THE INTRAEPIDERMAL INNERVATION OF THE SNOUT SKIN OF THE OPOSSUM

A Light and Electron Microscope Study, with Observations on the Nature of Merkel's *Tastzellen*

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ABSTRACT

The intraepidermal innervation of the snout skin of the opossum has been studied with the light and electron microscope. Numerous large nerve fibers loose their myelin sheath in the superficial dermis and pass into the epidermis. The basement membranes of the epidermis and Schwann cell become continuous at the point of entry of the neurite into the epidermis. Within the epidermis, the neurite is associated with a specialized secretory epidermal cell, termed a Merkel cell. This cell has many secretory granules apposed to the neurite. The Merkel cells are epidermal cells since they have desmosomes between them and adjacent epidermal cells. The neurite in the stratum spinosum is enveloped by Schwann cells in a manner analogous to the Schwann cell investment of unmyelinated neurites. In the upper stratum spinosum the nerve fiber evidences changes which can be interpreted as degenerative. The Merkel cell–neurite complex is interpreted as representing a sensory receptor unit.

The presence of neurites *within* a stratified squamous epithelium was first described by Cohnheim (11) in the corneal epithelium of mammals, in 1866, and in human epidermis by his student, Paul Langerhans (20), in 1868. Almost a century has elapsed since these original publications. Countless papers have been written on this subject, especially since such neurites would be related to mechanisms of cutaneous sensibility (43, 47, 49). A lack of enthusiasm in recent years for the thesis that neurites enter the epidermis is due in part to numerous internal contradictions present in the vast literature dealing with this subject (7, 9, 43, 49). Such contradictions might be expected on the basis of the methodology used during the past century; that is, metallic deposition or methylene blue staining for demonstrating neurites. Such technics can be capricious and the interpretations subject to the attitudes and competence of the observer. Recent improvements in the methods available for staining neurites in frozen section described by Richardson (39), together with the greater inherent resolution of the electron microscope, permits a reevaluation of the problem, which is the objective of the present study on the snout skin of the opossum.

In 1871 Eimer (14) described in the glabrous skin of the mole snout a truly phenomenal arrangement of intraepidermal neurites ensheathed in a column of specialized epidermal cells. This column of epidermal cells was in the core of large columnar rete pegs. This anatomical configuration has since been referred to as Eimer's organ. This system was used by Merkel (27, 28), Ranvier (35,

36), Retzius (37), Bielschowsky (2), Dogiel (12), Botezat (8), Kadanoff (17), Boeke (5–7), and Gronweg (15), as a test system for the study of intraepidermal innervation. As described by these investigators, myelinated neurites approach the epidermis, lose their myelin sheaths, cross the basement membrane, and course through the epidermis into the stratum lucidum where some beading occurs in the terminal portion of the neurite. The cytologic description of this intraepidermal innervation varies somewhat with the author. It is necessary to review briefly two concepts of historical importance which, on the basis of the present study, assume new importance. The first is that of Merkel's *Tastzellen.*

Merkel (27, 28) described specialized epidermal cells near the base of the rete peg in the snout skin of the mole which he termed *Tastzellen.* He considered these *Tastzellen* as cellular transducers of physical stimuli to the neurite and entirely analogous to the cellular transducers found in organs of special sensation; *i.e.*, hair cells in the organ of Corti, rods and cone cells in the retina, etc. *Tastzellen* were described as being present in Meissner's corpuscles, genital end bulbs, Grandry's corpuscles, Pacinian corpuscles, sensory hairs, and virtually everywhere in stratified squamous epithelia, especially in non-hairy skin. These cells were best visualized in osmium tetroxide-fixed preparations, and Merkel (27, 28) described them as large vesicular cells with large pale nuclei, the size and density of which were different from that of surrounding epidermal cells. When a neurite was found adjacent to the *Tastzelle* the complex was termed a *Tastkörperchen.* The neurite in this instance frequently appeared to be somewhat expanded adjacent to the cell and subsequently was termed Merkel's disc by other authors (5–7, 36, 41, 50). While the term Merkel's disc is used frequently in the literature today, the identity of this nerve ending is further confused by the use of the term hederiform ending. The same expansions of the neurite described by Merkel (27, 28) (in association with *Tastzellen*) have also been termed hederiform endings by Ranvier (35), and this type of ending has also been described by Dogiel (12), Retzius (37) and Botezat (8). These subsequent studies have buried the original concepts of Merkel (27, 28), supported by studies of Kadanoff (17) and Tretjakoff (44), with coining of new terms for the same morphologic entity or using the original terms with totally different implications. The

FIGURE 1 Opossum snout skin, stained with hematoxylin and eosin. Elongated rete pegs extend deeply into the dermis. A very thick stratum corneum is present, with no evidence of a stratum granulosum. × 200.

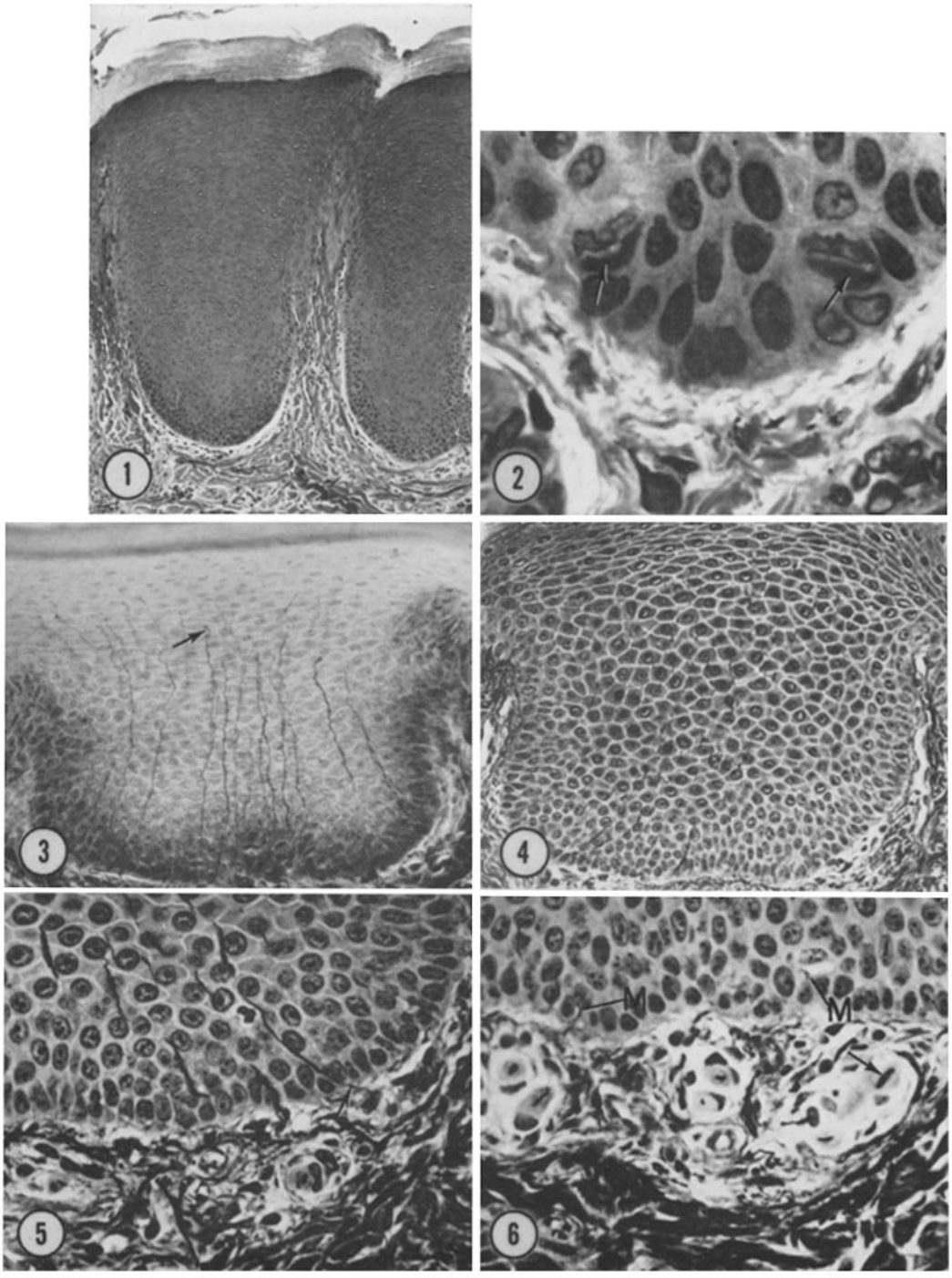
FIGURE 2 Opossum nose skin, OsO_4-fixed, Epon-embedded, stained with PAS and hematoxylin (29). Two Merkel cells are present with prominent PAS-positive cytoplasm (arrows). The nuclei of Merkel cells are irregular in profile as compared to those of the surrounding epidermal cells. The PAS-positive zones (arrows) are red in the original slide, and the nuclei blue-grey. × 800.

