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Stanley Powell Tompkins

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STANLEY POWELL TOMPKINS

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STANLEY POWELL TOMPKINS

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ABSTRACT

Electron microscopic investigations have revealed two types of cells populating normal skin of mammals: keratinocytes and nonkeratinocytes. The nonkeratinocytes were represented as melanocytes and epidermal Langerhans cells. This study reports the description and occurrence of another cell population found in both normal and neural crest-free skin from mice of the PET strain.

The cells of this population have been designated semikeratinocytes, characterized as dendritic cells possessing irregular nuclei, and found in both the basal and suprabasal cell layers. The cytoplasm of these cells possessed few to no tonofilaments and tonofibrils, a reduced number of ribosomes, mitochondria, numerous vesicles, and minidesmosomes along the plasma membrane.

Since the semikeratinocytes found in both normal and neural crest-free mouse skin possessed characteristics similar to both epidermal Langerhans cells and keratinocytes, the evidence presented suggests that the semikeratinocytes are divisional products of basal keratinocytes and differentiate into epidermal Langerhans cells.

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INTRODUCTION

The mammalian epidermis has been described as consisting of three different cell populations: keratinocytes, pigment cells, and Langerhans cells. Ultrastructural investigations of mammalian skin within the last decade have resulted in criteria whereby these cell types can be differentiated upon electron microscopic examination.

Ultrastructurally the keratinocyte is characterized by being a columnar or cuboidal epidermal cell which possesses a round to oval nucleus and a plasma membrane having numerous desmosomal attachments. Tonofilaments and tonofibrils, melanosomes, and keratohyalin granules are found in cells of the upper stratum spinosum and stratum granulosum (Odland and Reed, 1967; Snell, 1967). During their assent from the basal layer, the keratinocytes experience a flattening so that the long axis of the cells appears parallel to the surface of the integument.

Pigment cells are characterized by being dendritic basal cells which possess a round to oval nucleus which, at times, may have a slight indentation, and a plasma membrane which lacks desmosomal attachments (Birbeck, et al., 1961; Zelickson, 1967). The cytoplasm contains premelanosomes and melanosomes, lacks tonofilaments and tonofibrils, and appears relatively clear when compared with the cytoplasm of keratinocytes (Birbeck, et al., 1961).

Epidermal Langerhans cells are described as dendritic cells of the basal and suprabasal cell layers which possess a convoluted nucleus and a plasma membrane which lacks desmosomes. Their cytoplasm lacks tonofilaments and tonofibrils, and it appears relatively clear as compared with the cytoplasm of the keratinocytes (Birbeck, et al., 1961). In addition, the cytoplasm contains a characteristic organelle, known as the Langerhans granule, which appears rod-shaped or tennis racket-shaped in cross section, and disc-shaped in top view (Birbeck, et al., 1961; Breathnach, 1964; Zelickson, 1966; Wolff, 1967; Sagebiel and Reed, 1968).

The ultrastructural similarity of the epidermal melanocyte and the epidermal Langerhans cell led many investigators to believe that the Langerhans cells were derived from melanocytes as either effete melanocytes (Birbeck, et al., 1961), transitional products (Breathnach, et al., 1963; Zelickson, 1965; Mishima, 1966), or post-divisional products (Breathnach, 1964, 1965; Breathnach and Goodwin, 1965; Zelickson, 1966). The Langerhans cell-melanocyte relationship was challenged when organelles identical to Langerhans granules were found in the tumor histiocytes of histiocytosis-X, suggesting a mesodermal origin (Tarnowski and Hashimoto, 1967).

To add support to the speculation of a Langerhans cell-melanocyte relationship, Reams, et al., (1967) found no evidence for the presence of cells with characteristic Langerhans granules or melanosomes in murine skin experimentally deprived of its neural crest derivatives. In a more conclusive study, Breathnach,

et al., (1968) found nonkeratinocytes in the epidermis of mouse skin which had been deprived of its neural crest derivatives. Many of the nonkeratinocytes possessed granules within the cytoplasm, which were identical to the Langerhans granule. This evidence was purported to disprove all existing hypotheses which proposed that epidermal Langerhans cells were derived from melanocytes, and renewed speculation as to their origin and function.

Adding support to a possible mesodermal origin of the epidermal Langerhans cell was evidence by Kiistala and Mustakallio (1968) and by Pruniceras (1969). On the other hand, Wolff and Winklemann, (1967a), and Lessard, et al., (1968) suggested a possible relationship between the keratinocyte and the epidermal Langerhans cell. Another possible origin has been introduced by the report of an unidentifiable dendritic cell type in the epidermis of the guinea pig (Lessard, et al., 1967), and by the presence of an "unclassifiable nonkeratinocyte" in mouse skin by Quevedo (1968). Quevedo (1968) considers these cells to be precusors of Langerhans cells as well as melanocytes.

The present study was undertaken to investigate the occurrence of epidermal Langerhans cells in both neural crest-free and normal PET mouse skin, and to present evidence for a possible origin of the epidermal Langerhans cells of murine skin.

METHODS AND MATERIALS

The tissues used in this investigation were obtained from mice of the PET strain (Reams, 1967a). Mice of the PET strain were used because of availability and the background of information on the developmental history of their neural crest cells (Mayer and Reams, 1962; Reams, 1967b).

