalian skins (6).

In all the epidermal cells examined by ourselves (except the early embryonic human) and by others, electron-dense areas to which cytoplasmic filaments converge, are observed spaced along the cell edge facing the connective tissue. In larval amphibian skin Weiss and Ferris (5, 8) consider that these densities consist of two electron-dense plates connected by a more transparent middle piece and that these are attached to the inner surface of a distinct double contoured cell membrane which itself contains a layer of small granules. In the same skin types Porter (6) describes the layer of densities as thickenings of the cell membrane with striations across the associated bundle of filaments. The rodent tissues were not observed with sufficiently high resolution in this study to determine whether the fine structure in the densities resembles that described above or that observed in the human specimens. In the latter case, the discrete granules resolved within the dense areas appear to be rodlets. Only in one case (Fig. 15) were two distinct rows of these seen. Figs. 18 and 20 also indicate that in human specimens the less opaque granules, similar morphologically to those in larval amphibians (5), are found in the cellular ectoplasm.

Cytoplasmic filaments are not seen in the space between the rodlets and

the dermal membrane, and so appear to terminate at these dense areas (rodlets). This attachment is emphasized by their failure to attach to the other type of granules (the spherical less-opaque ones occasionally seen in the ectoplasm). The dense rod-like granules were not evident in the embryonic specimen in which keratinization was not yet extensive. This suggests that these granules are related functionally as well as morphologically to the tonofilaments.

It is difficult to speculate on this unusual method used by cells to anchor themselves to the dermis. The cell membrane is certainly adjacent to, if not continuous with, the granules terminating the tonofilaments (see Figs. 15, 19, and 20). Thus the situation is as if the granules anchor the filaments to the cell membrane while the latter is, itself, bound to the dermal membrane across the separating space presumably with the aid of amorphous material (polysaccharide ?) found in this space. The mechanism may be the same as at intercellular bridges (see below) where the cell membranes meet adjacent cell membranes at various points, rather than the dermal membrane.

Basal Epidermal Cells (Stratum Germinativum).—Basal cells of all the mammalian tissue studied were characterized by the same basic cytoplasmic components with adult human epidermis possessing pigment granules and intercellular bridges in addition. In adult human tissues the only variation in pattern of basal cells appears to be the presence of a perinuclear region completely devoid of filaments. Since this space usually contains both mitochondria and pigment granules, and filaments and other normal cell components are present in the rest of the cytoplasm, these cells do not necessarily follow the classical histological description of clear cells. The failure to observe anything more of typical clear cells in this investigation certainly suggests that they may be artefacts of conventional histological preparations. The alternative possibility that, because of the limited material included in ultrathin sections, true clear cells have not been observed, is certainly not denied but is considered less likely.

Several authors (1-3, 7) have observed the cytoplasmic filaments in various states of clumping and distortion following poor fixation or removal of the embedding material and have generally considered them as fixation artefacts. In view of phase microscopic evidence that there are fibrous arrays in living epidermal cells (28) and x-ray diffraction and chemical evidence (29) that there is a keratin-like fibrous protein in the lower layers of fresh epidermis it seems clear that, far from being fixation artefacts, the filaments are fibrous protein filaments present in living epidermal cells. They may be clumped and precipitated in varying degrees in fixed and stained histological preparations where they are visualized as tonofibrils. It therefore seems appropriate to call them *tonofilaments*. Without knowledge of the submicroscopic structure of tonofibrils, Giroud and Leblond (26) review the chemical, histological, and

x-ray data and also conclude that tonofibrils are probably the support for the characteristic protein (Rudall's "epidermin" (29)), an α -type protein, characteristically found in the epidermis and its derivatives. They correlate the extractability of epidermin with the ease with which tonofibrils may be modified by enzymes, acids, and autolytic processes, and suggest further that this protein is built up in the Malpighian layer and is the keratin precursor which becomes fully keratinized without change in fibrous skeleton in the region of passage from the Malpighian to the cornified zone. Changes in these filaments in the outer cell layers of the epidermis will be discussed in a following paper.

The diameter of the tonofilaments shown in the sections here is similar to that of extracted hair keratin (30), the protein which epidermin most resembles. Since there are, unfortunately, no studies of the macromolecular structure of epidermin itself, the tonofilaments cannot be correlated any more directly with it. Rudall describes a non-fibrous protein component of epidermis that is extracted together with epidermin and gives a β -type x-ray pattern. It has not been identified in tissue sections probably because it is non-fibrous and so is either combined with tonofilaments or other normal cell components or present in the soluble fraction or ground-substance of the cytoplasm.

The observations presented here should also make it clear that on the sub-microscopic level there can be no confusion of mitochondria with tonofilaments or Herxheimer fibers, as poorer resolution of light microscopic studies had suggested (11). When tonofilaments appear in distinct bundles extending from the nucleus to dermo-epidermal junction (Figs. 16 and 23) their identity as the Herxheimer fibers of the histological literature is clear. In common with the Herxheimer fibers (13) these bundles are most prominent in skin such as that of the footpad where there are also prominent dermal papillae.

The dense double discs in the intercellular bridges described here correspond to the nodes of Bizzozero or knots of Ranvier, since they naturally appear as a single granule under the light microscope. They contain phospholipide (31) which would react strongly with osmium tetroxide and which is therefore probably localized in the dense part of the double discs. This type of intercellular bridge is reminiscent of that recently found in the intercalated discs of heart muscle (32) and in nerve synapses (33, 34), in which there is accumulation of density at localized areas of adjacent cell membranes and close approximation of these areas with no continuity of cytoplasm. The three types of cells (muscle, epidermis, nerve) which employ this type of intercellular junction have in common the synthesis and maintenance of intracellular cytoplasmic filaments. The discontinuity of cytoplasm and tonofilaments at the bridges is certainly consistent with the ability of these cells to undergo mitosis.

The sections of pigment granules described here do not reveal any addi-

tional information apart from suggesting that granules from Negro skin are larger than granules from white skin and emphasizing the intense osmiophilia and inherent density of melanin which makes these granules so dense that no internal structure is revealed. The dimensions reported here correspond to those of granules isolated from Negro skin (35) considering that sections show the short dimension but not usually the full length. It is interesting that the granules may occur at any point in the cytoplasm and that the cytoplasmic structure of human epidermal cells with and without melanin appears the same with no change in the amount of endoplasmic reticulum. Contrary to other suggestions (2) the pigment granules are easily distinguishable from mitochondria or granules in the cell bridges.