FIGURE 3 Opossum nose skin, frozen section, 20 μ thick, Richardson silver method (39). Numerous neurites course through the rete peg and evidence beading in their terminal portions (arrow). × 300.

FIGURE 4 Paraffin section, 6 μ thick, silver method (42). Individual neurites can be traced over a relatively short course and are numerous throughout the rete peg. The neurites are black on a yellow-brown background in the original preparation. × 300.

FIGURE 5 Paraffin section, 10 μ, silver method (42). One neurite can be traced coursing through the basement membrane of the epidermis (arrow). The neurites appear to penetrate the cytoplasm of the epidermal cells (see also Fig. 11). × 400.

FIGURE 6 Paraffin section, 10 μ, silver method (42). At the base of the rete peg numerous small encapsulated nerve endings with central neurites (arrow) are present in the dermis. Merkel cells and associated neurites (*M*) are also present. The Merkel cells have a clear cytoplasm and the neurites are closely applied to one side of the cell. × 400.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recent publications of Cauna (9) and Weddell (49), however, are an exception to this rule and have correctly stated Merkel's thesis.

A second consideration of historical importance, and one that has also been shrouded by controversy, is the concept of Boeke (5–7) that neural-epithelial fusion (or intercellular cytoplasmic continuity) occurs regularly in specialized nerve endings and in intraepidermal innervation. In his excellnt drawings of 3 to 4 μ thick silver-stained sections, the neurites appear to penetrate the epidermal cells (confirmed by Kadanoff, reference 17), and he argued on this basis for neural-end organ syncytial formation. This view was subject to considerable skepticism, and Ranvier (35, 36), Dogiel (12), Tretjakoff (44), and Bielschowsky (2) were chief exponents of the view that these nerves were *inter*cellular. Cauna (9) has recently shown that in macerated epidermis of the pig's snout the neurites indeed seem to be intracytoplasmic.

The present study was undertaken to determine the nature of the relationships between neurite and epidermis in the snout skin of the opossum, the results of which are the basis of this report.

MATERIALS AND METHODS

The glabrous skin of the nose of five adult opossums, *Delphis marsupialis*, (3 female, 2 male) was removed under phenobarbital or ether anesthesia. Part of the tissue was prepared for light microscopy by fixation in Richardson's formalin sucrose (39) or in 10 per cent neutral buffered formalin (21). Portions of the formalin-fixed tissue were processed for paraffin embedding. The rest was stored in the fixative for frozen sections.

Frozen sections, 10 to 30 μ thick, of tissue fixed with Richardson's formalin-sucrose fixative were stained with the ammoniacal silver method of Richardson (39). Paraffin sections, 5 to 20 μ, of tissue fixed in neutral buffered formalin were stained with a modification of the ammoniacal silver method of Richardson as described in detail by Sevier and Munger (42).

Paraffin sections were also stained with hematoxylin and eosin, periodic acid–Schiff (PAS) reaction with and without diastase digestion, phosphotungstic acid–hematoxylin with and without prior $KMnO_4$ oxidation (21), aldehyde fuchsin with and without prior $KMnO_4$ oxidation, colloidal iron, alcian blue, chrome alum–hematoxylin, the ferrocyanide method for melanin, the diazo coupling method for enterochromaffin, the tetrazolium salt fast red for phenolic groups, methionine silver, and the chromate and periodate methods for chromaffin tissue (32). The DOPA reaction on fresh tissue was done according to Pearse (32).

For electron microscopy, small cylinders of skin were fixed in osmium tetroxide in White's saline with extra calcium (1, 40) or in 3 per cent glutaraldehyde followed by postfixation in OsO_4 and embedded in Epon 812 or in Swiss Araldite (Durcupan, Fluka). Thin sections were mounted on uncoated grids, stained with uranyl acetate, lead acetate or lead citrate (38), and examined in RCA EMU-3C and 3G electron microscopes.

OBSERVATIONS

Light Microscopy of Nerve Fibers

The epidermis of the nose skin of the opossum is organized into large and clearly circumscribed rete pegs approximately 0.2 mm in diameter (Fig. 1). These rete pegs are visible on the skin surface as discrete round elevations. (The term rete *peg* is appropriate in this case since the prolongations of epidermis are of cylindrical shape as opposed to rete *ridges* of human skin.)

Beneath the rete pegs are numerous small encapsulated nerve endings which can best be seen in silver-stained sections (Fig. 6). Endings of this type in cats have been termed sensory end organs by Winkelmann (50).

The large rete pegs of the opossum nose skin contain numerous neurites coursing from the base of the rete peg towards the surface of the skin (Figs. 3 and 4). These fibers in their course through the dermis are myelinated, but as they approach the epidermis they lose their myelin sheath, turn abruptly towards the skin surface and enter the epidermis. In the basal layers of the epidermis the neurites are associated with a vesicular PAS-positive cell with a large irregular nucleus identified as Merkel's *Tastzelle* (Figs. 2 and 6). This cell will be termed a Merkel cell in the remainder of the report, as suggested by Tretjakoff (44), and this terminology will be justified in the discussion. The Merkel cell always has a vacuolated cytoplasm in paraffin sections. The neurite usually conforms to the shape of the Merkel cell, and at the point of contiguity the neurite appears to be expanded. In paraffin section the cytoplasm, artifactitiously contracted to one side of the cell, is PAS-positive and diastase-resistant. In OsO_4-fixed preparations stained with PAS reaction (Fig. 2) the Merkel cell does not evidence this vacuolization artifact, and a diffuse PAS-positivity is present only on *one* side of the irregular nucleus (Fig. 2). This zone of PAS-