Epidermal tissue free of neural crest cells was obtained by implanting limb buds from 10-day-old mouse embryos into the coeloms of 72-hour White Leghorn chick embryo hosts. At the same time, limb buds possessing neural crest cells, from 12½-day-old mouse embryos were implanted into the coeloms of 72-hour White Leghorn chick embryos. These served as controls. After 15 days of intracoelomic cultivation, the grafts were recovered and small strips of skin were removed and immediately fixed. Under the steroscopic microscope, it was evident that the grafts from the 10-day-old embryos were deprived of their neural crest derivatives because colorless hairs were seen protruding from the grafts. In contrast, the grafts from the 12½-day-old embryos possessed black hairs, indicating the presence of neural crest elements.

Normal skin was obtained from 15 to 18-day-old embryos, newborn, and 3-day-old mice. Strips of skin, measuring 5 x 10 mm, were removed from the dorsal surfaces of the above, immediately placed into cold fixative, and sliced into smaller strips, 1 x 3 mm.

Samples of normal skin were removed from 15-day-old and adult mice which had been anesthetized with an intraperitoneal injection of Diabutal (sodium pentabarbital). As soon as the animals were immobile, a commerical depilatory, "Nair," was applied to their dorsal surfaces. The "Nair" was removed after five minutes leaving the surfaces hairless. While the mice were still alive, 5 x 10 mm strips of skin were removed and immediately placed into cold fixative where they were sliced into strips, 1 x 3 mm.

All of the strips of skin were fixed for two hours in 1% osmium tetroxide buffered at pH 7.4 with veronal acetate (Palade, 1952). Following two five-minute rinses in veronal acetate buffer, pH 7.4, the tissue was dehydrated through a series of acetones maintained at 4°C. The tissue remained for five minutes in each of 20%, 50%, 80%, and 95% acetone, and for fifteen minutes in each of two 100% acetone baths. It was then placed into 100% acetone at room temperature (25°C) for fifteen minutes.

The hardened strips of skin were carried through an infiltration and embedding process using an araldite compound (Durcupan-ACM, Fluka Company, Switzerland). The procedure required the tissue to be passed through the following series of acetone-Durcupan mixtures: 3:1 acetone to Durcupan, 1:1 acetone to Durcupan, and 1:3 acetone to Durcupan. The tissue remained in each mixture for one hour before being left overnight in 100% Durcupan. The tissue was then placed into flat molds (5 x 15 mm) filled with 100% Durcupan and allowed to polymerize in a 60°C oven for 48 to 72 hours.

Both thick and thin sections were obtained on a Reichert "OmU 2" ultramicrotome with either glass or diamond knives. The thick sections (0.5 to 1.0 μ thick) were mounted on glass slides for examination with a phase microscope to check fixation and the orientation of the tissue in the plastic blocks. Thin sections (60-90 mμ thick) were mounted on copper grids (100 mesh, 3 mm O.D.) which had been coated with a film of 0.10% parlodion.

The thin sections were routinely stained with a 1% aqueous solution of uranyl acetate for two minutes and with lead citrate (Reynolds, 1963) for three minutes. To emphasize such structures as basement membranes, desmosomes, and tonofibrils, some thin sections were stained with lead citrate (Reynolds, 1963) for one minute, followed by staining with potassium permanganate (Lawn, 1960) for fifteen minutes.

The thin sections were examined on a RCA EMU-3B electron microscope. Electron micrographs were taken on $3\frac{1}{4}$ x 4 inch glass Kodak projector slide plates from which prints were obtained following photographic enlargement.

OBSERVATIONS

The following observations were made while evaluating over 300 electron micrographs taken during the examination of over 200 sections from normal and neural crest-free PET mouse skin.

Normal skin. There appeared to be no significant ultrastructural differences between the various epidermal tissue types used as examples of normal skin. Keratinocytes and nonkeratinocytes were found in all age groups. Nonkeratinocytes were cells which did not possess the typical characteristics of keratinocytes and were represented as melanocytes and Langerhans cells.

The keratinocytes of the normal skin types were characterized as being cuboidal to columnar in shape and possessing nuclei which were either round, oval, or slightly indented. The plasma membrane possessed numerous desmosomes of normal appearance (Fig. 1). The cytoplasm contained numerous mitochondria, and tonofilaments and tonofibrils (Figs. 1 and 2). Ribosomes and vesicles were seen scattered throughout the cytoplasm. Cisternae of rough endoplasmic reticulum and Golgi complexes were also seen. Melanosomes were observed in cells of both the basal and suprabasal levels of the epidermis.. Keratohyalin granules were found only in the suprabasal layers of the upper stratum spinosum and stratum granulosum.

Pigment cells as melanocytes were observed only in the basal

cell layers of normal mouse skin. The nuclei appeared slightly indented (Figs. 1 and 2). The plasma membrane, in contrast to keratinocytes, lacked desmosomes. The cytoplasm appeared more electron dense than that of the adjacent keratinocytes and possessed numerous vesicles, mitochondria, and melanosomes, but lacked tonofilaments and tonofibrils (Fig. 2).

Nonkeratinocytes, which could be classified as Langerhans cells, were observed in skin from 15-day-old PET mice. These cells were observed in both basal and suprabasal cell layers. The nuclei were convoluted and the plasma membranes lacked desmosomes (Figs. 3, 4, and 5). The cytoplasm of these cells, like that of melanocytes, appeared more electron dense than that of the adjacent keratinocytes and lacked tonofilaments and tonofibrils. The cytoplasm also possessed numerous vesicles and mitochondria, Golgi complexes, and cisternae of the endoplasmic reticulum. Occasionally, centrioles and membrane-bound melanosomes were seen in the cytoplasm of these cells (Figs. 3 and 4).