The scarcity of vesicles of the endoplasmic reticulum (18) in the highly basophilic epidermal cells (11, 12, 29) supports the contention (19) that basophilia must be ascribed to the small particulate cytoplasmic component. In the sections described here there is no danger of confusing this component with cross-sections of the tonofilaments since the latter have considerably narrower diameters. The endoplasmic reticulum has been associated with synthetic activity (18). These epidermal cells are certainly involved in synthesis but their synthetic product (epidermin) has no need to leave the cells. Thus the system of canals of the endoplasmic reticulum may be necessary only in cells such as the pancreas, thyroid, and liver in which products must leave the cells. The absence of vesicles and membranes of the Golgi apparatus (36, 37) is difficult to understand in view of some evidence (11) that the apparatus may be present in epidermal cells, unless it is minimally or only occasionally developed in these cells and also requires as optimal a fixation as the mitochondria (see above).

Nuclear structure has appeared the same as in all cell types studied (16), one unusual feature in basal epidermal cells being the close association of its external membrane with tonofilaments.

SUMMARY

1. Basal epidermal cells and their junction with the dermis, as revealed in thin sections of osmium-fixed human and rodent skin, were studied with the electron microscope. Phosphotungstic acid staining was occasionally used to increase the electron density of membranous and filamentous structures.

2. Along the dermo-epidermal junction in all skin specimens there is a submicroscopic (~ 350 A thick) membrane following the basal contours of the epidermal cells, but separated from them by an ~ 300 A space. No epidermal or dermal filaments can be seen to cross it and except in embryonic skin it has no associated band of amorphous material. It is called the "dermal membrane" to distinguish it from the thicker membrane and associated material commonly called the "basement membrane."

3. In adult human skin the basal cell membrane facing the dermal membrane is continuous with, or adjacent to regularly spaced groups of small dense rodlets at which tonofilaments are attached and appear to terminate. Less dense spherical granules are also found in the cellular ectoplasm and are unattached to filaments.

4. The connective tissue fibers in the upper dermis were narrower (≥ 300 A) than, but displayed the same period (≥ 350 A) as the collagen fibers in the deeper dermis. The related fibers in embryonic human skin were even narrower (150 to 250 A). In accordance with the views of others that these are "young" collagen with great affinity for polysaccharide, they are called collagen fibers.

5. The same cytoplasmic components are found in all basal epidermal cells: mitochondria, many filaments, many submicroscopic particulates, and only very occasional vesicles of the endoplasmic reticulum. Adult human cells possess pigment granules and intercellular bridges, in addition. Before keratinization is evident, no intercellular bridges and little or no cytoplasmic filaments are visible. The scarcity of vesicles of the endoplasmic reticulum and the prevalence of submicroscopic particulates (80 to 150 A) distributed at random through the cytoplasm, support the view (19) that basophilia and cytoplasmic nucleoprotein are associated with the particulates.

6. Cytoplasmic filaments are < 100 A wide and are directed toward the dermo-epidermal junction and to intercellular bridges (when they occur). Because of their obvious identity as the submicroscopic constituents of tonofibrils, they were called "tonofilaments" and believed to represent the keratin-like fibrous protein "epidermin" (29). Bundles of them appear identical with Herxheimer fibers when, particularly in thick skin, they extend from the outer nuclear membrane to the granules spaced along the dermo-epidermal junction.

7. In human skin, adjacent basal cells are separated by simple cell membranes and connected by intercellular bridges. The filaments of one cell are attached to dense elongate granules in these bridges which are separated by a narrow less electron-dense space from a matching granule to which the filaments of the neighboring cell are attached. No filaments have been observed to cross the space in these bridges. In between the bridges adjacent cell membranes may separate from each other leaving an intercellular space. Although occasional basal cells possess a large perinuclear area devoid of cytoplasmic filaments, none following the classical histological description of "clear" cells were noted.

8. Pigment granules are extremely electron-dense with irregular angular outline. Their smallest diameter was significantly greater in Negro than in white skin while the lengths also appeared greater in the former skin type.

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EXPLANATION OF PLATES

PLATE 108

FIG. 1. Phase micrograph of a one micron section of white human breast skin that had been fixed in osmium tetroxide (pH 7.4) stained with 1 per cent phosphotungstic acid (pH 5.4) and embedded in methacrylate. The specimen block was the same one that was used for the ultrathin section illustrated in the electron micrograph of Fig. 12. The section was further stained in methylene blue before mounting on glass slides with diaphane and covered with a No. 1 cover glass (17). Micrograph taken at $727\times$ with a dark medium contrast oil immersion lens and enlarged photographically to $1450\times$. (Micrograph courtesy of Dr. John J. Biesele.)

FIG. 2. Phase micrograph of a one micron section of rat footpad skin that had been prepared in the same way as that of Fig. 1. The specimen block was the same one from which the ultrathin sections illustrated in the electron micrographs of Figs. 11 and 22 were obtained. The thick section illustrated here was further stained in methylene blue. Micrograph taken at $727\times$ with a dark medium contrast oil immersion lens and enlarged photographically to $1450\times$. (Micrograph courtesy of Dr. John J. Biesele.)

FIG. 3. Phase micrograph of a one micron section of rat footpad skin similar to that of Fig. 2 except that it had not been stained with methylene blue before mounting on the glass slide. Greater cytoplasmic and nuclear detail is evident in this specimen which had not undergone the solvent treatment necessary for staining, while the dermo-epidermal junction is unfortunately obscured by tearing of the section at this point. Micrograph taken with dark medium oil immersion lens at $727\times$ and enlarged to $1200\times$. (Micrograph courtesy of Dr. John J. Biesele.)

FIG. 4. Light micrograph of a section of human breast skin similar to that of Fig. 1. Less detail and contrast is seen than in the phase micrograph of Fig. 1. Taken at $700\times$ and enlarged photographically to $1000\times$.

FIG. 5. Phase micrograph of a section, thinner than one micron, of the same specimen of embryonic human skin that is illustrated in the electron micrographs of Figs. 6 to 9. The section was extremely thin so that the contrast of the resulting micrograph is low. The epidermis with its layer of cuboidal basal cells and superficial periderm can be distinguished together with scattered mesenchymal cells. Taken with dark medium oil immersion objective at $727\times$ and not enlarged photographically. (Micrograph courtesy of Dr. John J. Biesele.)