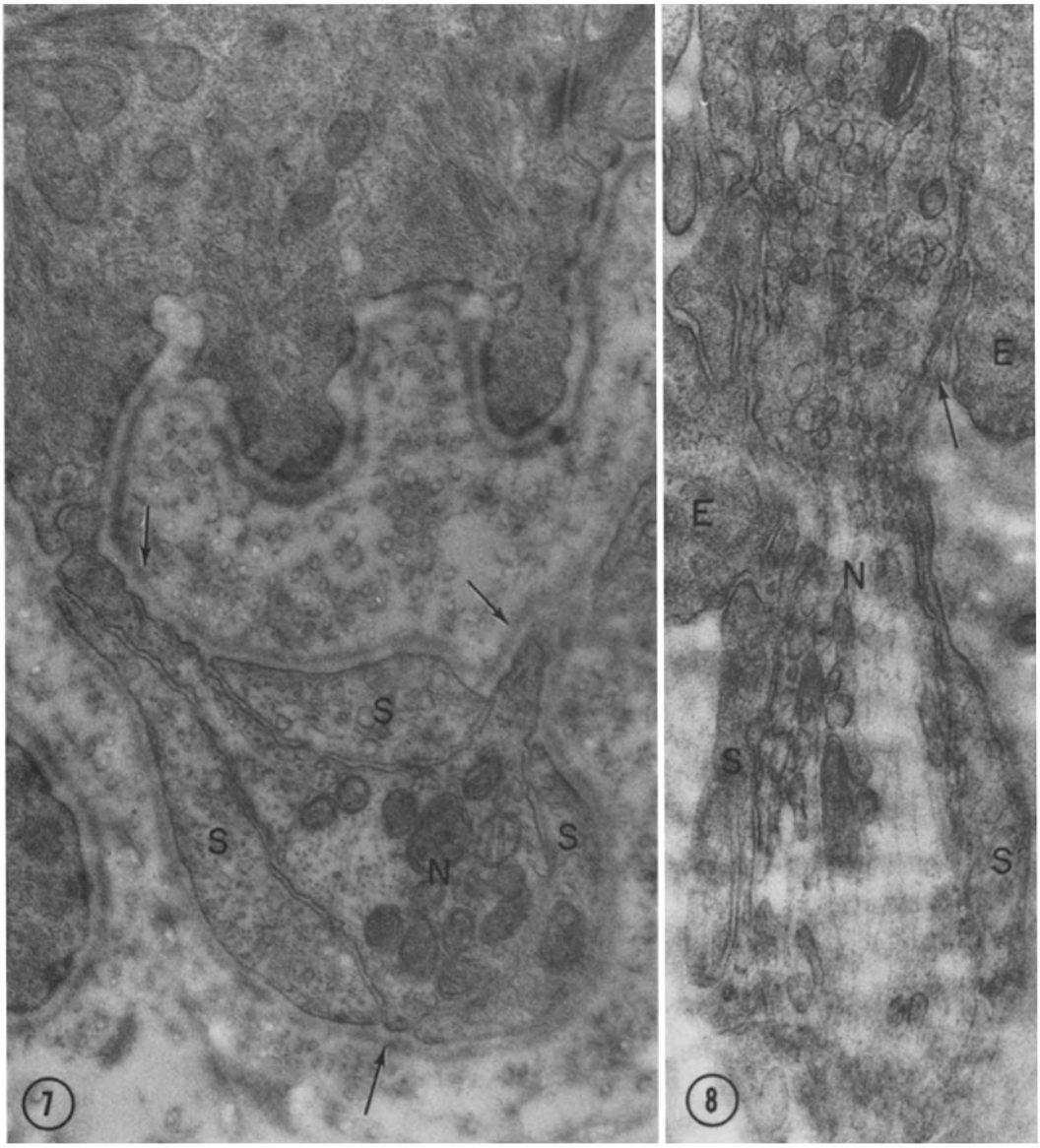


FIGURE 7 A dermal neurite (*N*) is in contiguity with the epidermis. The Schwann cell (*S*) surrounding the neurite (*N*) extends small fingers of cytoplasm towards the epidermis. The neurite itself is not covered by Schwann cell cytoplasm at three points (arrows), but the basement membrane, continuous with that of the epidermis, covers the neurite. Uranyl acetate and lead hydroxide. × 30,000.

FIGURE 8 A longitudinal section of a neurite (*N*) penetrating the epidermis (*E*). Delicate fingers of Schwann cell cytoplasm (arrow) extend into the epidermis and interdigitate with processes of epidermal cell cytoplasm. The neurite within the epidermis contains vesicles of varying size. Uranyl acetate and lead hydroxide. × 40,500.

positivity corresponds to the zone of secretory granules seen by electron microscopy in Figs. 9 and 10.

From the base of the rete peg the neurites pass in a relatively straight course to the upper limits of stratum spinosum where they undulate in a corkscrew path (Figs. 3 and 4). In silver-impregnated paraffin sections (42) the nerve fibers are

clearly seen to have *no* relationship in their course to the intercellular junctions between adjacent epidermal cells (Figs. 4, 5, and 11). Instead these fibers course apparently through the cytoplasm of the epidermal cells. The fibers vary in size, some of them being obviously smaller than others (Fig. 4).

As the neurites approach the stratum lucidum they show a beading (Figs. 3 and 16), which becomes marked in the lower stratum corneum where small bits of fibers appear to be disconnected from the nerve fiber itself. In the middle and upper stratum corneum no nerve fibers can be identified.

The course of the nerve fibers also can be visualized in paraffin sections stained with hematoxylin and eosin. In such preparations the nerve fibers are a negative image on an eosinophilic cytoplasm, again showing no relationship to intercellular junctions.

Electron Microscopy

In electron micrographs only small segments of the nerve fiber can be identified at any one time, and hence a total picture of the course and relationships of the neurites is a synthesis of many different preparations.

Numerous unmyelinated nerve fibers surrounded by a thin sheath of Schwann cell cytoplasm are present in the dermis immediately below the epidermis. These fibers approach the basement membrane of the epidermis and intermingle with processes of the epidermal cells extending into the dermis (Fig. 7). The neurites then pass into the epidermis *between* adjacent epidermal cells. The basement membrane of the Schwann cell fuses with that of the epidermis (Figs. 7 and 8), as processes of the cytoplasm of epidermal cell and Schwann cell intermingle at the level of the epidermal basement membrane. Delicate fingers of Schwann cell and epidermal cell cytoplasm interdigitate, encasing the neurite as it enters the epidermis (Fig. 8). In rare instances the presence of a gap in the Schwann cell sheath puts the neurite in contiguity with the basement membrane (Fig. 7).

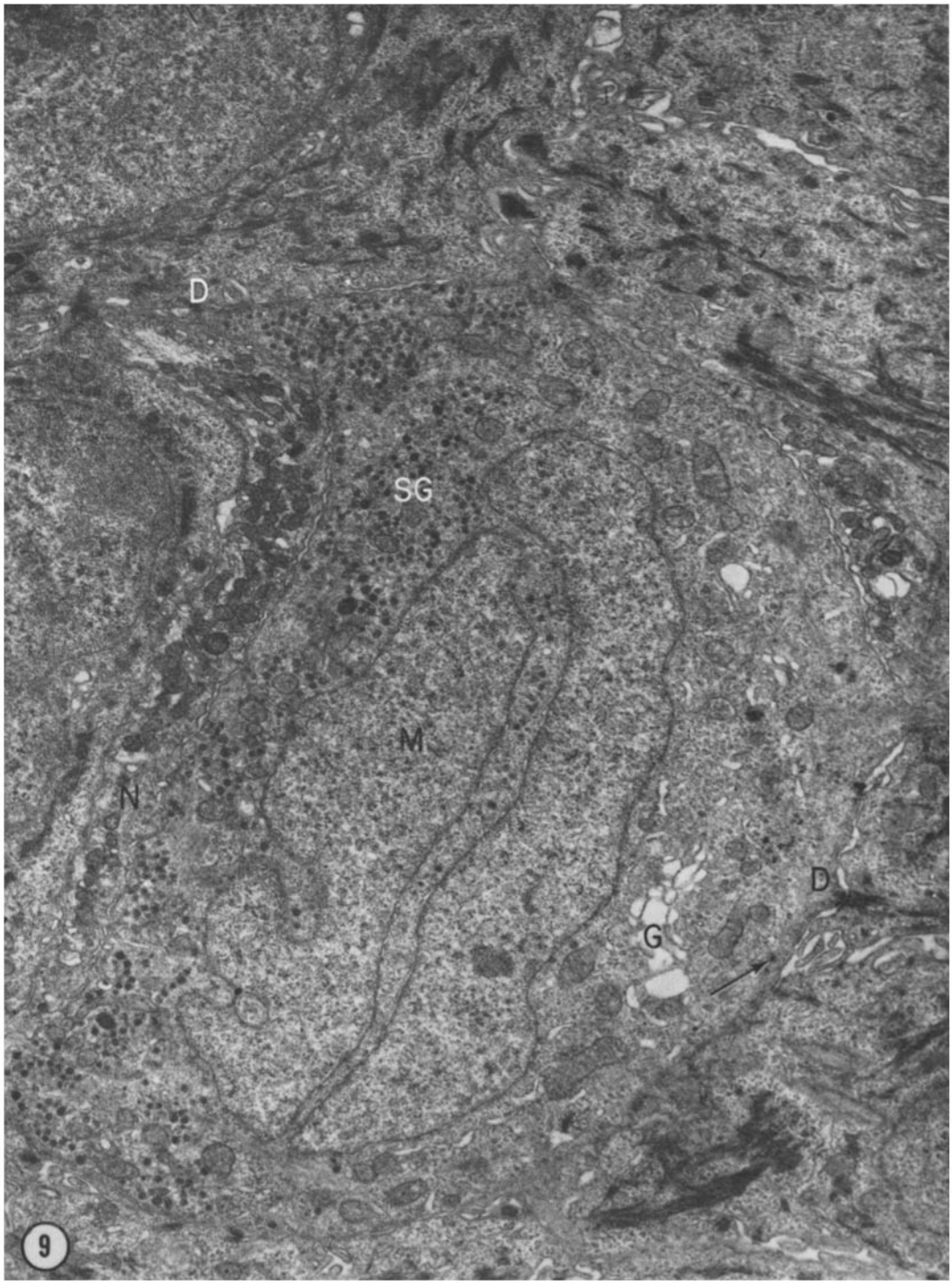
The axon proceeds a few microns into the epidermis and frequently becomes intimately associated with a cell of unique characteristics, which, on the basis of arguments presented in the discussion, is identified as Merkel's *Tastzelle* (27, 28), termed a Merkel cell (44) in the present report. This cell is characterized by the presence of many 100 mμ granules of moderate electron opacity surrounded by agranular limiting membranes, ultrastructurally similar in every respect to secretory granules (Figs. 9 and 10). The granules are segregated in the cytoplasm apposed to the nerve fiber. In some instances these presumptive secretory granules in the Merkel cell appear to be intimately associated with the plasma membrane adjacent to the nerve fiber, and in some cases they appear to fuse with it.

