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THE ULTRASTRUCTURE OF THE SKIN OF HUMAN EMBRYOS

III. THE FORMATION OF THE NAIL IN 16–18 WEEKS OLD EMBRYOS*

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The development of the human finger nail has been the subject of investigation for almost a century (1). Classically, the nail plate had always been considered a uniform sheet of cells originating solely from the ventral nail matrix until 1954 when Lewis (2) concluded, on the basis of histochemical findings, that the nail plate consisted of three layers, each of separate and distinct origin. Zaias (3) and Achten (4), however, in the two most recent studies of the embryology of the human nail, each arrived at different conclusions, Zaias finding the nail plate to be a unit structure derived entirely from the ventral nail matrix, and Achten that the nail plate consists of three layers: the first two layers originating from the ventral and dorsal matrices, and the third from the nail bed. The results of this investigation agree with those of Achten.

The present investigation is an attempt to answer in detail the frequently posed questions as to what type or types of keratinization processes cells undergo before forming the nail plate (3), and why nails grow out instead of up (5). This study follows for the most part the antecedent terminology of the adult nail structures except for the term "matrix primordium" which is more clearly understandable when referring to the entire nail matrix, ventral and dorsal matrices inclusive (Diagram I).

MATERIALS AND METHODS

The distal phalanges of five freshly aborted human embryos of approximately 12 to 15 cm crown-rump length (16 to 18 weeks old) were used. Specimens were prepared and examined with an RCA-3G electron microscope as was previously reported. Some thin sections were stained with the silver methenamine method of Movat (7) for detection of glycogen and mucopolysaccharides at the ultrastructural level. The DDD reaction for SH groups (8) and PAS stain (9) with and without previous diastase digestion were performed on fresh-frozen sections from each specimen.

RESULTS

The Matrix Primordium

A. *General growth pattern:* Longitudinal midline sections of the distal phalanx showed the nail matrix primordium to consist of a solid wedge of epithelial cells with the round apex directed proximally (Diagram I) (Fig. 1). The tall columnar basal cells forming the perimeter of the proximal matrix primordium (Fig. 1) rested upon a gently undulating, continuous basement membrane (Fig. 1). The long axis of each matrix cell forming the apex of the matrix primordium lay centrodistally (Fig. 1) and parallel to the central axis, the geometrical center line of the matrix primordium (Diagram I). At approximately one-fourth of the distance between the apex of the matrix primordium and the cuticle, the level which will be designated as the "vertical level of keratinization of the nail" in this report, squamous cells derived from the basal cells of the matrix primordium proximal to this level, flattened along the central axis, and became the nail plate (Fig. 2). These squamous cells, instead of accumulating keratohyaline granules, often became vacuolated and degenerated (Figs. 2, 5). At this same level the SH stain became positive, the thickness of the SH positive band continually increasing distally (Fig. 3), thus corresponding to the increasing distal thickness of the nail plate, as was revealed by the electron microscope (Fig. 4). The basal cells forming the ventral and dorsal perimeter of the matrix primordium were not perpendicular to the basement membrane; rather the long axis of these

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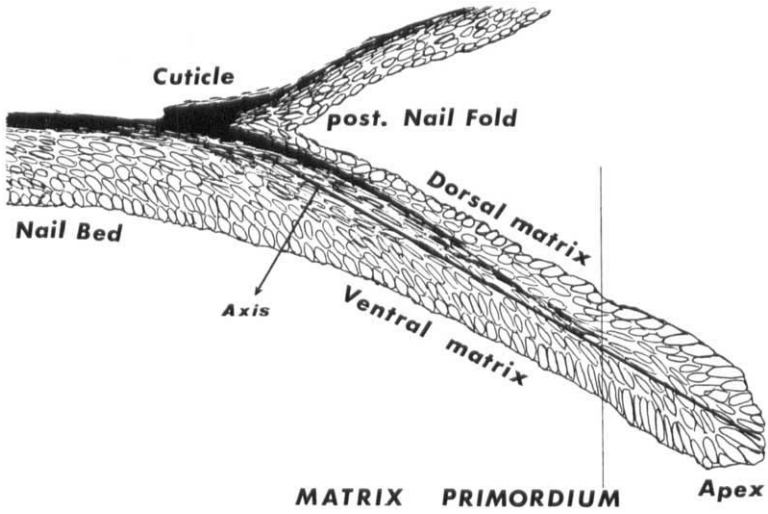

DIAGRAM 1. Schematic depiction of 18-week-old embryo nail. The line indicates the "vertical level of keratinization."

cells inclined centrodistally (Fig. 5). The squamous cells derived from these basal cells lay in a slightly more distal position with their long axes almost parallel to the central axis (Fig. 5) (Diagram I), and even distal to the vertical line of keratinization, some squamous cells demonstrated signs of degeneration (Figs. 2, 5).

B. *Nail plate:* Squamous cells of both ventral and dorsal origin started undergoing complete keratinization at the vertical level of keratinization through the formation of keratohyaline granules. While most of these keratohyaline granules did not differ morphologically from those of the epidermis with their attached bundles of tonofilaments (Fig. 4), some granules were thickly surrounded by, and often contained numerous ribonucleoprotein (RNP) particles, and were often completely devoid of the tonofilamentous component (Figs. 6, 7a). Up to the vertical level of keratinization the thicknesses of the ventral and dorsal parts of the matrix primordium with the central axis as a dividing line (Diagram I), were about equal. Distal to this level, however, the ventral half steadily thickened at the expense of the decreasing dorsal part; thus, the nail plate was shifted dorsally, and lay above the central axis (Diagram I). As the thickness of the ventral part increased, it supplied more horny (nail) cells, as is evidenced by the presence of more layers of granular cells with larger keratohyaline granules, than the dorsal counterpart (Figs. 4, 6) which eventually ceased to exist at the level of the cuticle (Diagram I) (Fig. 6). It must, however, be emphasized that the dorsal half of the matrix, though smaller than the ventral part, definitely contributed to the formation of the nail plate, as is evidenced by the continuous presence of keratohyaline granules up to the distal end of the proximal nail fold. It was impossible to distinguish between the nail cells contributed by the ventral matrix and those produced by the dorsal matrix once they had become keratinized. Past the vertical level of keratinization the dorsal squamous cells contained glycogen, which increased in amount distally (Fig. 6). Near the distal edge of the proximal nail fold in the dorsal half, melanocytes and Langerhans cells were often seen (fig. 6). These features suggested that the dorsal matrix was very similar to the surface epidermis near the cuticle.