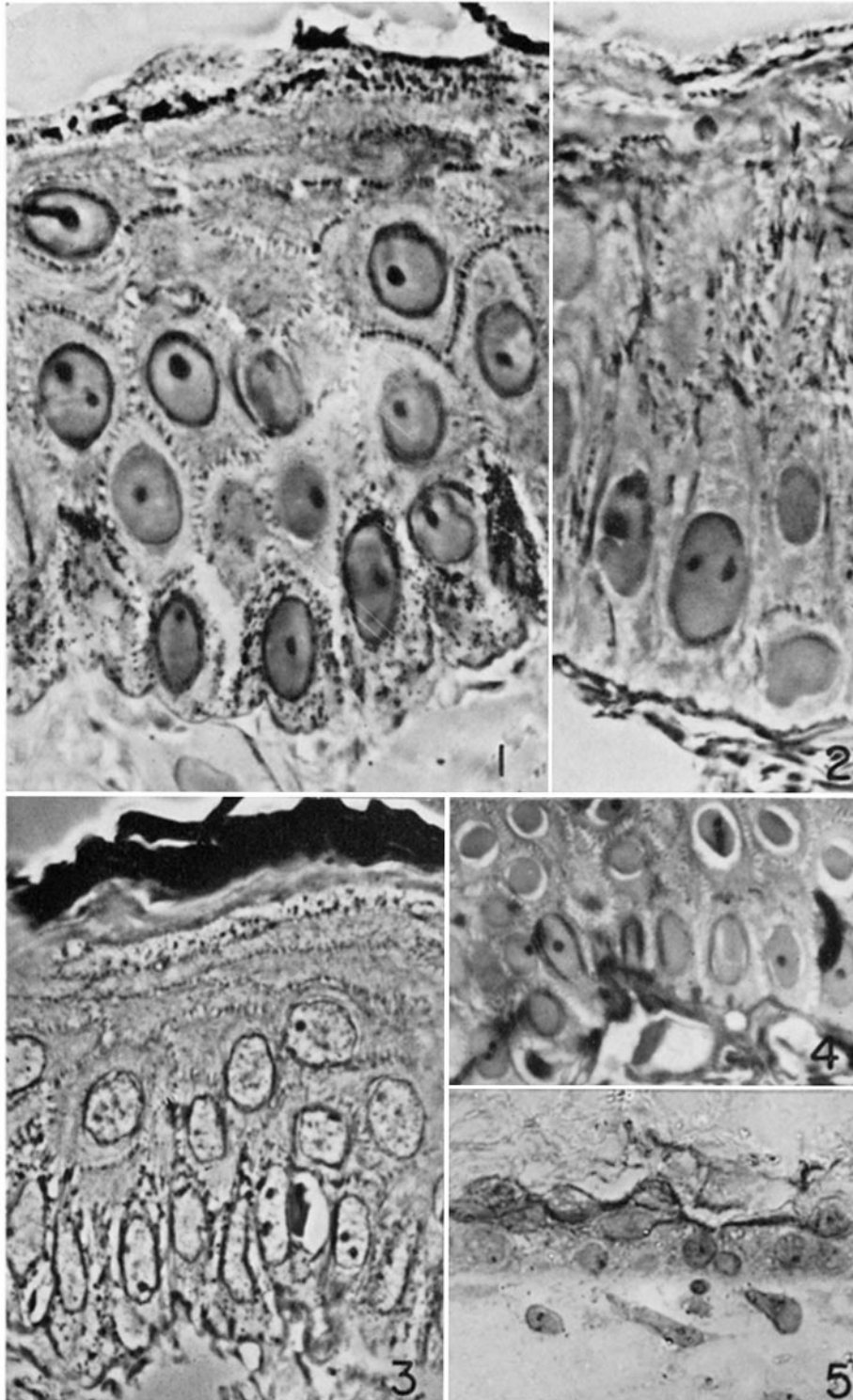


PLATE 109

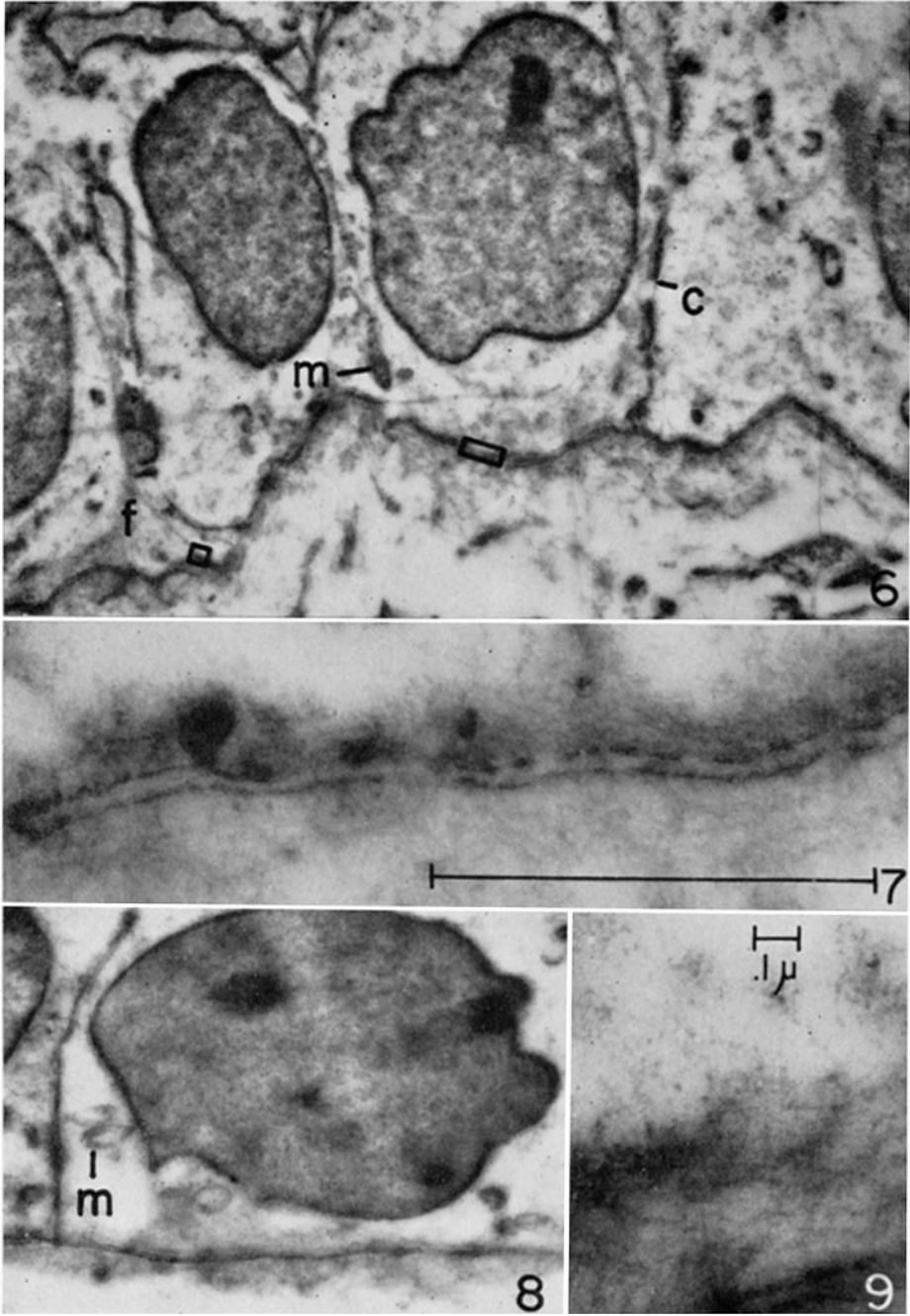
All micrographs illustrate sections of a specimen of abdominal skin from a 12 week human embryo. The skin had been fixed in 1 per cent osmium tetroxide buffered at pH 7.4 for 4 hours and then dehydrated in alcohols and embedded in methacrylate according to the usual procedure.