The Merkel cell itself is much larger and less electron-opaque than adjacent epidermal cells (Fig. 9), and its nucleus is lobulated and irregular in outline, unlike those of the surrounding epidermal cells. The irregular lobulation of the nucleus and the low over-all electron opacity of the cell permit recognition of the cell at low magnification. Other cells also are present in the epidermis with which Merkel cells can be confused. These will be described later.

The Golgi apparatus of the Merkel cell is large and prominent. It is invariably present on the side of the nucleus *away* from the nerve fiber and accumulation of secretory granules (Fig. 9). A few secretory granules are present near the Golgi apparatus (Fig. 9), and profiles presumed to be prosecretory granules can usually be identified associated with the Golgi apparatus. Mitochondria are scattered throughout the cell, as are free RNP particles and profiles of the granular endoplasmic reticulum (ER) (Fig. 10).

Numerous desmosomes are present between the

FIGURE 9 A Merkel cell (M) and associated neurite (N) are illustrated at low magnification. The nucleus of the Merkel cell presents two lobes with some intervening cytoplasm. The secretory granaules (*SG*) are accumulated on the side of the nucleus opposite from the Golgi apparatus (*G*) and they are subjacent to the neurite. A few secretory granules (arrow) are usually seen near the Golgi apparatus. Desmosomes (*D*) are present between the Merkel cell and adjacent epidermal cells. The neurite contains many mitochondria. Uranyl acetate. × 12,000.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Merkel cell and adjacent epidermal cells (Figs. 9 and 10). Bands of epithelial filaments (26) course through the cytoplasm of the Merkel cell (Fig. 10), although they are not grouped into obvious fibrils as is the case in epidermal cells.

The nerve fiber itself lies in very close apposition to the Merkel cell (Figs. 9 and 10). The adjacent plasma membranes of neurite and Merkel cell tend to be separated by a very narrow gap, even when intercellular spaces are present among the surrounding epidermal cells. A large expanse of the surface of the Merkel cell is frequently associated with the neurite. However, three-dimensional reconstruction of this relationship was not done to confirm the impression that the neurite is expanded as it apposes the Merkel cell. Such a conclusion is also supported by evidence from silver-stained paraffin sections (Figs. 6). At times the plasma membranes of neurite and Merkel cell are so close as to suggest a possible functional junction, but the plasma membranes evidence no areas of increased electron opacity.

The neurite in apposition to the Merkel cell frequently contains accumulations of mitochondria (Fig. 9) and lipoid material. Only in rare instances does the neurite in this location appear "normal" (Fig. 10). Similar accumulations of lipoid material and mitochondria are also seen in neurites high in the epidermis.

Neurites only rarely end on Merkel cells; usually they ascend into the epidermis of the rete peg after coming into apposition with the Merkel cell. The neurite bears a unique relationship to cells of the stratum spinosum. It becomes enveloped by processes of epidermal cell cytoplasm (Figs. 11, 12, 13, and 14). The epidermal cells thus "grip" the nerve fiber in a manner analogous to a Schwann cell investing an unmyelinated axon. The nerve fiber itself is only rarely in contact with the intercellular spaces between processes of the cells of the stratum spinosum. When the fiber passes from one epidermal cell to another, processes of the adjacent epidermal cells separate it from the intercellular space (Fig. 13). In preparations in which a degree of separation exists between adjacent cells of the stratum spinosum (spongiosis), separation only rarely occurs between the neurite and the adhering plasma membranes of the epidermal cells. In some sections a tangential cut of a junction between epidermal cells adjacent to the nerve fiber is found. In such cases myriads of desmosomes will be seen immediately adjacent to a nerve fiber (Fig. 12).

When a neurite within an epidermal cell is cut in cross-section, a mesaxon-like structure of the epidermal cell can usually be observed (Fig. 14). This mesaxon can be identified by tracing the intercellular space between epidermal cells to the neurolemma. However, in some instances no evidence of such a mesaxon can be seen (Fig. 15). The plasma membrane of the epidermal cell and the neurolemma appose one another with no evidence of discontinuity of the epidermal plasma membrane.

Expansions of the nerve fiber become evident in the upper regions of the stratum spinosum and stratum lucidum (Fig. 17). These undoubtedly correspond to the beading seen in the silver stains (Fig. 16). Such expansions contain masses of mitochondria, myelin figures, vesicles and vacuoles, and dense lipoid bodies. Remnants of nerve fibers have been difficult to identify within the stratum corneum itself, but in this layer are found bits of cell debris which could represent bits of nerve fibers.

A definite variability exists as to the structure of the nerve fiber itself. Some fibers appear perfectly normal (Figs. 12, 13, and 14), that is, contain central elongated mitochondria, neurofilaments, delicate microtubules, and a few membranous profiles of the agranular endoplasmic reticulum. However, many nerve fibers evidence alterations of this "normal" appearance. In some neurites the mitochondria are so numerous that they virtually fill the fibers (Figs. 9 and 17). In such instances myelin figures and dense lipoid bodies are usually present. Also, in some fibers the neurofilaments become clumped and more electron-opaque than usual. These types of change can be seen throughout the course of the nerve fibers from the dermis to the stratum corneum. Especially interesting is the rarity with which we have seen normal nerve fibers in association with the Merkel cell, whereas a short distance from the Merkel cell the neurites are usually normal in appearance. Also of interest is the observation that the central nerve fiber of encapsulated sensory nerve endings in the dermis frequently demonstrates alterations in structure similar to those seen in the epidermal nerve fibers.

Identification of the Merkel Cell

An attempt to identify the Merkel cell's secretory granules by light microscopy has yielded predominantly negative results.