C. *Individual cells and their organelles:* As far as the fine cellular structures were concerned, there were no differences between each corresponding layer of cells of the ventral and dorsal matrices except for glycogen and melanin. Therefore, the following descriptions, which emphasize only the salient features of the nail matrix cells, apply to both. *Basal cells* often contained dense lipid globules (Figs. 1, 5). The endoplasmic reticulum was poorly developed, though RNP particles were abundant. *Squamous cells.* Within the cytoplasm of the

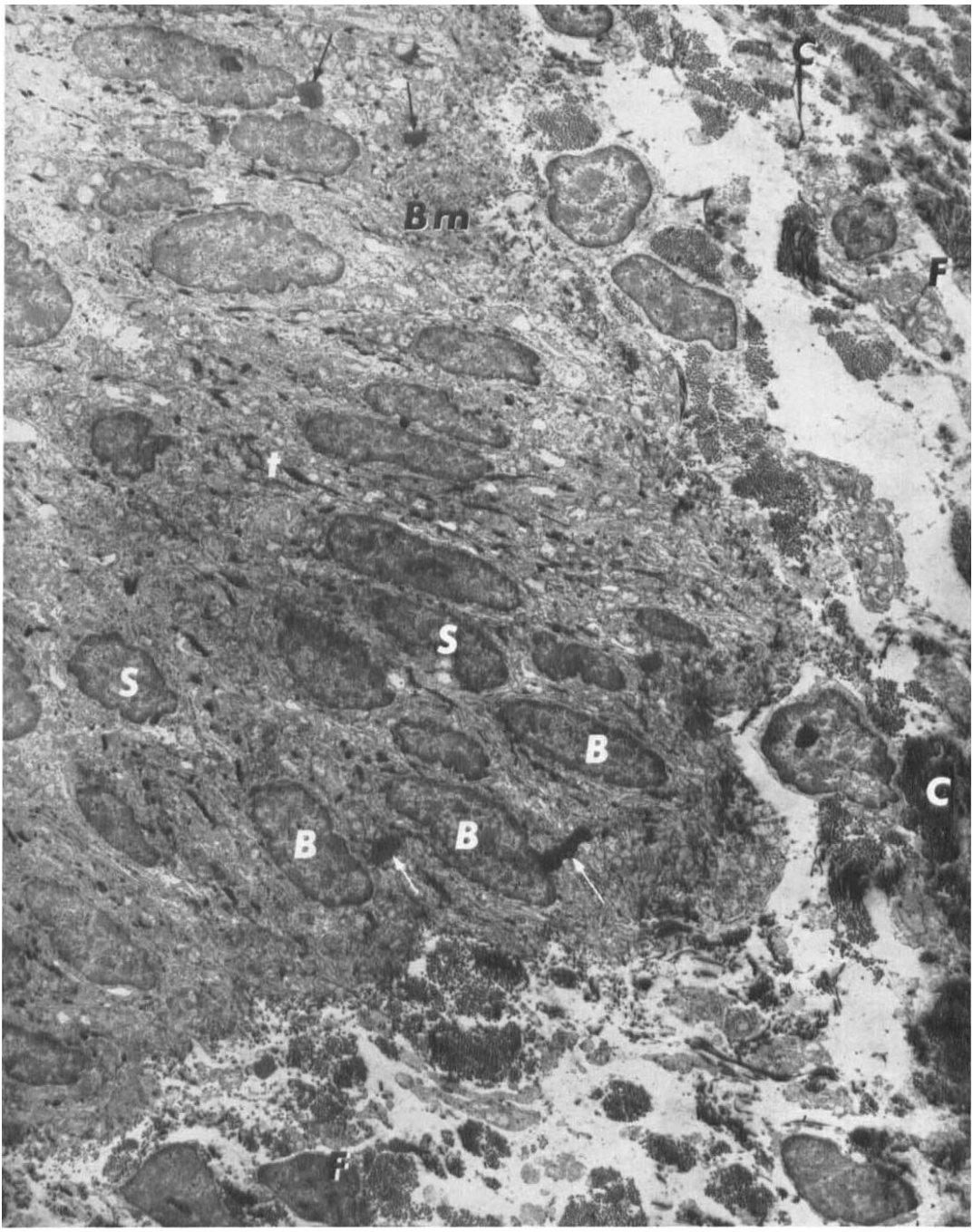

FIG. 1. *The apex of the matrix primordium* is surrounded by a continuous, undulating basement membrane (Bm). The tall columnar basal cells (B) composing the apex form a right angle with the underlying basement membrane and send out squamous cells (S) centrodistally. Arrows: lipid substance. C: collagen. F: fibrocytes. t: tonofilaments. (× 3,060).