FIG. 6. Taken at 2200 \times and enlarged photographically to 6600 \times to show a cross-section of the epidermis at low magnification. The dermo-epidermal junction runs across the lower half of the picture and is characterized by two parallel membranes with connective tissue filaments on one side and basal cell cytoplasm on the other. The section includes portions of four basal cells illustrating their mitochondria (*m*), cytoplasmic particulates and occasional cytoplasmic filaments (*f*); simple cell membranes (*c*) separate adjacent cells. The outlined portions are enlarged in Figs. 7 and 9.

FIG. 7. Taken at 7500 \times and enlarged photographically to 58,500 \times to show the portion of the section of Fig. 6 that is indicated by the rectangular outline. This enlarged view illustrates the double membranes separating cell cytoplasm from connective tissue.

FIG. 8. Taken at 2200 \times and enlarged photographically to 6600 \times to show another portion of the same epidermis. Individual cell membranes separate adjacent cells and are continuous around the cell so that they also face the dermo-epidermal junction. The dermal membrane separating the connective tissue from the basal cells is continuous across the junction between the two adjacent cells. A mitochondrion (*m*) with evidence of internal membranes is indicated.

FIG. 9. Taken at 7500 \times and enlarged photographically to 58,500 \times to show the portion of the section of Fig. 6 that is outlined by a square. This enlarged view illustrates submicroscopic cytoplasmic particulates in the upper half of the picture and submicroscopic cytoplasmic filaments in the lower half.

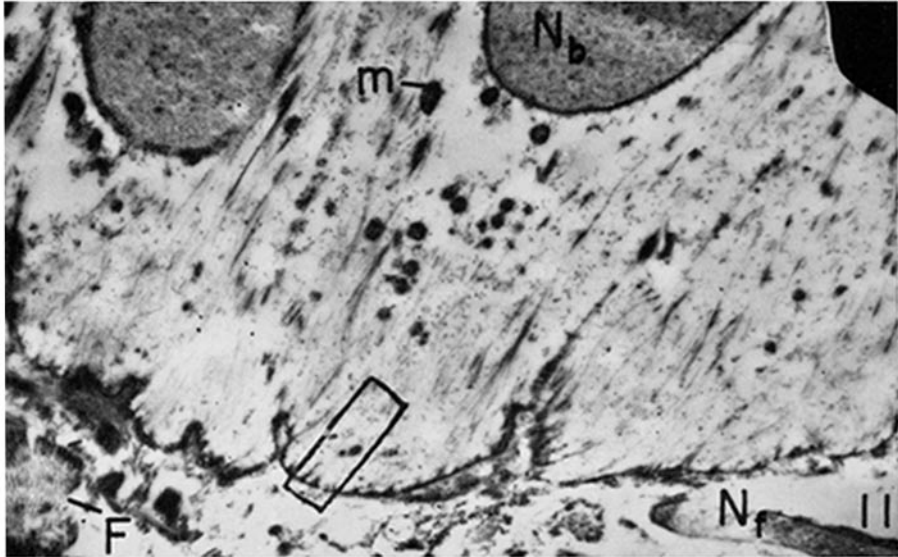
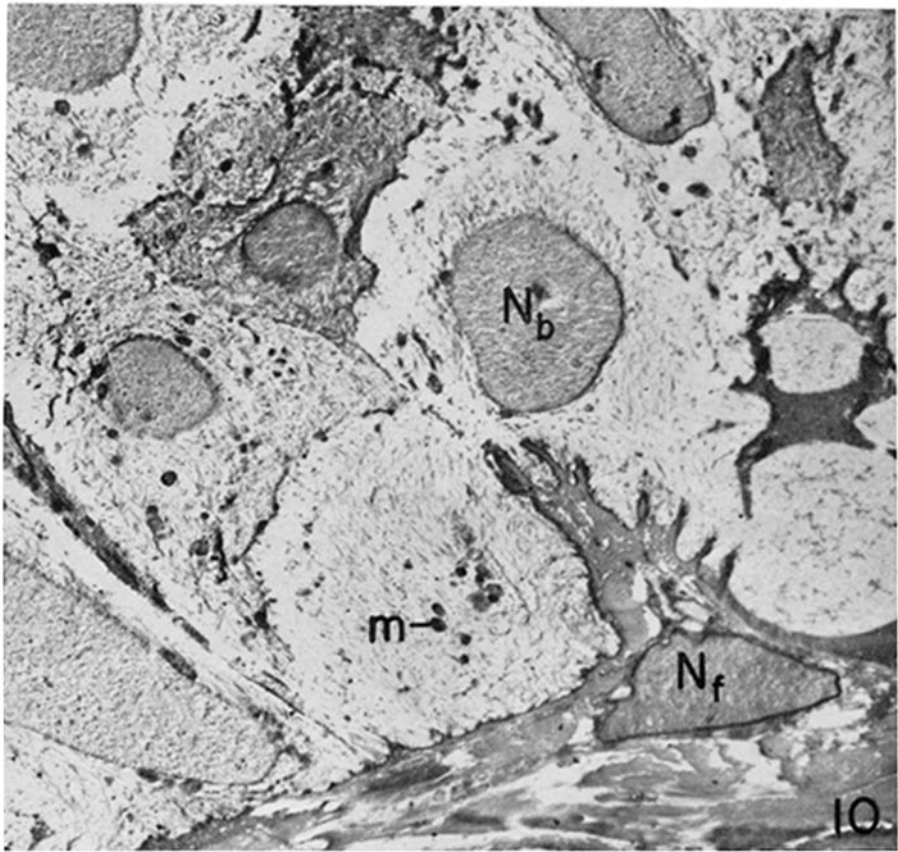


(Selby: Ultrastructure of the epidermis)

PLATE 110

FIG. 10. This low magnification view of a relatively thick section of a specimen of rat footpad skin, from which the plastic embedding had been removed by xylol prior to electron microscopic observation, illustrates the dense packing of connective tissue filaments immediately below the dermo-epidermal junction (which runs across the lower right hand corner of the photograph). Finer and less densely packed filaments are seen in the epidermal cells and run predominantly in a direction toward the dermis. Mitochondria (*m*), a nucleus (*N_b*) of a basal epidermal cell, and a nucleus (*N_f*) of a fibroblast are indicated. Taken at 2400 × and enlarged photographically to 7200 ×.

FIG. 11. Low magnification view of a thinner section of rat footpad skin from which the embedding matrix had not been removed prior to electron microscopic examination. The tissue was taken at the same time as that of Fig. 10 and fixed in osmium tetroxide for the same period of time but then stained in 1 per cent phosphotungstic acid (pH 5.4) for 24 hours prior to dehydration and embedding. Nuclei of an epidermal cell (*N_b*), and a fibroblast (*N_f*), and mitochondria (*m*) are indicated. The dermo-epidermal junction runs across the lower half of the picture separating the filaments in the epidermal cells from the thick filaments (*F*) in the connective tissue. Submicroscopic cytoplasmic particles are also visible in the epidermal cell cytoplasm. The outlined portion of this section is shown at higher magnification in Fig. 22. Taken at 2400 × and enlarged photographically to 6150 ×.