Cytoplasmic Langerhans granules, organelles characteristic of Langerhans cells were observed (Figs. 3, 4, and 5). However, no more than one granule per section per cell was seen. Most of the granules appeared to be rod-shaped with rounded ends and a central dense line. A rod-shaped organelle with what appeared to be a vesicle attached to one end was seen to be continous with the plasma membrane (Fig. 5). Two Langerhans cells were observed adjacent to one another and appeared to be the products of a recent mitotic division (Fig. 4).

Often nonkeratinocytes were observed which could not be definitely identified as either melanocytes or Langerhans cells due to the absence of characteristic granules (Fig. 6). This was particularly true for cells located in the basal cell layer where both types of nonkeratinocytes were found (Fig. 6). These cells possessed characteristics applicable to both types of nonkeratinocytes: they lacked desmosomes and fibrillar elements, and had a cytoplasm that was more electron dense than the cytoplasm of adjacent keratinocytes.

Another type of cell frequently encountered was characterized as a dendritic cell with an irregular nucleus (Fig. 7). In this cell there was a scarcity of desmosomes along the plasma membrane. Those which were seen appeared as minidesmosomes when compared to the size of the average desmosome possessed by keratinocytes (Figs. 4 and 7). The cytoplasm of these semikeratinocytes was relatively clear compared with adjacent keratinocytes. It possessed numerous mitochondria and vesicles, and cisternae of the endoplasmic reticulum. A few tonofilaments were scattered throughout the cytoplasm (Fig. 7). The number of ribosomes was greatly reduced as compared to a typical keratinocyte (Fig. 7).

Neural crest-free skin. The skin recovered from the limb bud transplants after 15 days of intracoelomic incubation was equivalent in age to skin from the limbs of 4-day-old PET mice which had developed normally. As seen with the light microscope, the skin from the 10-day-old limb bud grafts appeared to be free of its neural crest derivatives as colorless hairs were seen protruding

from the surface of the grafts. In contrast, the presence of black hairs protruding from the surface of the 12½-day-old limb bud grafts used as controls indicated that these grafts were not deprived of their neural crest derivatives.

Ultrastructurally, the general appearance of the epidermis from the graft tissue was similar to the epidermis from normal skin. Typical keratinocytes were seen throughout the entire epidermis. No cells were observed which could be identified as nonkeratinocytes, melanocytes or Langerhans cells. However, two types of cells were seen which were classified as semikeratinocytes.

One type of semikeratinocyte, type A, was characterized as being a dendritic cell with an irregular to convoluted nucleus (Figs. 8-14). The cytoplasm of these cells was relatively clear when compared with the adjacent keratinocytes, and possessed mitochondria, vesicles, cisternae of the endoplasmic reticulum, a reduced number of ribosomes, and few to no tonofilaments. The plasma membrane had only a few minidesmosomes (Figs 8-14). Several cells were seen with dendritic processes (Figs. 11 and 12). These cells were ovserved in both the basal and suprabasal cell layers. One cell of this type possessed a vesicle which appeared to contain the remnant of an autophagocytized desmosome (Figs. 13 and 14). Other semikeratinocytes of type A appeared to be in the process of forming convoluted nuclei. One cell was located in the basal layer (Fig. 9), while another appeared to be migrating out of the basal layer (Fig. 10).

The second type of semikeratinocyte, type B, was also characterized as being a dendritic cell with an irregular nucleus (Figs. 15 and 16). The cytoplasm of these cells was more electron dense than that of the adjacent keratinocytes. It possessed mitochondria, numerous vesicles, cisternae of the endoplasmic reticulum, and scattered ribosomes (Figs. 15 and 16). The plasma membranes of these cells possessed a few minidesmosomes, as did the cells of type A. Several of these cells had dendritic processes extending from the main body of the cell into the superficial cell layers. Cells of type B were found in close proximity to one another in both basal and suprabasal levels. In the suprabasal cell layers, two cells were observed separated by two keratinocytes. In the basal layers, these cells were found, at times, to be separated by six to ten keratinocytes. Occasionally, two cells of type B were seen adjacent to one another which suggested that they were the products of a recent mitotic division.

DISCUSSION

Early investigations into the nature of the epidermal Langerhans cells were conducted solely by histochemical methods. Fan, et al., (1959) described epidermal Langerhans cells in the guinea pig as gold positive, high-level branched cells of the epidermis. They considered these as effete or worn out melanocytes because these cells lost their affinity for gold when melanocyte proliferation was stimulated. Mishima and Miller-Milinska (1961) demonstrated not only high-level branched cells, but also basal melanocytes with osmium iodide in human skin. Since both cell types were osmium iodide positive, they considered this as positive proof for a Langerhans cell-melanocyte relationship. Because of the non-specificity of many histochemical methods, e. g. gold chloride, and osmium iodide, a more positive method of identifying epidermal Langerhans cells was needed.