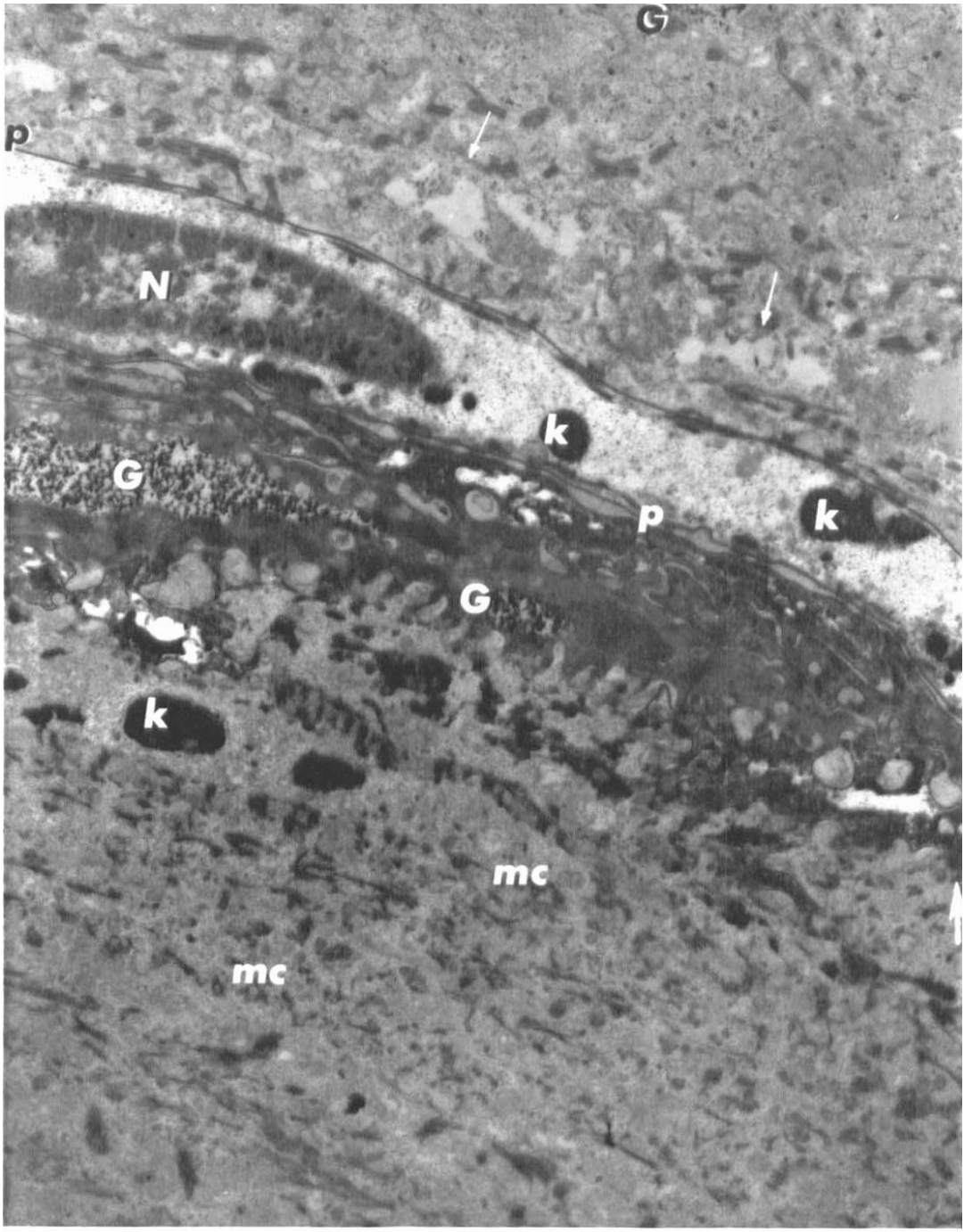

FIG. 2. *At the vertical level of keratinization* (large arrow) the nail plates began to form by keratinization of the squamous cells derived from the basal cells situated proximally to this level. Keratinization took place through the formation of keratohyaline granules (k). Also note the thickened plasma membranes (p) of granular and horny (nail) cells. G: glycogen particles in the dorsal matrix cells and within nail plates. mc: membrane-coating granules. N: nucleus of a granular cell. Small arrows indicate degeneration of upper squamous cells. (× 6,205).

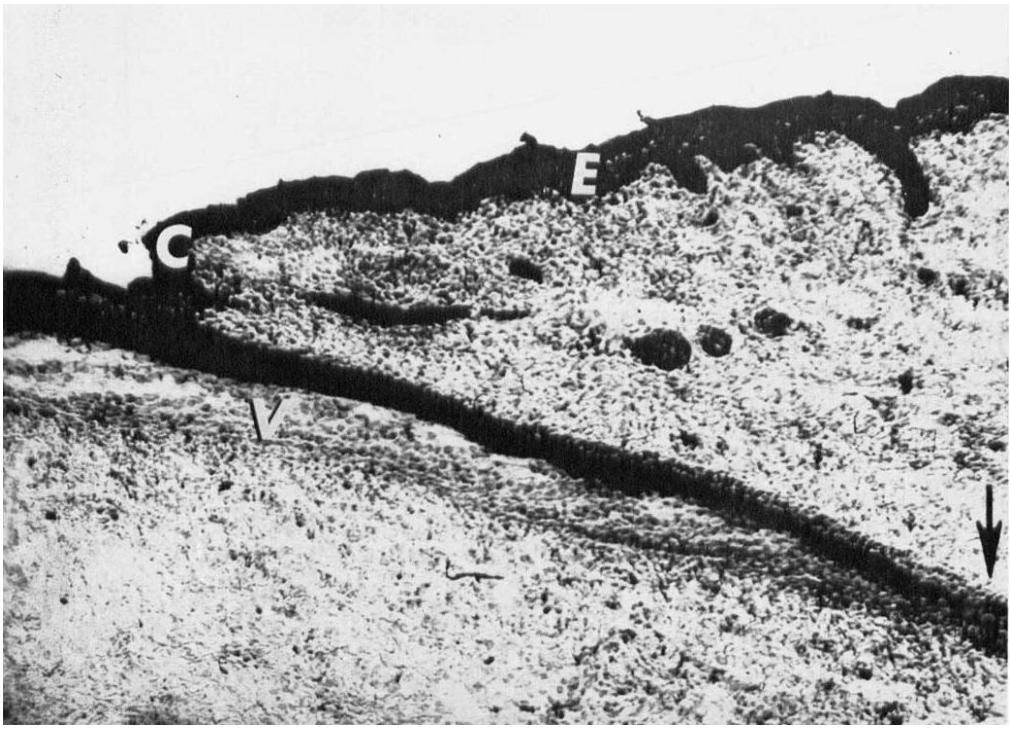

FIG. 3. *Sulfhydryl groups* are strongly positive in the nail plate starting from the vertical level of keratinization (arrow). The thickness of this positive band is increased upwardly and distally. Epidermis (E) also shows a positive reaction. V: ventral matrix cell layers which are definitely thicker than the dorsal counterpart towards the level of the cuticle (C). (× 50).