(Selby: Ultrastructure of the epidermis)

PLATE 111

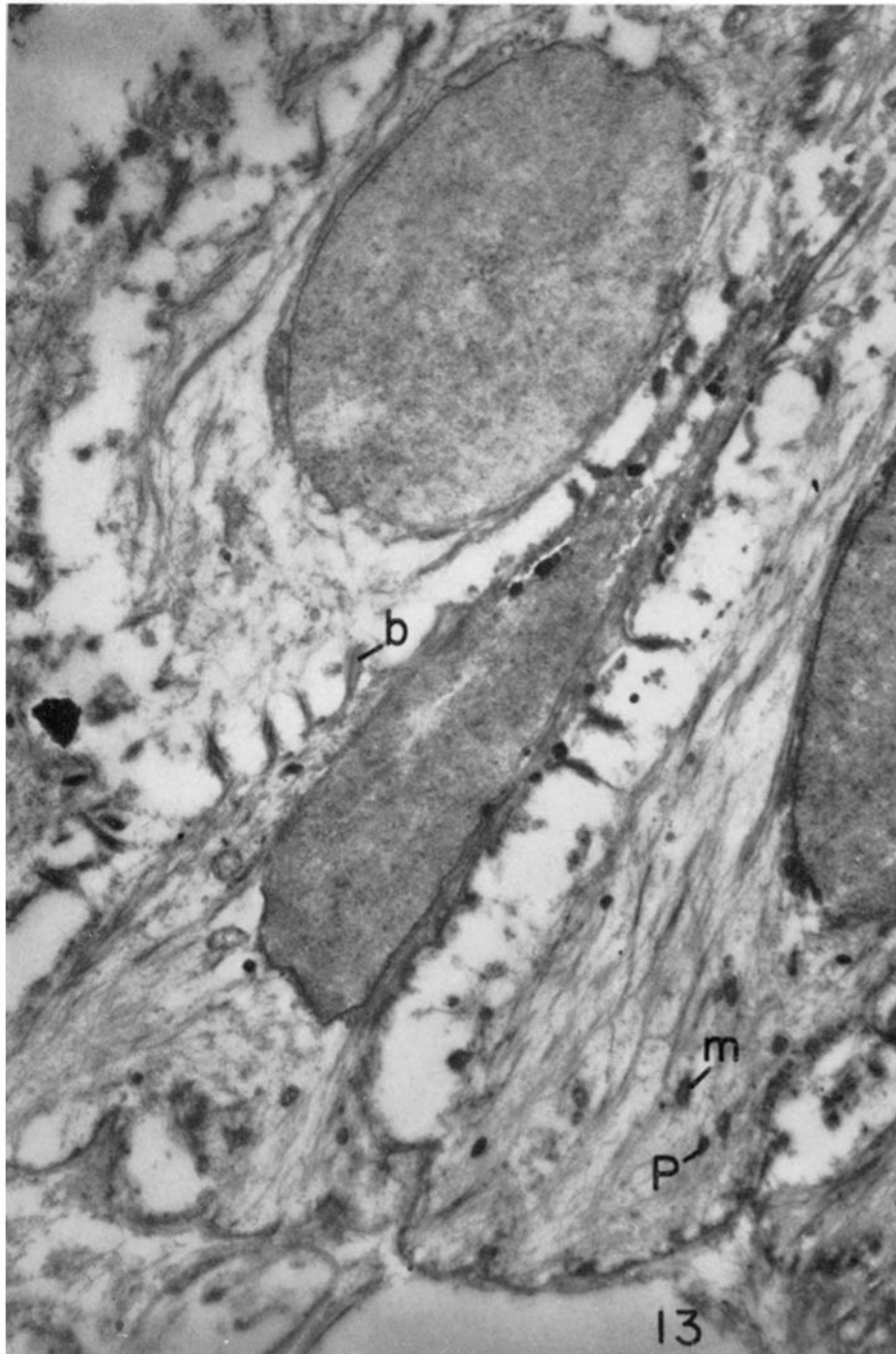
FIG. 12. Montage of two low magnification electron micrographs to show a section through the connective tissue and two cell layers of the epidermis of white human breast skin. The dermo-epidermal junction runs diagonally across the lower photograph separating the basal cells from the connective tissue filaments, while portions of two prickle layer cells are included in the upper photograph. Mitochondria (*m*), pigmented granules (*P*), and a portion of a nucleolus (*n*) are indicated. Cytoplasmic filaments run toward the dermis in basal cells and toward cytoplasmic bridges in prickle cells. The prickle cell appears to have separated from its neighbors at the cytoplasmic bridges (*b*) while the basal cells are still closely attached to each other at the bridges with no intercellular spaces evident. Basal cell filaments also are closely associated with the nuclear membranes. Taken at 2400 \times and enlarged photographically to 6150 \times .



(Selby: Ultrastructure of the epidermis)

PLATE 112

FIG. 13. Low magnification view of portions of three basal cells in human breast skin and their junction with the connective tissue. The specimen was taken at the same time and prepared in the same way as that of Fig. 12 except that after osmium fixation it was stained with 1 per cent phosphotungstic acid (PTA) (pH 5.4) for 24 hours and dehydrated in solutions also containing 1 per cent PTA. Mitochondria (*m*), occasional pigment (*P*), and cytoplasmic bridges connecting adjacent basal cells (*b*) are indicated. The bridges appear as oblique cuts through two dense granules which are separated from each other by a less dense space. After staining with PTA the cytoplasmic filaments and bridges appear denser than in Fig. 12, although there is no significant change in the density of other cell components. Taken at 2400 \times and enlarged to 11,800 \times .