Birbeck, et al., (1961) described the ultrastructure and the occurrence of epidermal Langerhans cells in vitiliginous and normal human skin. This ultrastructural description of Langerhans cells established the criteria with which to distinguish these cells from all other cells populating the epidermis. The Langerhans cells were characterized as dendritic cells, occurring in both basal and suprabasal layers and possessing convoluted nuclei. The plasma membrane of these cells lacked desmosomes, and the cytoplasm was

clear compared with adjacent keratinocytes due to the lack of fibrillar elements. The cytoplasm also possessed conspicuous rod-shaped organelles or granules. The granules were described as disc-shaped, possessing a "two-dimensional array of particles." In cross-section these disc-shaped granules appeared as rods with a central dense line representing the lattice of particles. Birbeck, et al., (1961) noted that the ultrastructure of the epidermal Langerhans cells was similar to that of the epidermal melanocyte, except for the appearance of the nuclei and the structure of the characteristic granules. The nuclei of epidermal melanocytes did not possess the degree of indentation or convolution, and the premelanosomes and melanosomes had a different structure compared with that of the Langerhans granule. In normal skin, melanocytes were found in the basal layer, whereas Langerhans cells occurred in the suprabasal layers. In vitiliginous skin, which lacked melanocytes, Langerhans cells occurred in both the basal and suprabasal cell layers. Due to the strong similarities between the melanocytes and the epidermal Langerhans cells, and the occurrence of basal Langerhans cells in vitiligo, Birbeck, et al., (1961) considered the epidermal Langerhans cells effete melanocytes, agreeing with Fan, et al., (1959).

In human skin treated with thorium-X to increase melanocyte turnover, Breathnach, et al., (1963) reported the occurrence of basal melanocytes, suprabasal epidermal Langerhans cells, and a third cell type, lacking granular structures common to the other two. The third cell type was considered to be a transitional cell type between the melanocyte and the Langerhans cells. Thus it was suggested

that the suprabasal Langerhans cells were transitional products of basal melanocytes.

With the reported occurrence of epidermal Langerhans cells in both basal and suprabasal layers of normal ginea pig skin (Breathnach, et al., 1964; Breathnach and Goodwin, 1965), Breathnach, (1965) suggested that Langerhans cells were post-divisional products of basal melanocytes. One of the daughter cells, having the characteristics of a Langerhans cell, migrated into the suprabasal layers never to become melanogenic. The other daughter cell remained in the basal cell layer, became melanogenic, and eventually divided, forming the next generation of basal and suprabasal dendritic cells.

Reports by Zelickson (1965), investigating normal skin, and by Mishima (1966), examining stripped human skin, of transitional cells containing both melanosomes and Langerhans granules, renewed speculation of a transitional relationship between melanocytes and Langerhans cells. This was challenged, however, by Breathnach and Wyllie (1965) who showed membrane-bound melanosomes within the cytoplasm of Langerhans cells in both normal and vitiliginous human skin. This suggested that the melanosomes had been phagocytized by the Langerhans cells.

Evidence to support a melanocyte-epidermal Langerhans cell relationship was tendered by Reams, et al., (1967) who found no nonkeratinocytes which could be identified as either melanocytes or Langerhans cells in neural crest-free murine skin equivalent in age to skin from 3-day-old post natal mice. The neural crest-free skin was obtained from 10-day-old mouse limb buds which had been

grafted into the coelom of chick embryos. In a more extensive investigation, Breathnach, et al., (1968) found nonkeratinocytes possessing Langerhans granules in neural crest-free mouse skin equivalent in age to skin from 10 and 25-day-old post natal mice. The neural crest-free skin was also from 10-day mouse embryo limb buds, but had been grafted onto the spleen of adult mice. The results of this study disproved existing hypotheses concerning a melanocyte-Langerhans cell relationship.

Evidence challenging a neural crest origin for the epidermal Langerhans cell was presented before the report by Breathnach, et al., (1968). Tarnowski and Hashimoto (1967) showed Langerhans granules in tumor histiocytes, macrophages, of human skin with histiocytosis-X, suggesting a mesodermal origin for epidermal Langerhans cells. Hashimoto and Tarnowski (1968), and Kiistala and Mustakallio (1968) support this hypothesis by showing histiocytes containing Langerhans granules crossing the basement membrane. They suggest that the cells were moving from the dermis into the epidermis. This was contrary to an earlier report by Zelickson (1965), which stated that cells containing Langerhans granules were passing through the basement membrane of normal skin, while moving from the epidermis into the dermis. The concept of mesodermal origin for the epidermal Langerhans cells has been presented also by Prunieras (1969) in reviewing the history and current concepts regarding the epidermal Langerhans cells.

Reams and Greco (unpublished) have found evidence against a mesodermal origin for epidermal Langerhans cells as a result of

their fluorescent-antibody studies in normal mouse skin. After they injected tagged anti-lymphocyte serum into mice, they examined skin samples and found no tagged cells within the eipdermis.

The possibility that epidermal Langerhans cells are derived from yet another cell population has been presented (Lessard, et al., 1967, 1968; Quevedo, 1968). An unidentifiable dendritic cell was demonstrated with ATPase in regenerating epidermis of the guinea pig following epidermal stripping (Lessard, et al., 1967). In a later report from the same study, Lessard, et al., (1968) reported that an increase in the number of epidermal Langerhans cells first appeared on the sixth day following epidermal stripping, and attained its normal cell population by the 15th day. Quevedo (1968) reported the occurrence of "unclassifiable nonkeratinocytes" from birth in normal mouse skin, while epidermal Langerhans cells and melanocytes were not identified until the 6th day after birth. Because of this evidence he felt that the "unclassifiable nonkeratinocyte" gave rise to both melanocytes and epidermal Langerhans cells.