upper squamous and granular cells, distal to the vertical level of keratinization, lay numerous small round bodies of variable density (Fig. 7a), some of which showed several cristae (Figs. 7a, 7b). They were found both in the ventral and dorsal halves of the matrix, and were similar to those described by Selby (10), Odland (11), Frei and Sheldon (12), and more recently by Matoltsy and Parakkal (13) in keratinizing epithelia. These bodies seemed to be formed in the Golgi apparatus (13) and were often seen attached to the plasma membrane and discharging their contents into the intercellular spaces (Fig. 7b). These bodies will be called the membrane-coating granules in accordance with the terminology of Matoltsy and Parakkal (13). The plasma membranes of the upper granular and horny (nail) cells became definitely thickened in the areas where an active discharge of the membrane-coating granules was seen (Fig. 7a). The PAS stain after diastase digestion revealed a strong positive reaction in the restricted central area along the axis (Figs. 8a, 8b) starting approximately at the vertical level of keratinization and gradually increasing in thickness distally (Fig. 8b). The silver methenamine stain of Movat (11), which stains the same substances as does PAS for light microscopy (7), showed a heavy deposit of silver grains on these bodies, on or near the plasma membrane and intercellular spaces (Fig. 9). *Horny cells* were made up of filamentous components and an amorphous substance which filled the spaces between these filaments (Fig. 7b).

The Nail Bed

The nail bed of the 18 cm-long embryos, at a point just distal to the lunula, was covered by a nail plate of approximately 20 keratinized cells. Beneath the nail plate were several layers of flattened squamous cells, within whose cytoplasm both tonofibrils and keratohyaline granules were seen. As the basal layer was ap-

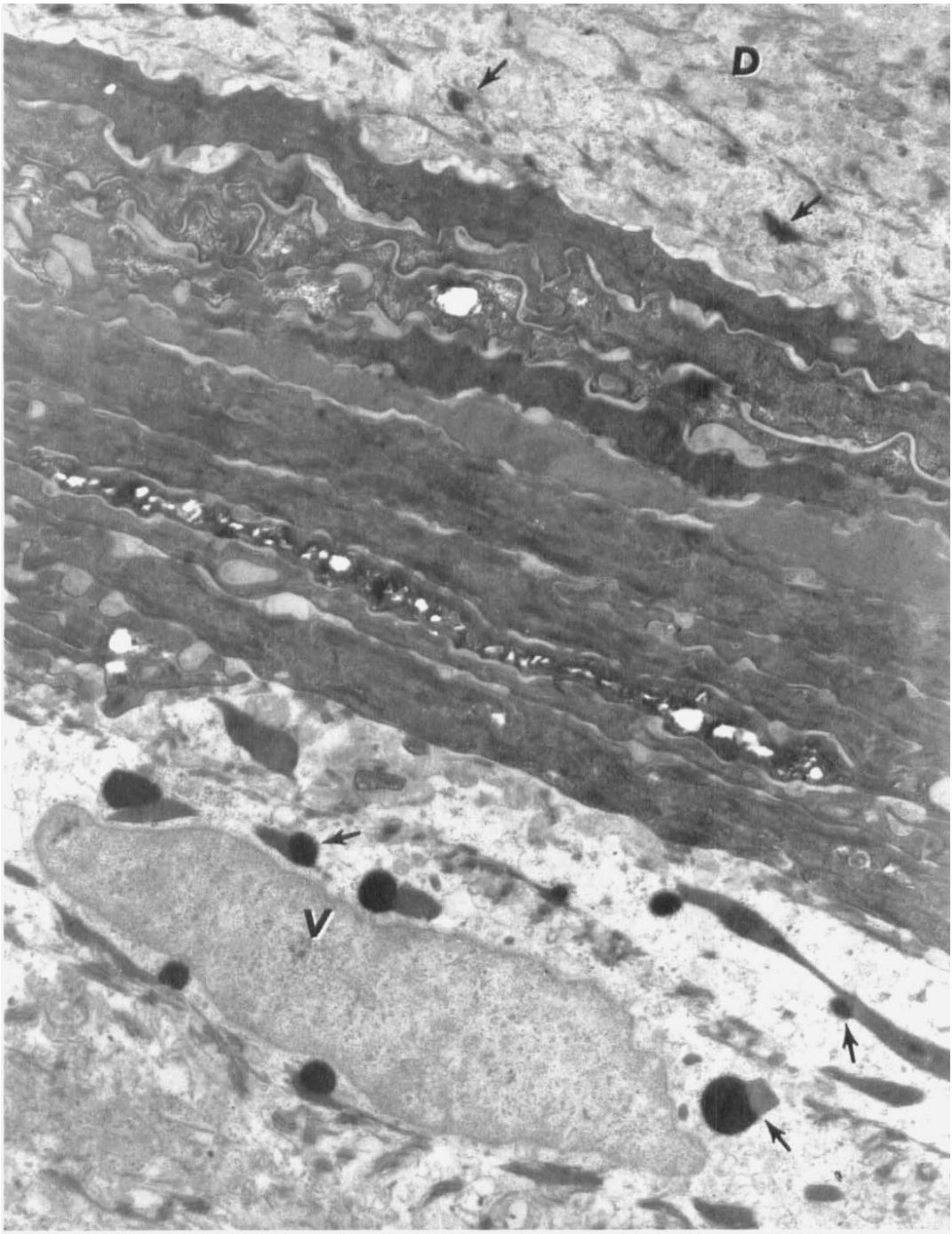

FIG. 4. *The nail plate at about ½ the distance between the apex and the cuticle.* The nail plate has thickened considerably and consists of about 12 layers. Keratinization takes place through the formation of keratohyaline granules which deposit on the bundles of tonofilaments (arrows). Note that the ventral granular cell (V) contains more and larger keratohyaline granules than the dorsal ones (D). It is, however, difficult to distinguish horny (nail) cells produced by the ventral matrix from those contributed by the dorsal matrix. (× 32,560).