In the opossum, the skin of the nose is albino

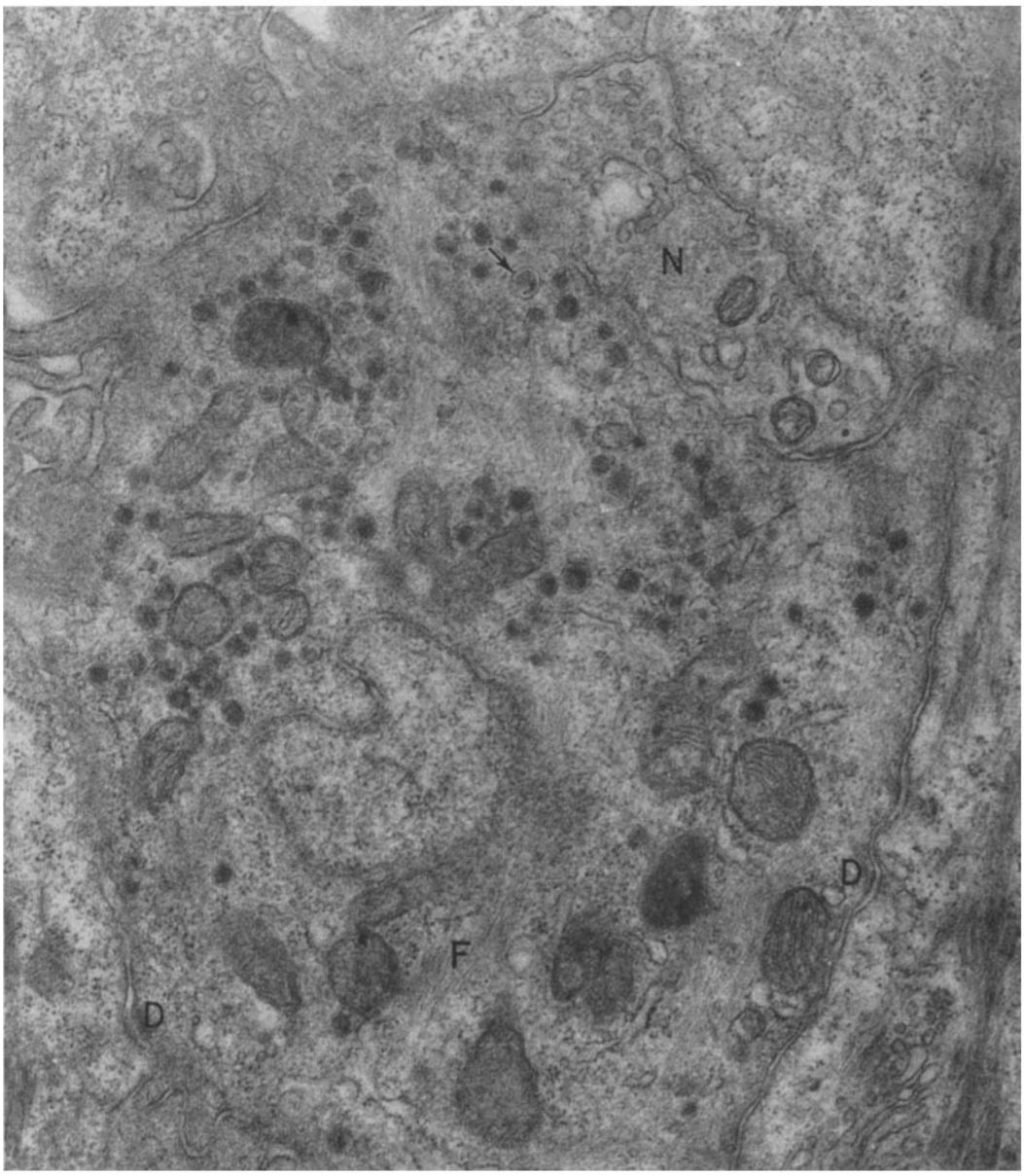


FIGURE 10 A portion of a Merkel cell and associated neurite (*N*) are depicted at higher magnification. The Merkel cell has several desmosomes (*D*) between it and the surrounding epidermal cells. Numerous epithelial filaments (*F*) course through the cytoplasm of the Merkel cell. The secretory granules of the Merkel cell have an electron-opaque internal core and an agranular limiting membrane (arrow). Scattered sacs of the granular endoplasmic reticulum and mitochondria are present throughout the cytoplasm. A small portion of the nucleus of the Merkel cell appears in the center of the micrograph. Lead acetate. × 32,000.

but that of the remainder of the body is predominantly pigmented, with scattered albino areas in the hairless skin of the feet. The possibility was considered that Merkel cells are melanocytes and that the granules are melanin. These cells are negative to weakly positive when stained with methenamine silver and ammoniacal silver. The ferrocyanide reduction for melanin and the DOPA reaction were negative in the entire epidermis of the snout, but positive in skin elsewhere in the body.

The only stains which selectively demonstrated these cells were PAS and aldehyde fuchsin. The granules are distinctly PAS-positive and diastase resistant, best seen in sections of OsO_4-fixed, plastic-embedded tissue. Aldehyde fuchsin positivity is much more intense after prior $KMnO_4$ oxidation, but even without prior oxidation the reaction was definitely positive. All other stains for secretory granules, carbohydrates, adrenalin, chromaffin tissue, and catecholamines were negative.

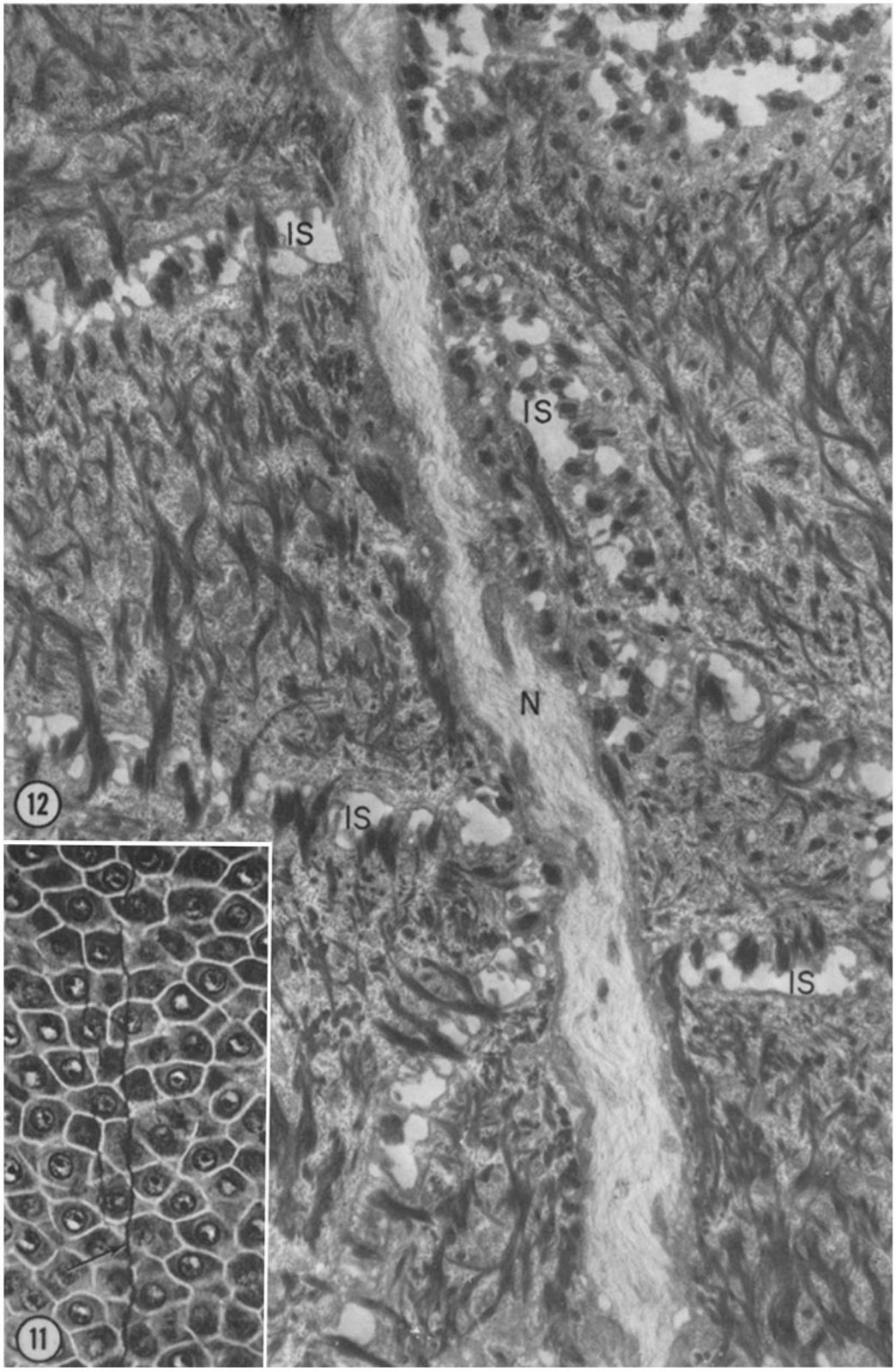
Merkel cells in paraffin section consistently evidence "ballooning" of the cytoplasm, a fixation artifact not seen after OsO_4 fixation and epoxy resin embedding (27, 28). This ballooned appearance resembles that of melanocytes in paraffin sections of other types of epidermis. In paraffin sections cells other than Merkel cells (identified by their PAS-positive granules) also evidence ballooning of the cytoplasm. Cells which are not epidermal cells and yet are not Merkel cells can also be identified with the electron microscope. Such cells are similar in size to epidermal cells, have a lobulated nucleus, lack any specific granules, have *no desmosomes* between them and adjacent epidermal cells, and have no bands of epithelial filaments (26) in the cytoplasm. Their Golgi apparatus is scant, and no ergastoplasmic sacs are present. The cytoplasm appears to be predominantly granular. These cells can be found near the stratum basale or the lower stratum spinosum. In the nose skin of the opossum, cells other than epidermal cells are either Merkel cells or cells of the category just described which are considered to be amelanotic melanocytes or Langerhans cells (3), depending on their position in the epidermis. Arguments to validate the identification of these cells will be given in the discussion.