In the present study, epidermal Langerhans cells were not identified in normal skin from PET mice varying in age from late term embryos to 3-day-old post partum. However, cells which could be identified as epidermal Langerhans cells, by the presence of characteristic granules, were seen in both basal and suprabasal cell layers of the epidermis in PET mice 15-days post partum and older (Figs. 3, 4, and 5), agreeing with Quevedo (1968).

No nonkeratinocytes were observed in murine skin which had been deprived of its neural crest derivatives, substantiating work

by Reams, et al., (1967). This find was contrary to the report by Breathnach, et al., (1968) which indicated the presence of epidermal Langerhans cells in the epidermis of neural crest-free murine skin. Two factors, the length of the incubation period, and the nature of the host tissue, must be considered when comparing the reports by Reams, et al., (1967) and Breathnach, et al., (1968) with the present study. Breathnach, et al., (1968) grafted neural crest-free limb buds from 10-day-old embryos onto the spleens of adult mice. The splenic site, they noted, had been proven to contain cells which posess Langerhans granules. After 3 or 5 weeks of incubation, the graft skin was equivalent in age to skin from 10 and 25-day-old post partum mice, respectively, ages at which Langerhans granules have been identified within nonkeratinocytes of normal mouse epidermis (Quevedo, 1968). In contrast, the present study found no nonkeratinocytes in neural crest-free skin equivalent in age to skin from a 4-day-old mouse, indicating that nonkeratinocytes containing Langerhans granules appear in mouse skin between the 4th and 6th days following birth.

The semikeratinocytes observed in normal skin of all ages and in neural crest-free skin (Reams and Tompkins, 1969) are believed to be similar to, if not identical to, the "unclassifiable keratinocytes" described to constitute a constant population within mouse skin from birth (Quevedo,1968), and to the unidentifiable dendritic cells common in regenerating epidermis (Lessard, et al., 1967).

Present evidence suggests that the semikeratinocytes are divisional products of basal keratinocytes which have the potential

to differentiate into epidermal Langerhans cells. Following mitotic division of a basal keratinocyte, the semikeratinocytes have the appearance of cells designated as type A. They possess a relatively clear cytoplasm, an irregular to convoluted nucleus, a reduced number of ribosomes, and a few minidesmosomes along the plasma membrane (Fig. 8). These type A cells either remain in the basal layer and undergo differentiation (Fig. 9), or they migrate out of the basal cell layer and undergo differentiation in the suprabasal cell layers (Fig. 10).

During the early phases of differentiation, the semikeratinocytes begin to acquire dendritic shapes (Figs. 11 and 12), the nuclei appear irregular in shape (Figs 11 and 12), and autophagocytosis of the minidesmosomes is initiated (Figs. 13 and 14). The autophagocytized desmosome lining one side of the vacuole in Figures 13 and 14 is similar to the late stages of desmosome autophagocytosis in trypsinized embryological epidermal cells described by Overton (1968).

In the later phases of differentiation, the semikeratinocytes have the appearance of cells designated as type B. These are more dendritic in shape, the cytoplasm becomes more dense, and few to no minidesmosomes remain along the plasma membranes (Figs. 15 and 16). By the sixth day following the start of differentiation, the semikeratinocytes have acquired all of the characteristics of epidermal Langerhans cells (Quevedo, 1968).

It has been suggested that since epidermal Langerhans cells constitute such a constant population within the epidermis, a close

relationship exists between an epidermal Langerhans cell and the surrounding keratinocytes, forming an epidermal Langerhans cell unit, which may be analogous to the epidermal melanin unit (Wolff and Winklemann, 1967b). If this is true, and if the epidermal Langerhans cells are related to the keratinocytes, then once the correct ratio between epidermal keratinocytes and epidermal Langerhans cells has been reached, differentiation of semikeratinocytes stops and they remain in the epidermis to be exfoliated with the other epidermal cells (Fig. 7). The population of semikeratinocytes is perpetuated by the basal keratinocytes.

The presence of centrioles in epidermal Langerhans cells (Fig. 3), the observation of two epidermal Langerhans cells juxtaposed (Fig. 4), the report of DNA synthesis by Langerhans cells (Giacometti and Montagna, 1967), and the report of mitotic activity by Langerhans cells (Hashimoto and Tarnowski, 1968) suggest that epidermal Langerhans cells constitute a self-sustaining cell population under normal conditions. However, in the event of epidermal injury, disrupting the epidermal Langerhans cell unit (Wolff and Winklemann, 1967), semikeratinocytes are stimulated to differentiate into epidermal Langerhans cells to return the cell population to normal. Following epidermal stripping of the guinea pig, an increase in the number of epidermal Langerhans cells was not observed until the sixth day (Lessard, et al., 1968), which agrees with the first appearance of identifiable epidermal Langerhans cells in mouse skin (Quevedo, 1968).