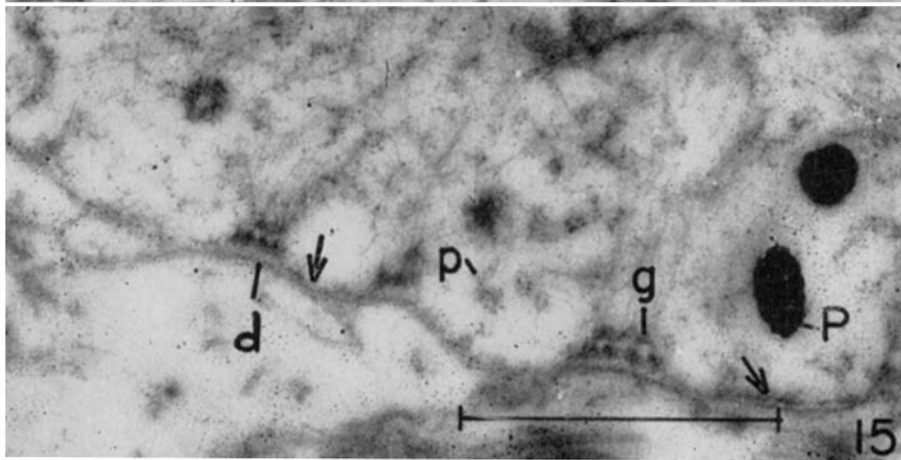
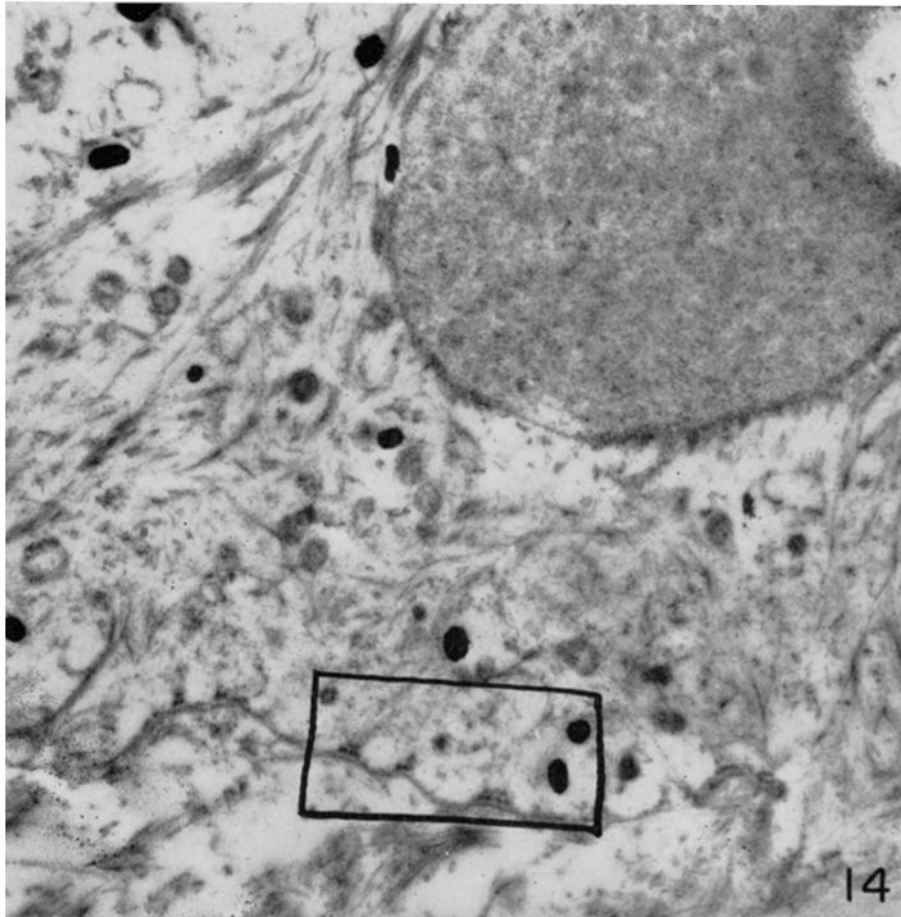


(Selby: Ultrastructure of the epidermis)

PLATE 113

FIG. 14. Ultrathin section through the basal portion of a basal epidermal cell in a specimen of Negro breast skin that had been fixed and prepared in the usual manner. The dermo-epidermal junction runs across the lower portion of this view while the cell nucleus is seen in the upper right hand corner. Cytoplasmic filaments run primarily from the nucleus to the dermis and lie in close approximation to the nuclear membrane. Taken at 5200 \times and enlarged photographically to 16,000 \times .

FIG. 15. Enlargement of the indicated portion of Fig. 14 to demonstrate detail at the dermo-epidermal junction. Cytoplasmic filaments terminate at the rows of granules (*g*) which are lined up in groups along the basal cell membrane. This cell membrane is indicated by arrows and has a small separation from the dermal membrane (*d*). Cytoplasmic particulates (*p*) are clearly seen here and pigment granules (*P*) indicated. The many dense approximately spherical dots superimposed on this view are artefacts due to sublimation of pigment or to dirt on the photographic plate. Taken at 5200 \times and enlarged photographically to 42,000 \times .

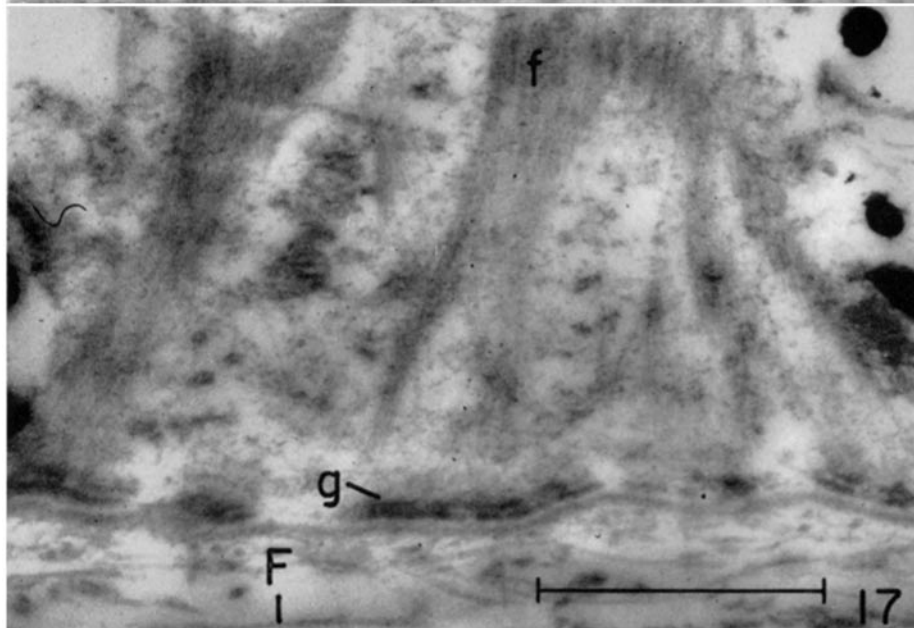
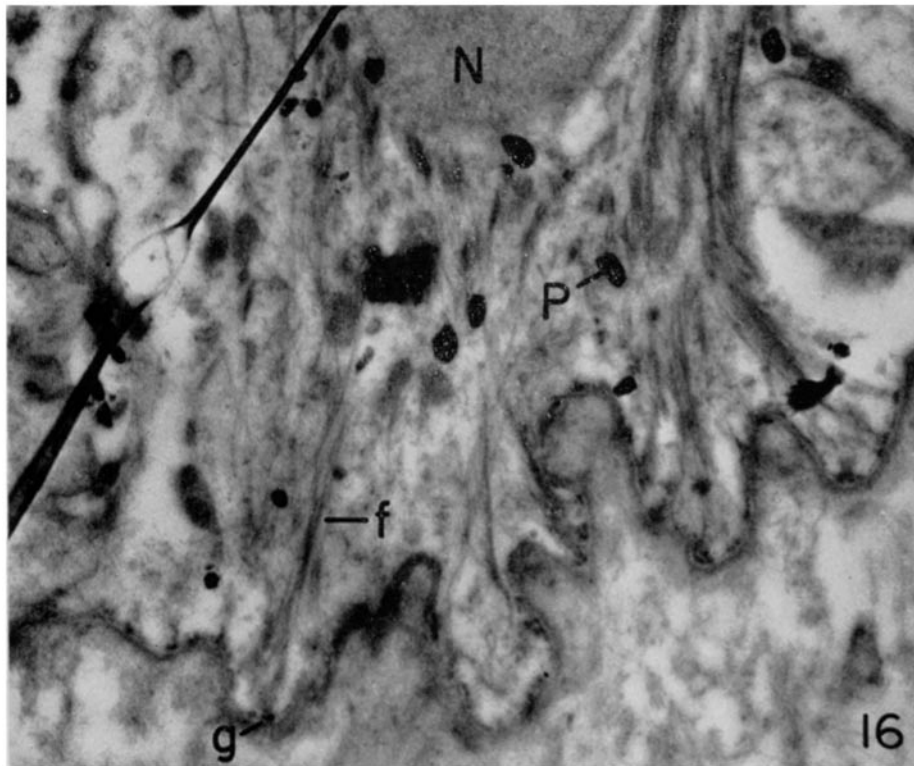