DISCUSSION

This study has confirmed the existence of nerves in epidermis in one highly specialized system and has revealed some singular relationships which these neurites possess when they are present in epidermis. The prominent investment of these nerve fibers by epidermal cells, as seen by electron microscopy, explains the findings of Boeke (5–7) who claimed that these nerves were intracytoplasmic and that the substance of the nerve fiber fused with the cytoplasm of the epidermal cell. As observed in the present work and also in the light microscope studies of Cauna (9), the neurites course through the epidermis without regard for intercellular spaces, apparently penetrating the epidermal cells. The light microscope studies have been confirmed by electron microscopy. The epidermal cell, in many instances, performs the function of a Schwann cell. The neurite in cross-section is enveloped by cytoplasmic processes analogous to those of the Schwann cells of unmyelinated axons. The junction between two epidermal cells is characterized by processes of epidermal cell cytoplasm covering the neurite, being similar to the junction of two Schwann cells at a node of Ranvier. Hay (16) has also observed neurites in the salamander epidermis by electron microscopy, but these neurites are always intercellular. In mammalian species studied to date (discussed in detail later), neurites can always be found penetrating the epidermal cells.

FIGURE 11 Paraffin section, silver method (42). The neurites (arrow) appear to pass through the epidermal cells without regard for intercellular spaces. × 400.

FIGURE 12 A neurite (*N*) is cut in longitudinal section as it passes through the stratum spinosum, corresponding to the area of the silver impregnation illustrated in Fig. 11. The neurite does not pass through the intercellular space (*IS*); rather it is ensheathed by epidermal cell cytoplasm, best illustrated by the cell at the lower right, where the boundaries of one cell can be clearly defined. Numerous desmosomes are present in the areas containing prominent intercellular spaces. Uranyl acetate. × 12,000.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The fact that these epidermal cells can perform a function similar to that of Schwann cells raises several interesting questions for which definitive answers are not presently available. First, how does the neurite regulate its cellular ensheathment? The use of epidermal cells for investing the neurite implies that cells other than Schwann cells can perform this function. This may create difficulties in interpreting Schwann cell function in degeneration and regeneration of peripheral nerves.

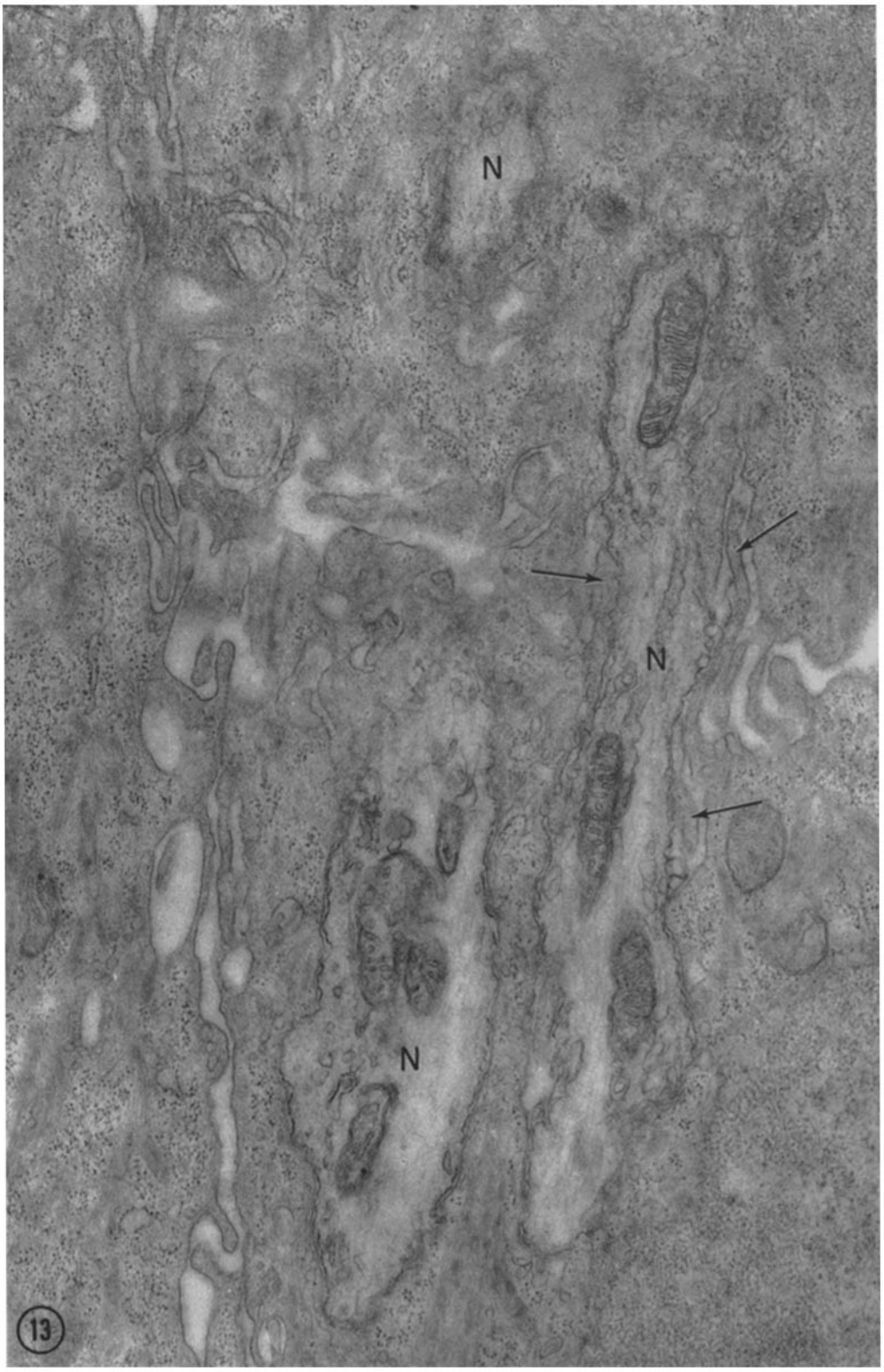
Secondly, how is the course of the nerve fiber growth controlled? As the neurite grows toward the skin surface it must establish new cellular relationships with neighboring epidermal cells. And furthermore, it grows high enough in the epidermis so that its supply of oxygen and nutrients most likely is limited. The forces within the epidermis which promote this phenomenal nerve growth are not known. Ramon y Cajal (34) has postulated that trophic factors in the epidermis force the growth of nerve fibers into the epidermis. The secretory granules of the Merkel cell are an intriguing correlate to this hypothesis.

Thirdly, how are nutrients supplied to the neurite within the epidermis? The intimate association of neurite and epidermal cells would imply that the epidermal cells are responsible for nutrition of the neurite. The neurite is not exposed to the intercellular spaces and thus it is not exposed to a direct supply of nutrients. The problem of nutrition in the upper stratum spinosum most likely is particularly critical.

With respect to the nerve fiber itself, by electron microscopy these fibers appear to exhibit some cytologic features resembling those seen in regenerating or degenerating axons (10, 45). Ranvier (35) has also argued that intraepidermal neurites in the mole's snout must also be constantly growing, and he based his view on arguments relative to the growth of the epidermis itself. Although direct attachment devices are not present between the nerve fiber and adjacent epidermal cells, the association as seen by electron microscopy is intimate. This might indicate that as the epidermis grows it carries the nerve along with it, an opinion expounded by Ranvier (35). The vesiculation and beading of the fiber near the stratum corneum, and the bits of presumptive neural substance seen in the stratum corneum support the thesis that the fiber is pinched off bit by bit as the epidermal cells differentiate into keratinized cells. The nerve fiber must therefore be continually growing or possibly having alternating phases of growth and degeneration. Neurites within the encapsulted endings in the dermis of the opossum nose also show similar accumulations of mitochondria, and these have also been observed by Cauna and Ross (10) in neurites of Meissner's corpuscles. Such neurites in some receptors have been considered by Cauna (9, 10) to be continually growing. The neurite within the epidermis of the opossum nose also may be continually growing and these accumulations of mitochondria might be a reflection of this activity.