Any study into the nature and origin of the epidermal Langerhans

cell must also include consideration of its characteristic cytoplasmic granule first described by Birbeck, et al., (1961). The presence of cytoplasmic organelles having similar descriptions in various tissues has been reported by a number of investigators. The first three-dimensional description was made by Wolff (1967), immediately followed by Sagebiel and Reed (1968). Both have designated the organelle to be known as the Langerhans granule, and suggested that the granule is formed by the collapse of vesicles. Two sites have been suggested for the origin of these granules: a. they are formed by the collapse of Golgi vesicles which move to the periphery of the cell (Breathnach, 1964, 1965; Zelickson, 1965, 1966); and b. they are formed by an infolding of the plasma membrane (Breathnach, 1964). Support for the latter mode of formation was presented when granules identical with Langerhans granules were shown being formed by a cytoplasmic villus folding back onto the plasma membrane forming organelles continuous with the plasma membrane (Tarnowski and Hashimoto, 1967; Hashimoto and Tarnowski, 1968). The presence of Langerhans granules in lysosome-like vacuoles (Breathnach, 1965; Tarnowski and Hashimoto, 1967), strengthens the hypothesis presented by Hashimoto and Tarnowski (1968) suggesting that once the granules have been formed by the plasma membrane, the granules and their contents are digested by the lysosomes. Added support for the infolding theory was presented by Cancilla (1968) who found that only the Langerhans granules continuous with the plasma membrane were stained when exposed to lanthanum.

From the above, the author is inclined to agree with the theory which suggests that Langerhans granules are formed by an infolding of the plasma membrane. However, the real significance of these organelles is questioned since they have been found in a variety of mammalian tissues, as well as in invertebrates (Tarnowski and Hashimoto, 1967; Hashimoto and Tarnowski, 1968).

CONCLUSION

The presence of four different cell populations in both normal and neural crest-free mouse epidermis is described. These cell populations consist of keratinocytes, pigment cells, Langerhans cells, and semikeratinocytes. The latter possess characteristics of both keratinocytes and Langerhans cells. They are characterized by the presence of an irregular nucleus, few to no tonofilaments and a reduced number of ribosomes in the cytoplasm, and minidesmosomes along the plasma membrane. Evidence is presented which suggests that the semikeratinocytes are derived from the basal keratinocytes and differentiate into epidermal Langerhans cells.

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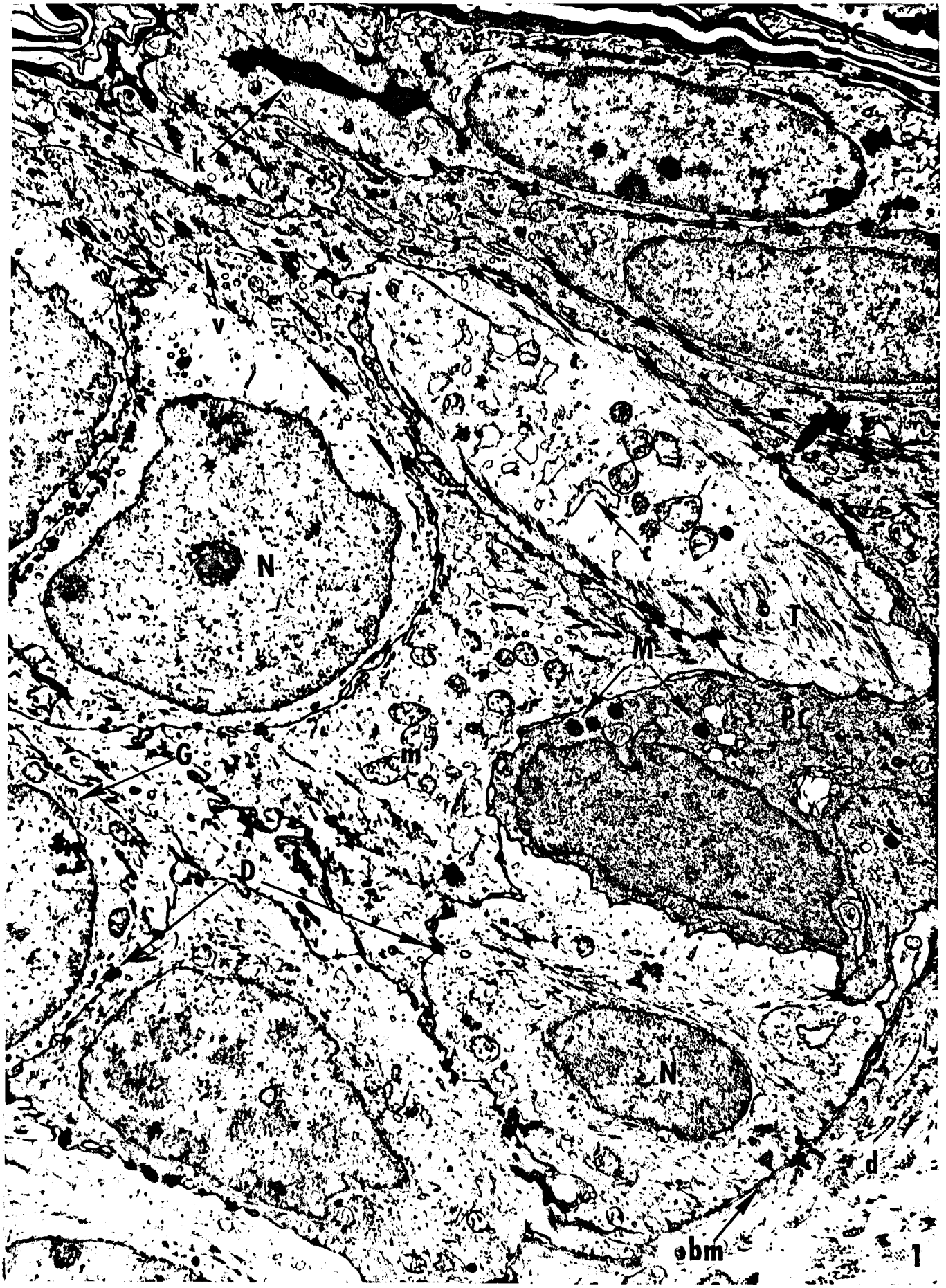
Figure 1 -- Electron micrograph of the epidermis from 15-day-old normal PET mouse skin. All of the cells between the basement membrane and the stratum corneum are keratinocytes, except for a basal melanocyte. Stratum corneum, SC; keratohyalin granule, k; nucleus, N; desmosomes, D, mitochondrion, m; tonofilaments and tonofibrils, T; vesicles, v; cisternae of the endoplasmic reticulum, c; Golgi complex, G; melanosomes M; pigment cell, PC; basement membrane, bm, dermis, d. Magnification: 11,460x.