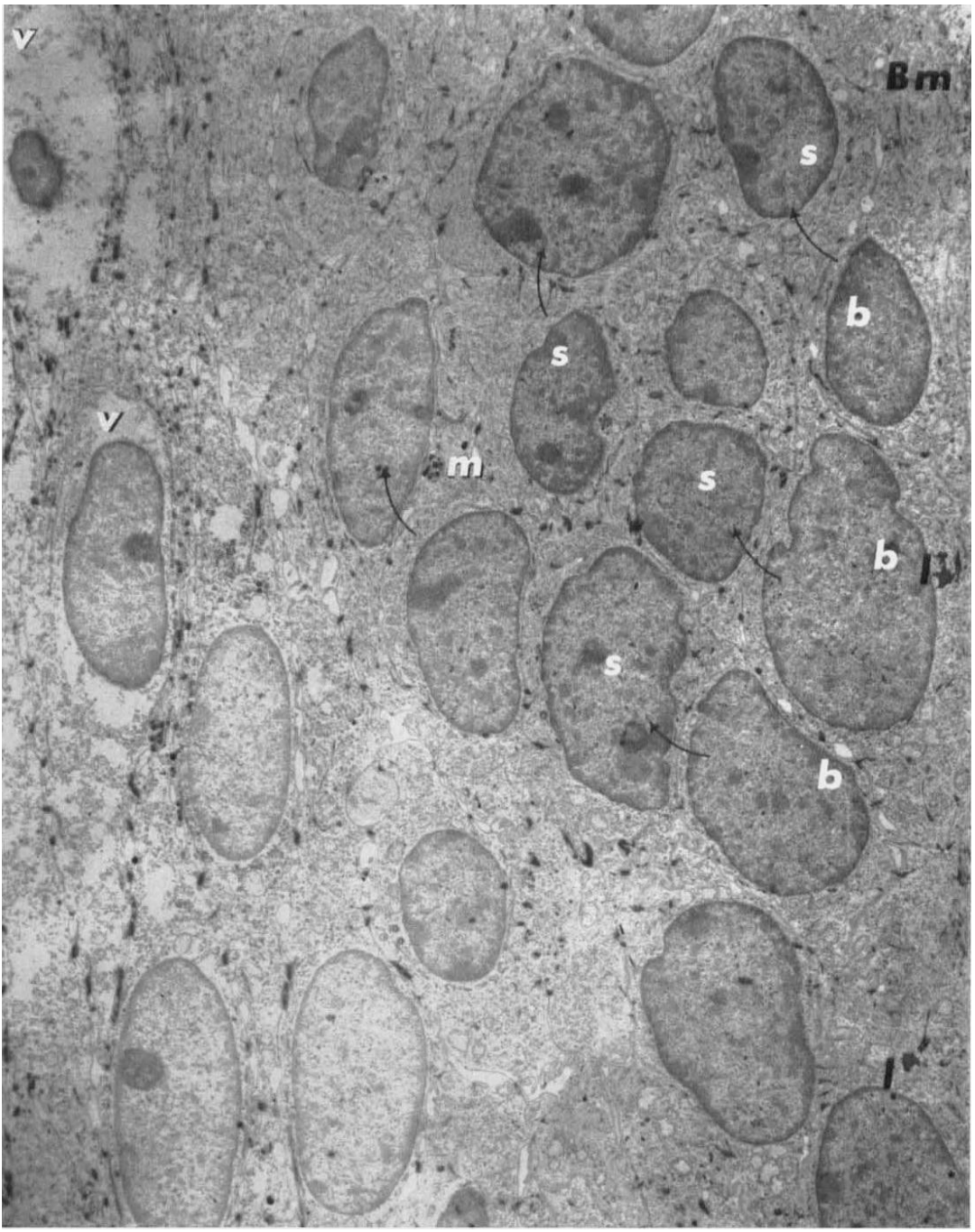

FIG. 5. *Growth directions of the dorsal matrix cells* proximal to the vertical level of keratinization. Each basal (b) cell is inclined centrodistally as well as the squamous cells (s) which derived from them. Arrows indicate a general direction of each cell line. Vacuolated and degenerating cells (v) are seen near the central axis, while the basal cells (b) and the lower squamous cells (s) are well preserved. Bm: basement membrane. l: lipid substance. m: dendrite of melanocytes. (× 4,197).

FIG. 6. *Near the cuticle* the nail plate is increased in thickness. More and larger keratohyaline granules are seen in the ventral matrix (K) than in the dorsal counterpart (*). Glycogen particles (G), melanocytes and Langerhans cells (L), on the other hand, are abundant in the dorsal matrix, whereas the ventral matrix contains none of them. The most distal portion of the connective tissue of the posterior nail fold is seen containing collagen (c) and fibrocytes (f). The basal cells covering this connective tissue send out squamous cells dorsally (S.d), which form the surface epidermis of the cuticle (Ep), distally (S.dis.), which become the cuticle, and ventrally (S.v), which form the most superficial layers of the nail plate. (× 3,060).