Another unique property of these nerve fibers is their constant association with a specialized, secretory, epidermal cell which we have identified as Merkel's *Tastzelle*. The Merkel cells in the present study correspond in every respect to the *Tastzellen* described by Merkel (27, 28). These peculiar cells were seen to best advantage, according to Merkel, in OsO_4-fixed preparations. Only under these circumstances could the cytologic details of this cell be preserved. *Tastzellen* were larger, had less opaque cytoplasm, and had larger and more irregular nuclei than surrounding epidermal cells, and were intimately associated with a neurite. The present study confirms Merkel's description. In paraffin sections the cytoplasm is always empty, pulled away to one side (33). In PAS-stained preparations, only after OsO_4 fixation (Fig. 2) can the PAS-positivity be resolved to a definite zone in the cytoplasm. We have not been able to confirm the observation of Dogiel (12) and Kadanoff (17) that a nerve net surrounds the Merkel cell. Rather, the neurite in all of our preparations is always seen only on one side of the Merkel cell. However, to term this cell a *Tastzelle* is presently

FIGURE 13 A neurite sectioned longitudinally passes from the cytoplasm of one epidermal cell to a second. Delicate fingers of epidermal cell cytoplasm (arrows) separate the neurite from the intercellular spaces between the adjacent epidermal cells. Two other profiles of neurites are also seen within the epidermal cells, probably representing irregularities in the course of the neurite. Lead acetate. × 30,500.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unjustified since we do not know its function. The term used by Tretjakoff (43) is more appropriate under these circumstances; *i.e.*, Merkel cell.

The nature of the secretory granules of the Merkel cells has not been elucidated. The granules appear structurally similar to secretory granules of endocrine organs, and they possess an affinity for aldehyde fuchsin and show PAS-positivity, as do some endocrine secretory granules. The granules in Merkel cells have been considered as possibly being unusual melanin granules, and the cells thereby melanocytes. This conclusion is unjustified on the basis of the following arguments: (*a*) Melanocytes are present in the *basal layer* of the epidermis (3); Merkel cells are present above the basal layer. (*b*) Melanocytes have never been described as having desmosomes between them and adjacent epidermal cells (3); Merkel cells have desmosomes. (*c*) Melanocytes have numerous cytoplasmic processes; Merkel cells have few processes. (*d*) Merkel cells are DOPA negative, indicating an inability to synthesize melanin. (*e*) If these granules were melanin they should be demonstrable with stains to demonstrate melanin; these were negative. (*f*) Cells which are presumptive amelanotic melanocytes are present in this albino skin of the opossum nose. These amelanotic melanocytes lack desmosomes, have no specific granules, and have blunt cytoplasmic processes, thus appearing similar to other amelanotic melanocytes (3).

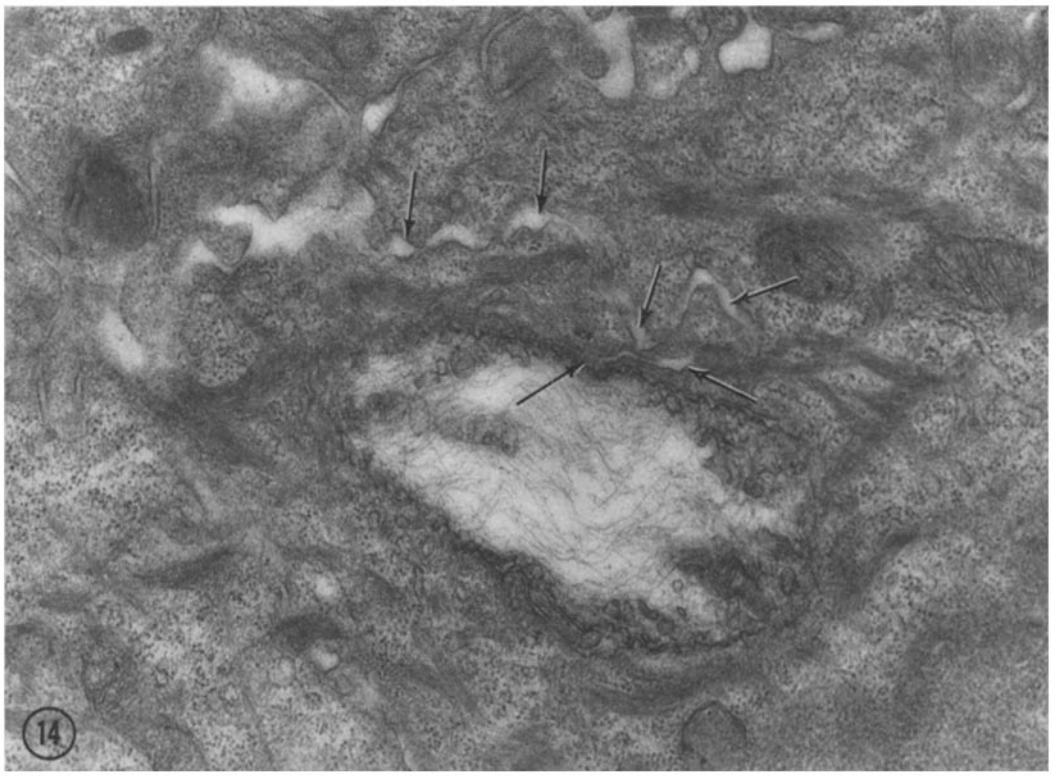
Some features of these cells as described above were not observed by Merkel (27, 28) in his study of peripheral nerve endings and in his exposition on the nature of *Tastzellen*. Considerable confusion now surrounds the concept of the *Tastzellen* which in part is derived from the use of the terms hederiform endings, touch discs, and Merkel's discs for the expanded portion of the neurite in the epidermis (5–8, 12, 36, 41, 50). These terms imply that the complex described by Merkel was an expanded bouton of a terminal nerve partially encircling an epidermal cell. Nothing could be further from Merkel's original description (27, 28), as has been correctly pointed out by Boeke (7), Cauna (9), Kadanoff (17), Stohr (43) and Weddell (49).

Historically, the presence of nerves in the epidermis of animals is much more frequently described (2, 5–9, 15, 17, 36) than in man (7, 9, 13, 17, 46, 51). In fact, most investigators claim that intraepidermal innervation does not exist to any appreciable extent in man (7, 43). However, observations of nerves in human epidermis by light microscopy have been described repeatedly (13, 20, 22, 41, 46, 51), and recently McGavran (23, 24) has seen two examples of a neurite-Merkel cell complex by electron microscopy in skin of two different individuals, identical in every respect with the Merkel complexes described in the present study. Studies in progress in this laboratory have also demonstrated Merkel cells in the skin of the nose and foot pad of the cat (19), the nose skin of the rat (42), the foot pad skin of the guinea pig (30), and associated with the intraepithelial neurites of sinus hairs of the rat (31). Thus, these complexes are widely distributed in biological systems.

The thesis of Merkel must also be examined closely with respect to the function of these neurite-cellular complexes. Merkel (28) believed that these cells were the transducers of physical energy to the nerve, in this case tactile sensibility. The skin studied in the present report may well be exquisitely sensitive. Eimer (14) made such a proposition in his original description of mole snout skin. He proposed that the mole, being without vision, would need an exquisitely sensitive tactile apparatus leading the way. The opossum is

FIGURE 14 The mesaxon-like configuration of the epidermal cell plasma membrane can be traced in this micrograph. The folded plasma membrane of the epidermal cell is continuous from the intercellular space to the neurite (arrows). At the lower left arrow the plasma membrane turns back on itself and surrounds the neurite. Lead acetate × 32,500.

FIGURE 15 A neurite (*N*) cut in cross-section surrounded by epidermal cell cytoplasm. There is no evidence of the mesaxon-like structure seen in Fig. 14. The plasma membrane of the epidermal cell surrounds the axolemma without any sign of interruption. The lack of mesaxon-like structure could not be due to tangential sectioning since a considerable expanse of epidermal cell cytoplasm is visible with no evidence of invagination of the plasma membrane towards the neurite. Lead hydroxide and uranyl acetate. × 32,000.