(Selby: Ultrastructure of the epidermis)

PLATE 114

FIG. 16. This section through the basal portion of a basal epidermal cell in human breast skin that had been fixed and embedded in the usual manner, illustrates the manner in which bundles of cytoplasmic filaments (*f*) extending from the nucleus to dermo-epidermal junction resemble the histological description of Herxheimer fibers. They start in bundles from the nucleus (*N*) and terminate at granules (*g*) at the junction. A fold in the section runs across the upper left hand corner. Pigment granules are indicated (*P*). Taken at 2400 \times and enlarged photographically to 18,500 \times .

FIG. 17. An enlarged view of the dermo-epidermal junction in Negro breast skin that had been stained in 1 per cent PTA after the usual osmium fixation. The density of collagen fibers is clarified by the staining and the difference in diameter between the dermal membrane and these filaments (*F*) is clearly seen. Cytoplasmic filaments (*f*) lead to granules (*g*) at the cell membrane while the rest of the cytoplasm appears considerably washed out after the staining procedure. Taken at 7500 \times and enlarged photographically to 37,500 \times .

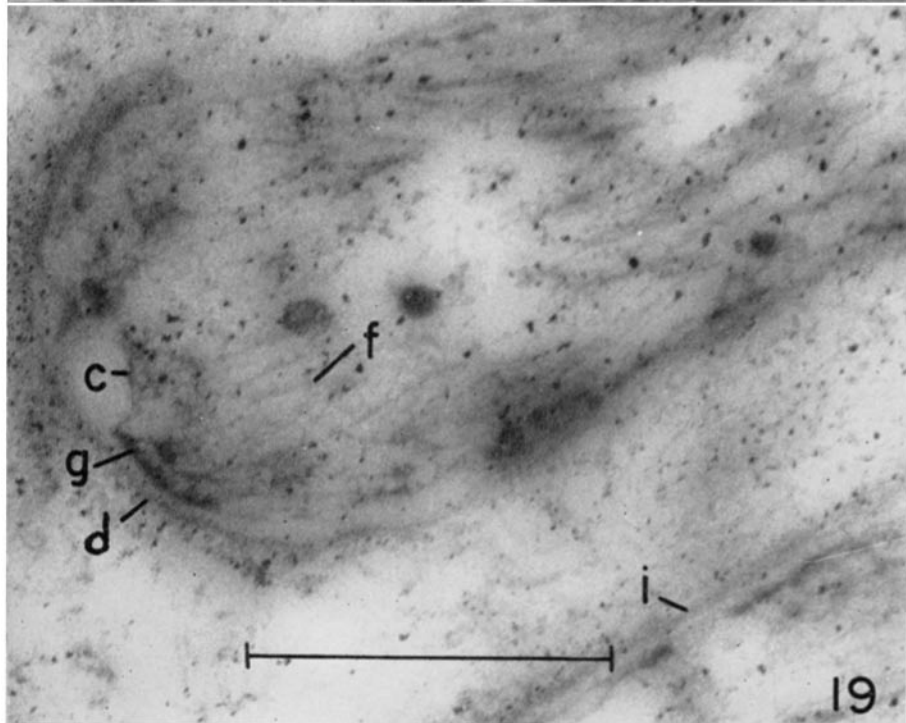
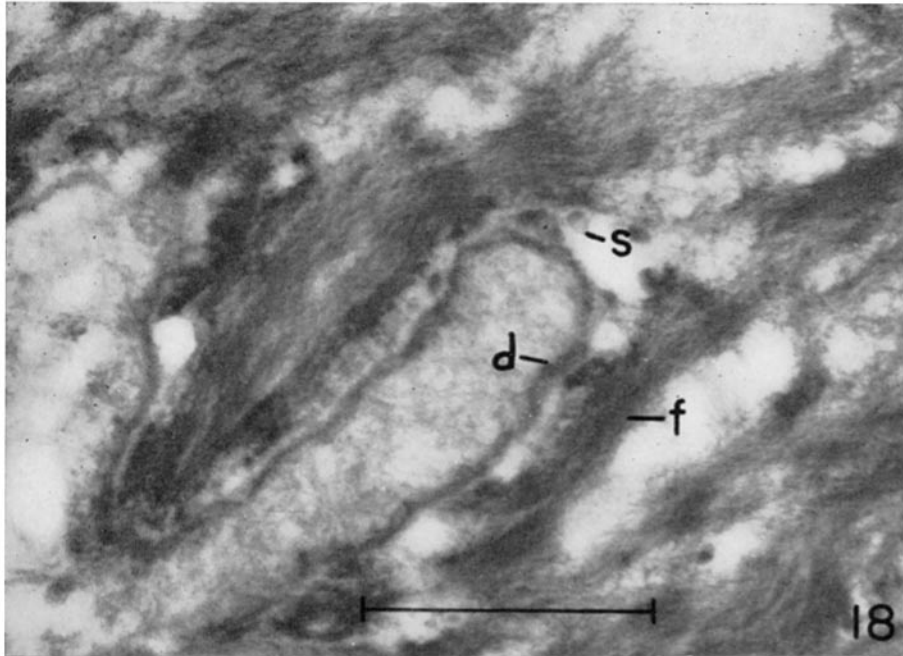


(Selby: Ultrastructure of the epidermis)

PLATE 115

FIG. 18. Enlarged view of a section across the dermo-epidermal junction in a specimen of human footpad skin which had been stained 24 hours in 1 per cent PTA after the usual osmium fixation. Small projections of basal cell cytoplasm containing particularly dense bundles of cytoplasmic filaments extend into the dermis. The dermal membrane (*d*) is broad and dense. The filaments (*f*) terminate at certain dense granules while less dense spherical granules (*s*) unassociated with filaments, also appear. Taken at 7500 \times and enlarged photographically to 37,500 \times .