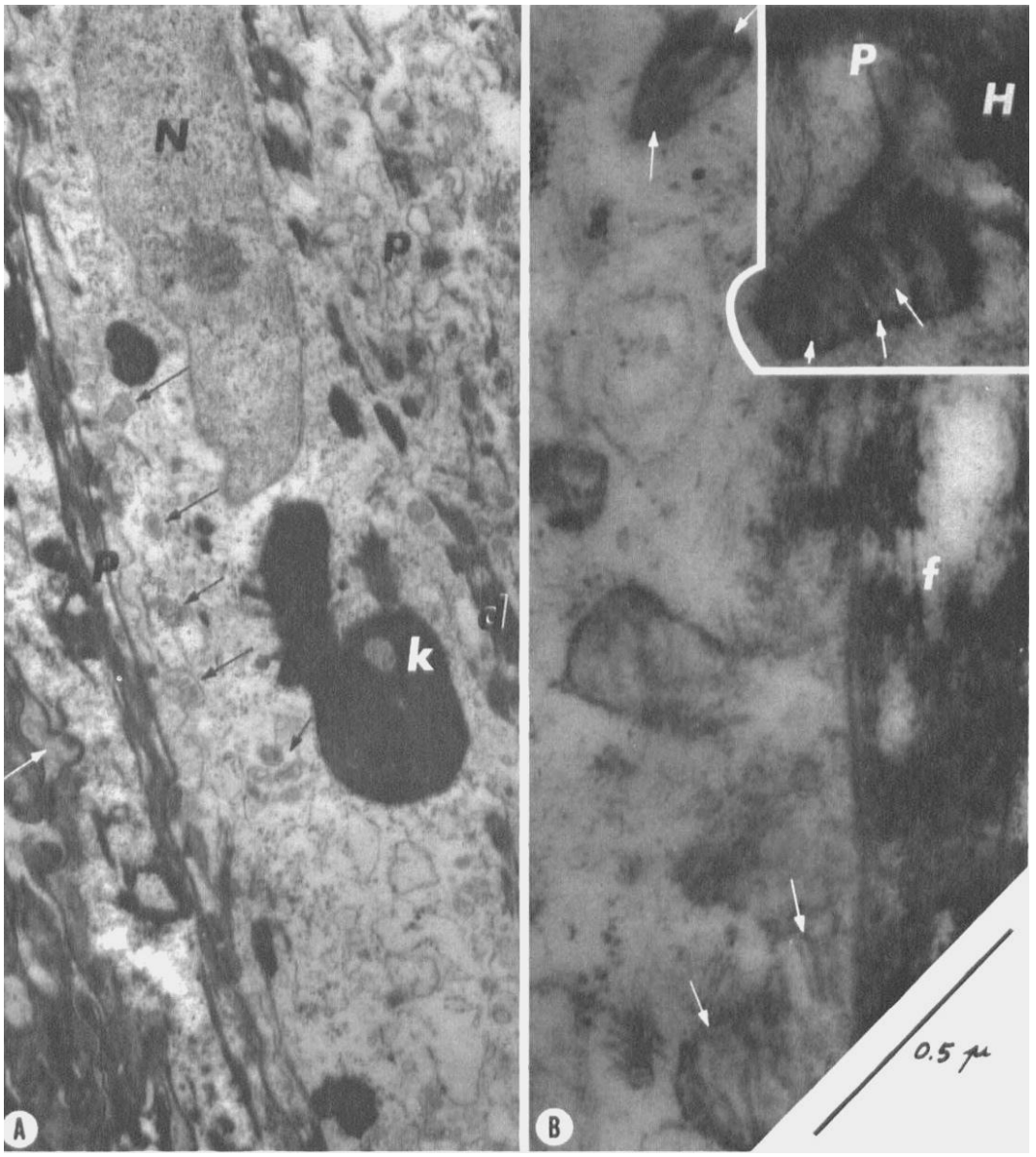

FIG. 7a. *A ventral granular cell* contains a number of membrane-coating granules (black arrows) which show several cristae. Note that the upper plasma membrane (P) of this cell shows a considerable increase of its thickness while the lower border of the same cell is lined by an inconspicuous plasma membrane (p). A large keratohyaline granule (k) is surrounded by numerous RNP particles which are also contained in it. White arrow: discharged membrane-coating granules. d: desmosomes. N: nucleus. (× 34,320).

FIG. 7b. *Membrane-coating granules* are being discharged from granular cells. They clearly show several cristae (arrows). The upper borders (P) of these cells which face the horny (nail) cell (H) are considerably thickened. Horny cells are composed of filaments (f) and an amorphous substance. (× 66,300).