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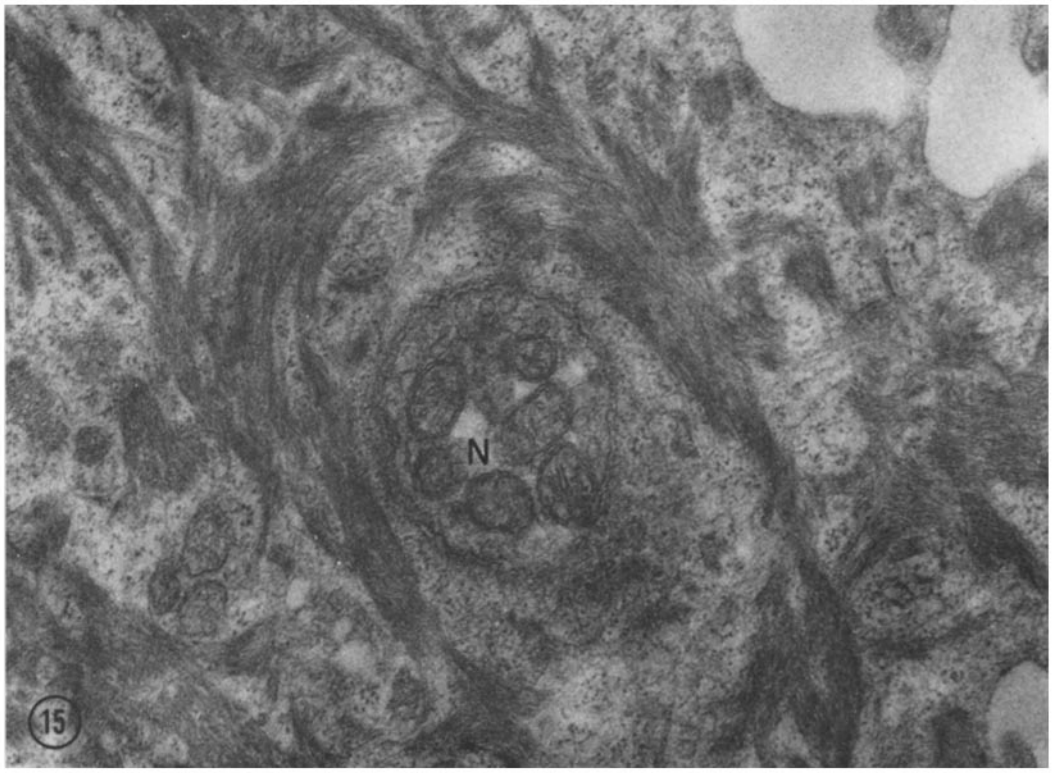

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predominantly nocturnal and could use this system in a similar manner. Another view commonly held is that of Winkelmann (50) who has suggested that intraepidermal neurites are found whenever the epidermis is thick, as thick skin would insulate the dermal networks from physical stimuli applied to the surface. However, this will not account for the intraepithelial innervation of the vibrissae (28, 31, 37). Such intraepidermal nerves can also be found in the epidermis of the rat snout which does not have excessively thick epidermis (42). Weddell (46) has also observed nerves penetrating the human epidermis in psoriasis, but not exclusively within the thickened plaque. The distinct possibility exists that the sensory function of this system is highly specialized and, as Eimer (14) suggested, does assist in sensory discrimination in the absence of, or as a supplement to, visual information. In this context it is interesting to note the very rich intraepidermal innervation of the ampulla of Lorenzini (18) which has been regarded as a mechano-receptor (47). How similar this system is to mammalian intraepidermal innervation is not presently known.

The function of the Merkel cell in this system is thus not known. Before its function can be ascertained, the chemical nature of the secretory granules must be determined. This has not been possible to date with histochemical methods. However, the cell's relationship to the neurite is certainly suggestive for modulating the growth or function of the neurite. Especially intriguing is the fact that adjacent to the Merkel cell the neurite always contains many mitochondria, whereas a short distance from the Merkel cell the neurite appears "normal." This association could reflect intense functional activity at the point of junction of neurite and Merkel cell.

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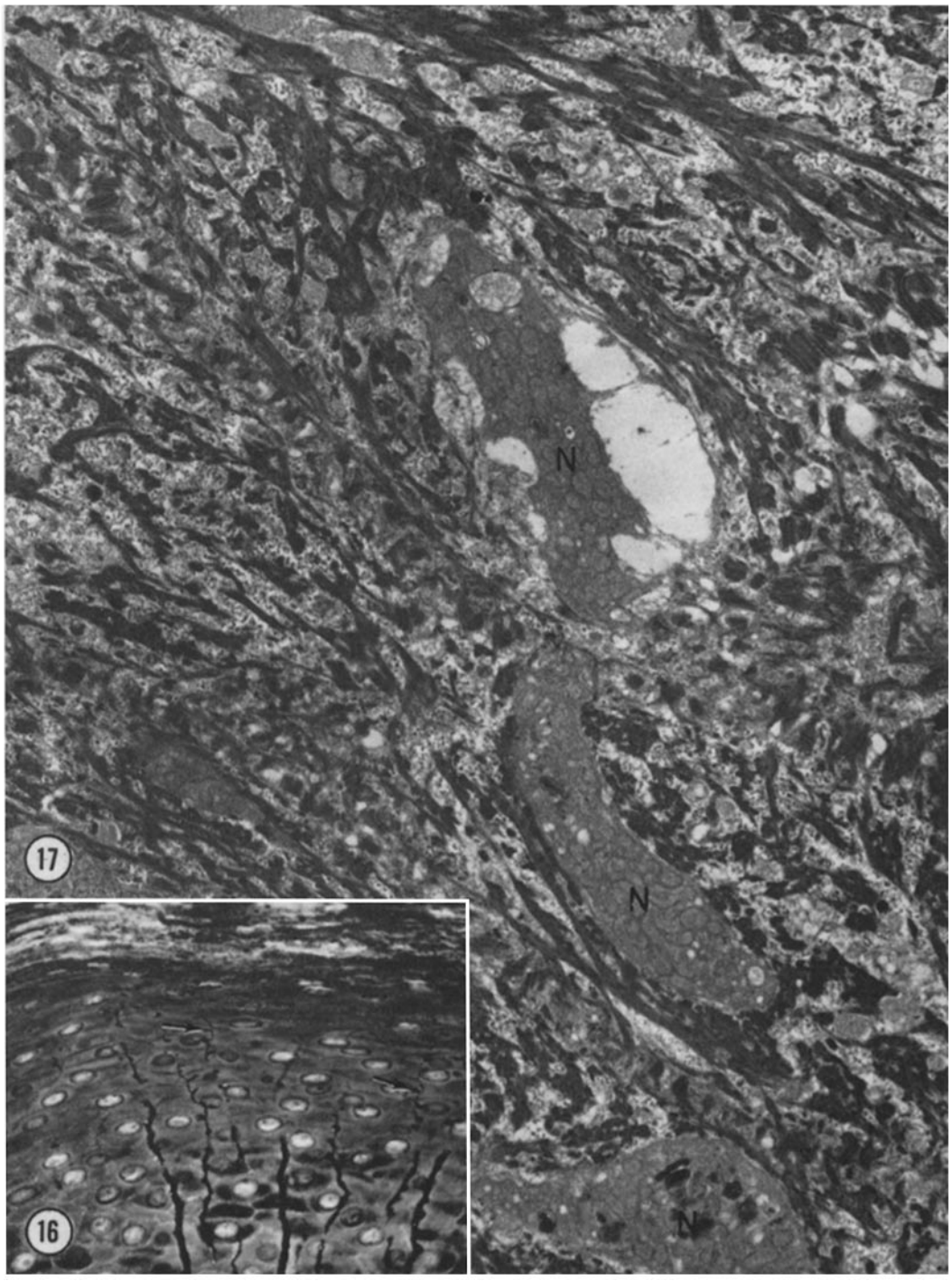
FIGURE 16 Paraffin section, silver method (42). The stratum corneum is present at the top of the micrograph. The neurites as they approach the stratum corneum evidence vesiculation and beading (arrows). × 400.

FIGURE 17 Section taken from the high stratum spinosum just beneath the stratum corneum. Several expanded portions of neurites are present corresponding to those seen in Fig 16. The neurites (*N*) are filled with mitochondria and dense lipoid material. The upper neurite also evidences vesiculation which could be fixation artifact but could also be a sign of degeneration of the neurite. Uranyl acetate. × 9,500.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