1

Figure 2 -- Electron micrograph of a dendritic basal melocyte of the epidermis from 15-day-old normal PET mouse skin. The nucleus is irregular, and the cytoplasm is more electron dense than the adjacent keratinocytes. The cytoplasm lacks tonofilaments and tonofibrils, but contains melanosomes. The plasma membrane lacks desmosomes. Nucleus, N; melanosomes, M; tonofilaments and tonofibrils, T; keratinocyte, K; dermis, d; desmosome, D. Magnification: 19,680.

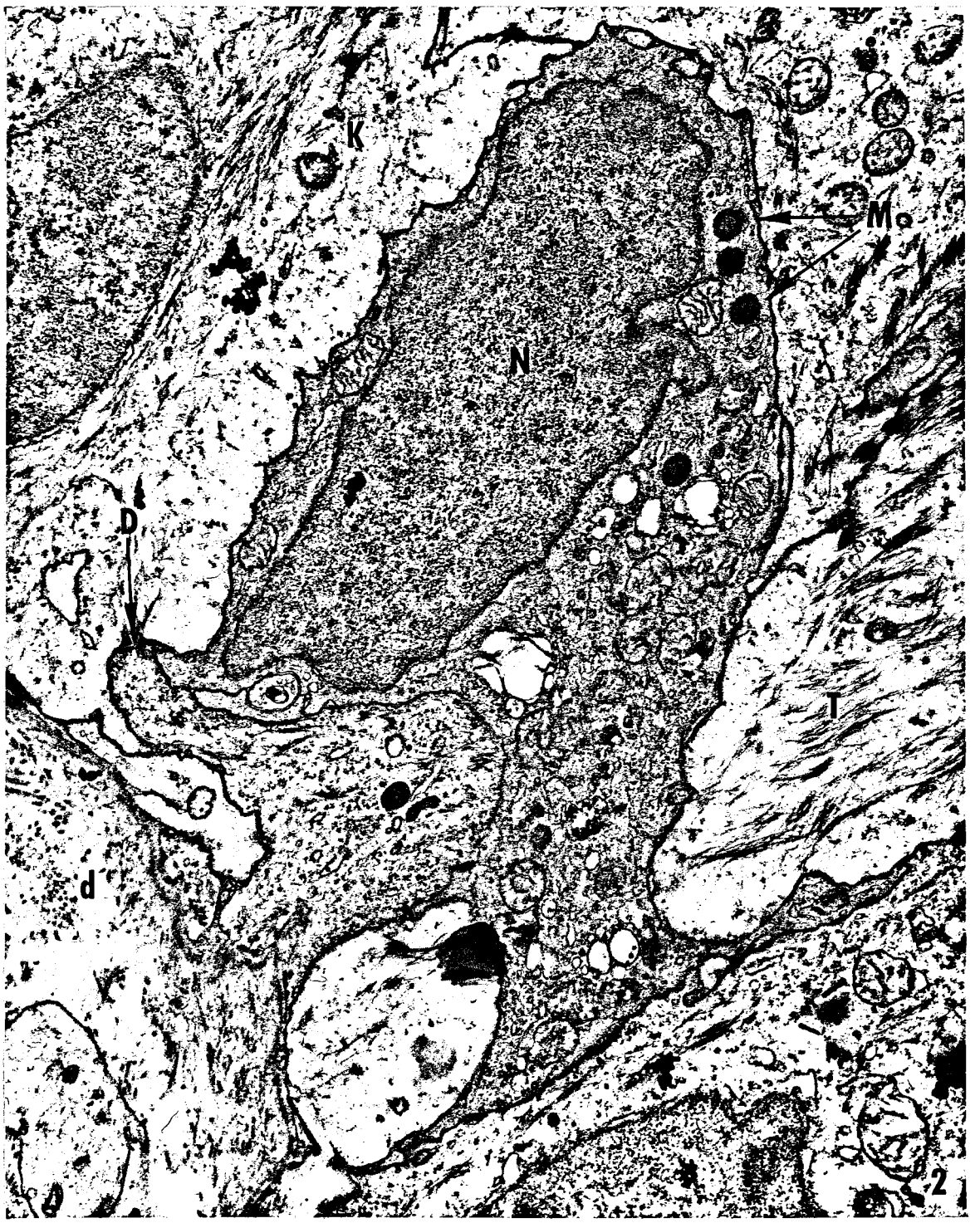

K
M
N
D
I
d
2

Figure 3 -- Electron micrograph of a basal epidermal Langerhans cell from 15-day-old PET mouse skin. The nucleus is convoluted, and the cytoplasm is more electron dense than the adjacent keratinocytes. The cytoplasm lacks tonofilaments and tonofibrils, and the plasma membrane lacks desmosomes. A characteristic Langerhans granule and a centriole are present in the cytoplasm. Nucleus, N; Langerhans granule, G; centriole, C; keratinocyte, K; dermis, d. Magnification: 46,100x.