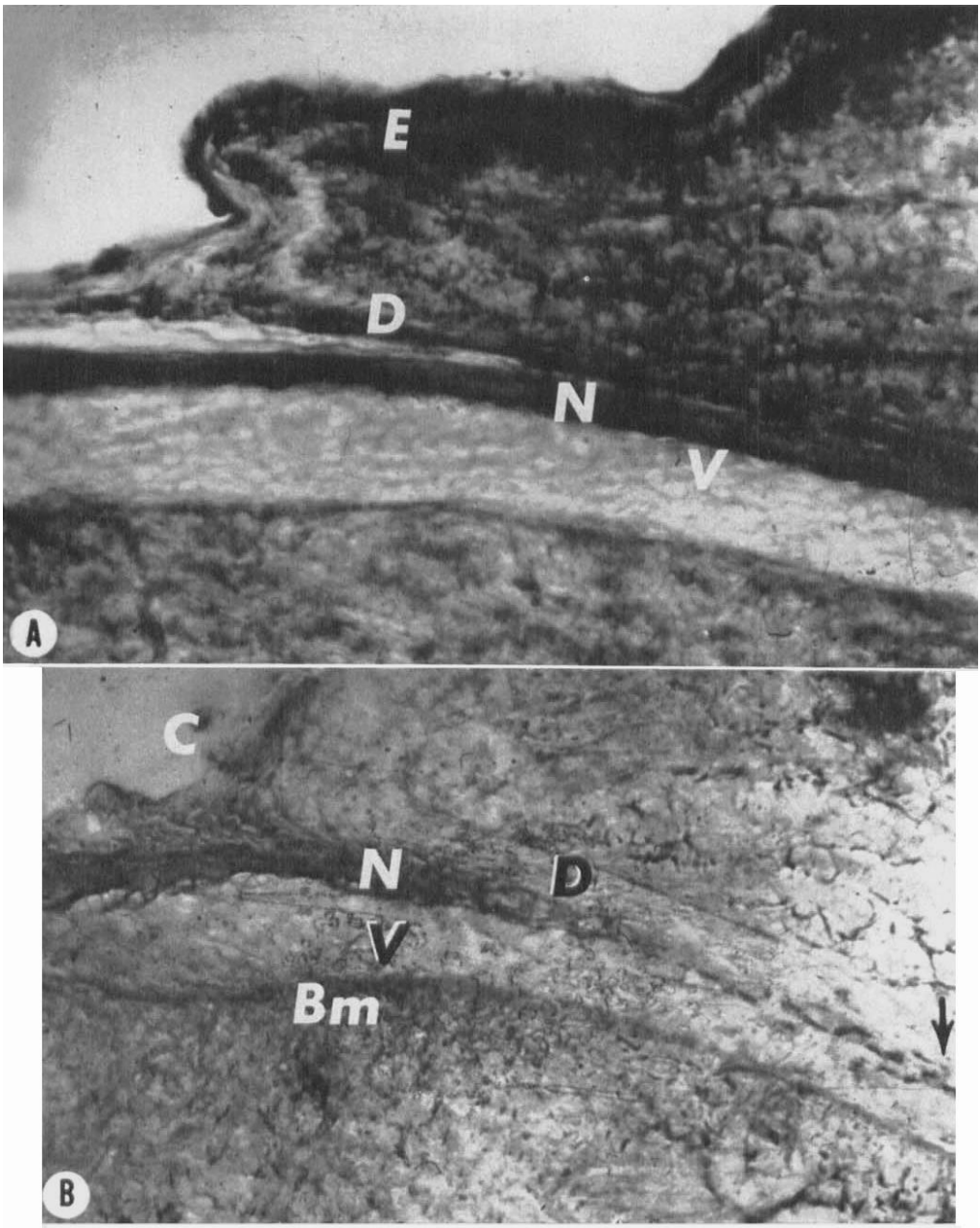

FIG. 8a. *PAS stain* reveals positive reactions in the epidermis (E), in the nail plate (N), and in the dorsal matrix (D), but not in the ventral matrix (V). (× 200).

FIG. 8b. *The diastase-resistant PAS reaction* is seen in and near the nail plate (N). Bm: basement membrane. C: cuticle. D: dorsal matrix. V: ventral matrix. Arrow: vertical level of keratinization. (× 200).

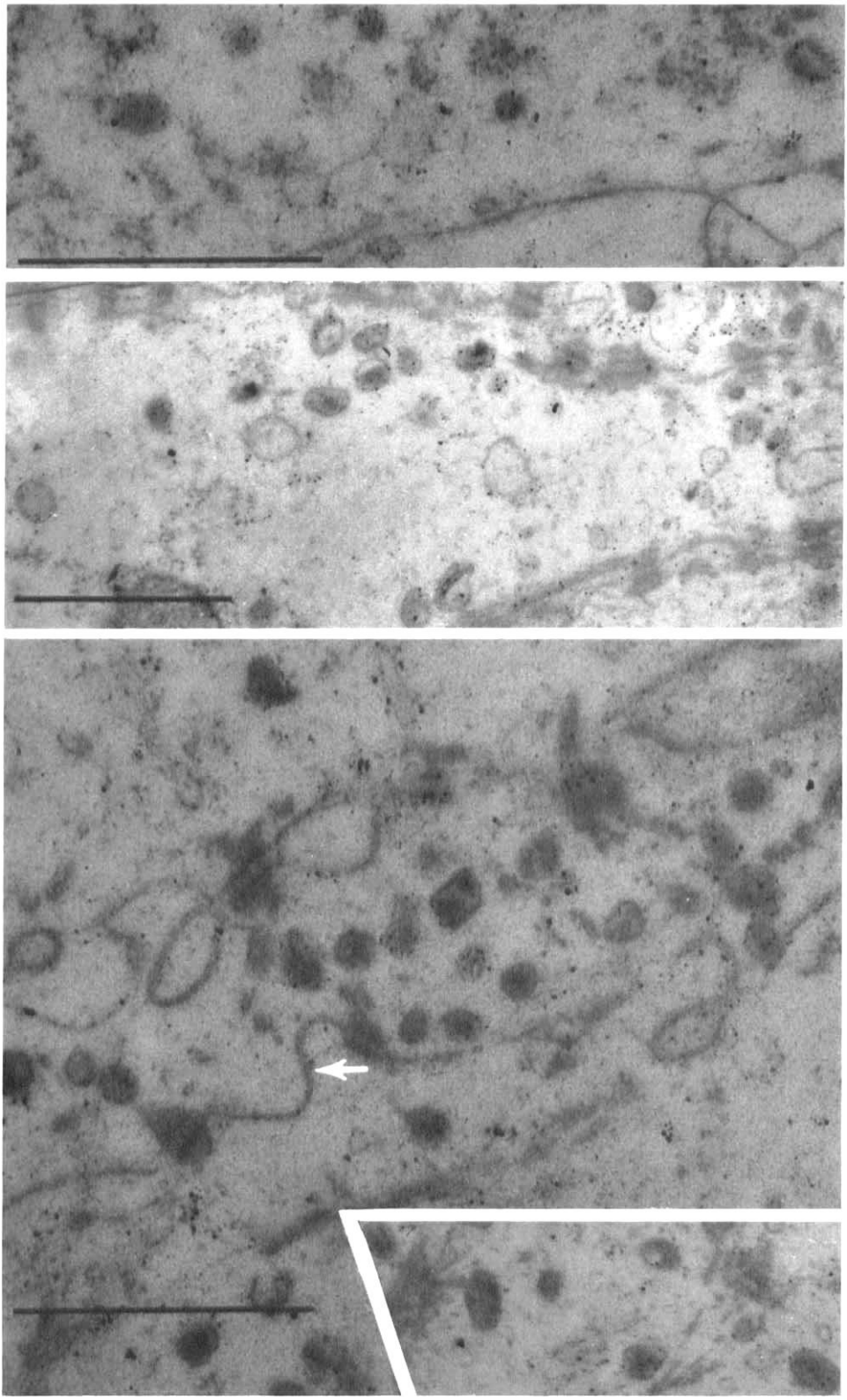

FIG. 9. *Silver methenamine stain of the membrane-coating granules.* Fine silver grains are deposited on these granules. Some deposits are also seen on the plasma membranes (arrow). Lines in each picture represent 1μ.

proached, fewer tonofibrils and keratohyaline granules were observed. The basal cells of the nail bed rested upon a basement membrane which was continuous with the basement membrane of the ventral matrix primordium. The details of the keratinization of the nail bed will be given elsewhere (14).