FIG. 19. This electron micrograph illustrates an area comparable to that of Fig. 18 but in a specimen that had been left in distilled water for the same period of time that the specimen of Fig. 18 had been in 1 per cent PTA. There is considerably less density in the cytoplasm and in the connective tissue although the dermal membrane *d*, cytoplasmic filaments (*f*), and junctional granules (*g*) are still visible. The cell membrane (*c*) can also still be distinguished joining neighboring granules. The many dense dots covering the photograph are artefacts possibly due to osmium tetroxide precipitated after the extended washing. Taken at 9500 \times and enlarged photographically to 47,500 \times .



(Selby: Ultrastructure of the epidermis)

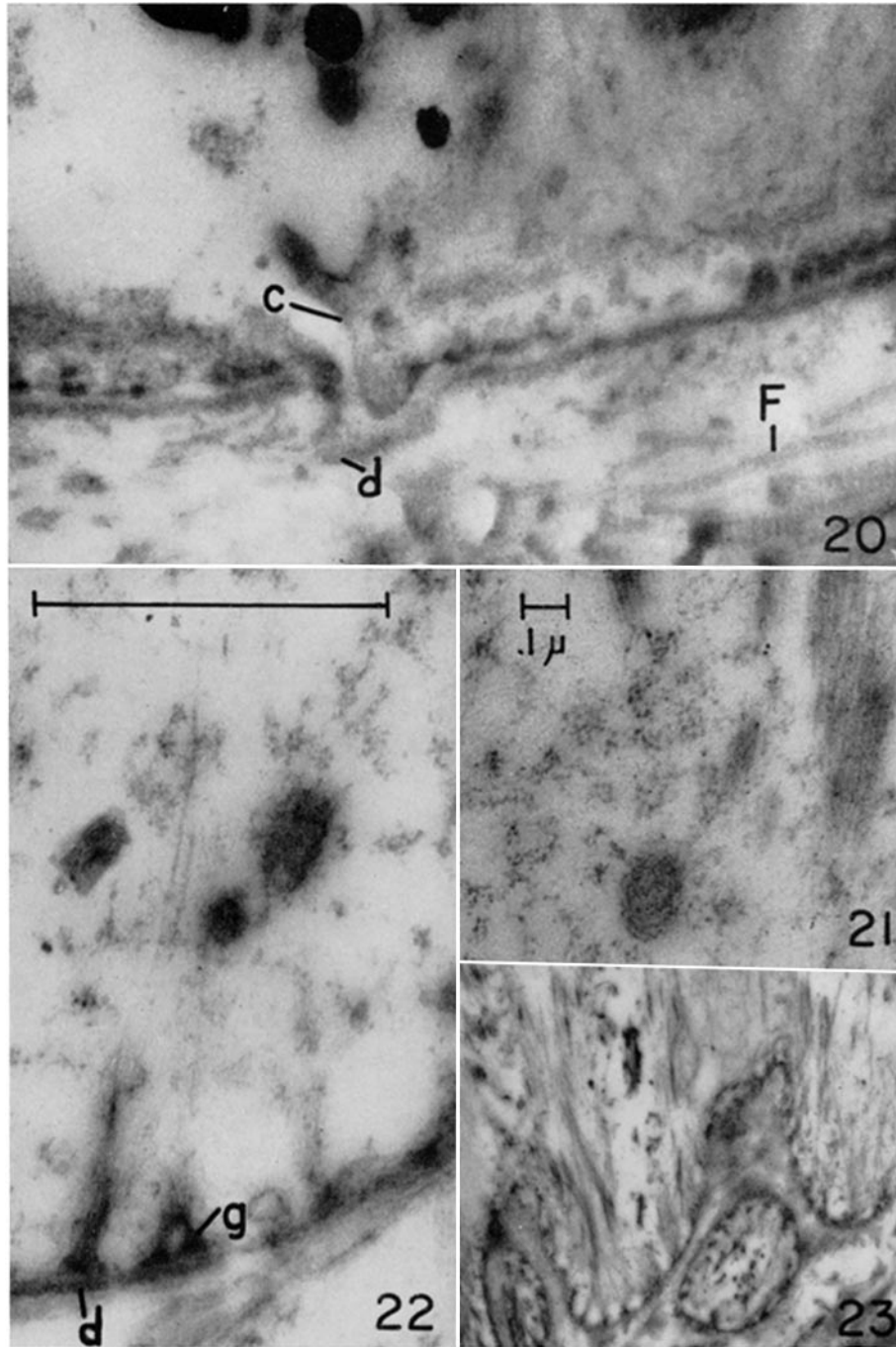
PLATE 116

FIG. 20. This view includes the junction between two adjacent basal cells and the dermis in a specimen of Negro breast skin that had been stained in 1 per cent PTA in addition to the osmium fixation. The staining has enhanced the striation in the connective tissue filaments (*F*) although washing out a considerable amount of the cellular cytoplasm. The dermal membrane (*d*) is continuous across the junction between the two basal cells and is distinguishable from the connective tissue filaments by its density and lack of striation. The cytoplasmic membrane (*c*) of one cell may be followed along the dermal interface in close approximation with various granules, and then as it meets the adjacent basal cell and leaves the dermal junction. Taken at 5400 \times and enlarged photographically to 16,200 \times .

FIG. 21. High magnification view through a portion of the cytoplasm of a basal epidermal cell in Negro breast skin that had been fixed in osmium tetroxide in the usual way without staining. Submicroscopic cytoplasmic particules are distributed at random and clearly have diameters larger than the cytoplasmic filaments grouped into bundles at the right hand side of this view. Taken at 7500 \times and enlarged photographically to 58,500 \times .

FIG. 22. An enlarged portion of Fig. 11 to demonstrate the identical cell components illustrated in Fig. 21 (particulates and filaments) as well as the dermal membrane (*d*) and junctional granules (*g*). Taken at 9500 \times and enlarged photographically to 47,500 \times .

FIG. 23. A low magnification view of the dermo-epidermal junction in human foot-pad skin to demonstrate the grouping of cytoplasmic filaments into fiber bundles that terminate at junctional granules. The gap between neighboring granules creates the appearance of prickles that may be recognized under the light microscope. This orientation is preserved even after 24 hour, washing in distilled water, to which this specimen was subjected as a control to the parallel specimen which was stained with PTA. Taken at 2400 \times and enlarged to 7200 \times .



(Selby: Ultrastructure of the epidermis)