DISCUSSION

The entire matrix primordium and the nail bed can be regarded as the matrix of the nail plate. The basal cells at the apex of the matrix primordium are oriented in a straight line with the axis of the matrix primordium. Because some of the cells which derived from these basal cells showed signs of degeneration, it is quite possible that these degenerations occurred during fixation, embedding and microtoming, although these changes were also observed in the best preserved specimens in which the ultrastructure of the basal cells (usually the most difficult cell type to preserve) remained intact (Fig. 5). Zaias (3), in his light microscope study of the human embryo nail, reported that many of the matrix cells became vacuolated before reaching the nail plate, the vacuolization and degeneration being to some extent natural processes and not artifacts. The nail plate, which was initially located in the center of the matrix primordium in the proximal portion, was gradually shifted distally upward as the contribution of the ventral matrix became more than that of the dorsal half. Cells derived from the basal cells of both the dorsal and ventral matrices matured in long parabolic arcs converging distally on or above the axis to meet each other. Once the cuticle was reached, the nail plate no longer added to its thickness on the dorsal surface. From this point to the hyponychium, only the nail bed contributed to the nail plate. Although it is not certain whether or not the portion contributed by the dorsal matrix would eventually shed off, the dorsal matrix plays a definite productive role in the formation of the embryonic nail plate.

The process of keratinization was identical in the dorsal and ventral matrices and in the nail bed; it occurred through the formation of dense round or oval-shaped keratohyaline granules and did not differ from the keratinization process of the epidermis or of the inner root sheath of the hair. Keratinization did not occur through the gradual accretion or cementing of the tonofilaments or fibrous keratin, as seen in the so-called hard keratin formation of the hair cortex (15). Morphologically, the keratin of both the epidermis and the embryonic nail plate were identical, being composed of a fibrous element and an amorphous material.

The keratinization began at approximately one-fourth of the distance between the apex of the matrix primordium and the cuticle. This level was critical in that the immature matrix cells began to show all signs of keratinization such as formation of keratohyalin, accumulation of sulfhydryl groups, discharge of membrane-coating granules, and thickening of the plasma membranes. This level deserves the special designation, "vertical level of keratinization," as does the "keratogenous zone" of the hair.

The membrane-coating granules contained silver methenamine-positive substances. This stain corresponds to the PAS stain of light microscopy and stains both glycogen and mucopolysaccharides. Since the lead citrate stain of Reynolds, which has demonstrated the glycogen particles in the squamous cells of the distal dorsal matrix (Figs. 7, 8), was completely negative in these granules, it was concluded that this substance was mucopolysaccharide. The finding that a PAS-positive, diastase-resistant substance was seen in the distal paraaxial region of the matrix, and that this material, along with a silver methenamine-positive substance, were also seen between the cells and on the plasma membranes where the membrane-coating granules were seen in abundance, corroborates our statement that these granules contained mucopolysaccharides, as Matoltsy and Parakkal had assumed in other keratinizing epithelia (13). It may be that the reaction occurred because mucopolysaccharides were deposited by these granules on the plasma membrane.

Kligman (5), on the other hand, believed that the dorsal matrix of the adult nail produced desquamating horny cells, and did not contribute to the nail, but that the cul-de-sac formed by both the ventral and dorsal matrices, which he regarded as a non-productive roof, determined mechanically the outward growth of the nail (5). In this investigation, however, it became certain that the nail, at least in early embryonic life, was formed by

the dorsal, apical, and ventral matrices, and that all the matrix cells were oriented in a centrodistal direction. It now appears obvious why the nail plate grows distally.

SUMMARY

1. An electron microscopic study of the matrix primordium of 16 to 18 weeks old human embryos revealed that it consisted of two horizontal layers, *i.e.*, ventral and dorsal matrices. Proximal to the vertical level of keratinization, which is about one-fourth of the distance from the apex of the matrix to the cuticle, matrix cells did *not* undergo keratinization. Some of these proximal matrix cells were even vacuolated and degenerated.

2. Distal to the vertical level of keratinization, the basal cells of both the ventral and dorsal matrices became mature squamous cells, accumulating tonofibrils, growing into granular cells, containing keratohyaline granules, and finally being transformed into horny cells (nail cells) with densely packed keratin which did not appear to differ from the keratin of the epidermis. This process of maturation took place while the basal cells of both ventral and dorsal halves traveled distally towards the axis of the matrix. At the vertical level of keratinization, horny cells independently produced by ventral and dorsal matrices met on the axis (the geometrical center line) of the matrix primordium. As this band of horny cells, *i.e.*, the nail plate, increased in thickness distally the ventral half contributed more horny cells than its dorsal counterpart, and subsequently the nail plate became localized above the axis.

3. Beyond the vertical level of keratinization the dorsal matrix began to show glycogen and melanocytes in increased amounts nearer the cuticle. The thickness of the ventral matrix gradually increased and reached the plateau beyond the level of the cuticle where the ventral matrix transformed itself into the nail bed and continued alone to contribute nail cells to the nail plate.

4. At the vertical level of keratinization SH groups were stained with DDD stain in the initial formation of the nail plate. At the same level a PAS-positive, diastase-resistant substance also began to appear in the vicinity of the nail plate. By electron microscopy numerous membrane-coating granules were detected at the same level. Silver methenamine stain performed on ultrathin sections was positive in these granules while lead citrate stain did not reveal glycogen particles, thus allowing the conclusion that these granules contained mucopolysaccharides which probably are responsible for the PAS-positive, diastase-resistant substance.

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