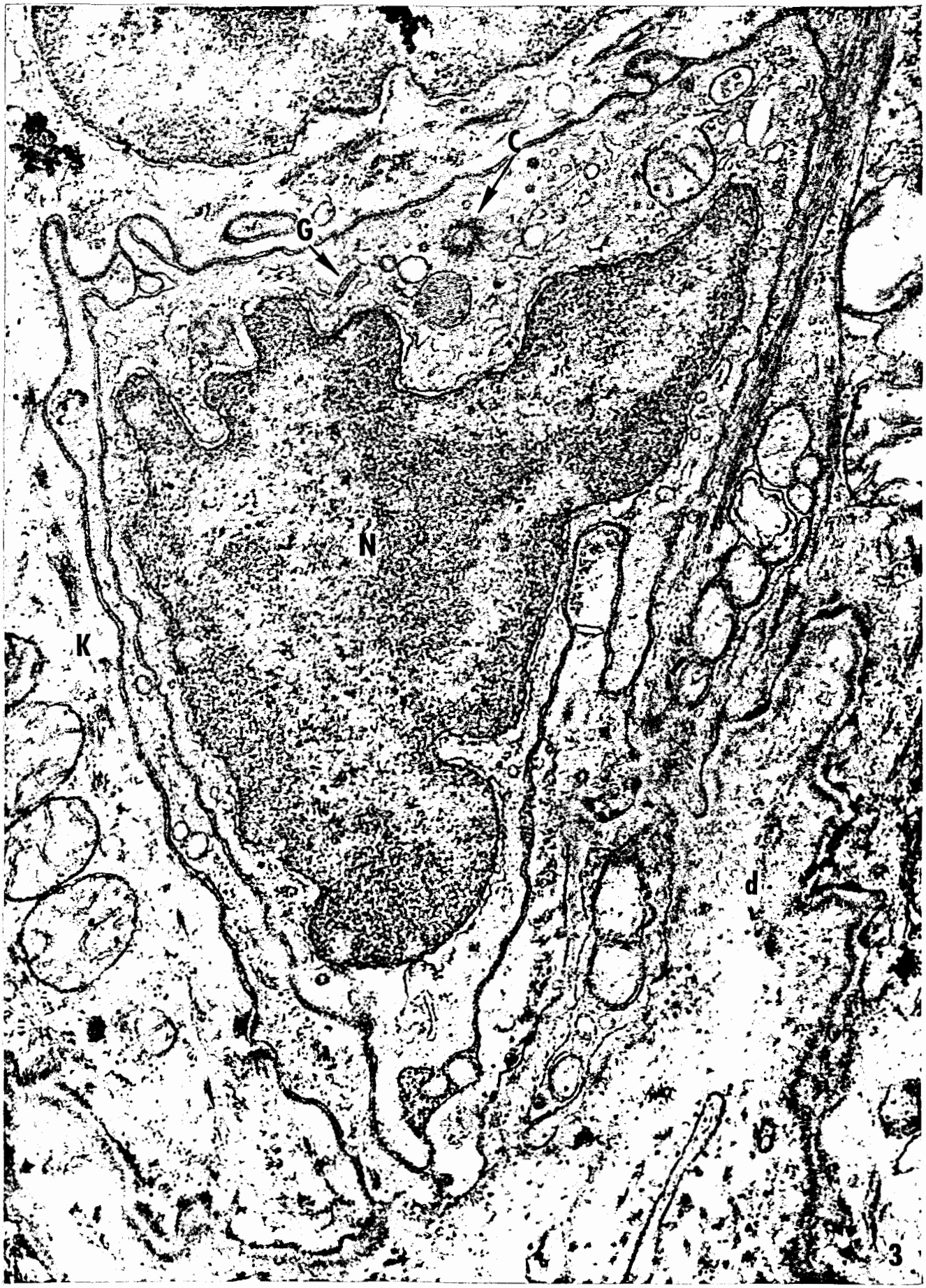

C
G
N
K
d
3

Figure 4 -- Electron micrograph of two juxtaposed suprabasal epidermal Langerhans cells from 15-day-old PET mouse skin. The nucleus is convoluted and the cytoplasm is more electron dense than that of the surrounding keratinocytes. The cytoplasm lacks tonofilaments and tonofibrils, and the plasma membrane lacks desmosomes. A Langerhans granule is present in the cytoplasm of the lower cell, and membrane bound melanosomes are present in the cytoplasm of the upper cell. Nucleus, N; Langerhans granule, G; membrane bound melanosome, M; keratinocyte, K; tonofilaments and tonofibrils, T; desmosomes, D. Magnification: 22,960x.

K
M
T
D
N
G
4

Figure 5 -- Electron micrograph of two juxtaposed suprabasal epidermal Langerhans cells from 15-day-old PET mouse skin. The nucleus is convoluted and the cytoplasm is more electron dense than that of the surrounding keratinocytes. The cytoplasm lacks tonofilaments and tonofibrils, and the plasma membrane lacks desmosomes. A rod-shaped granule, attached to what appears to be a vesicle, is continuous with the plasma membrane. Nucleus, N; tonofilaments and tonofibrils, T; Langerhans granule, G; keratinocyte, K. Magnification: 48,405x.

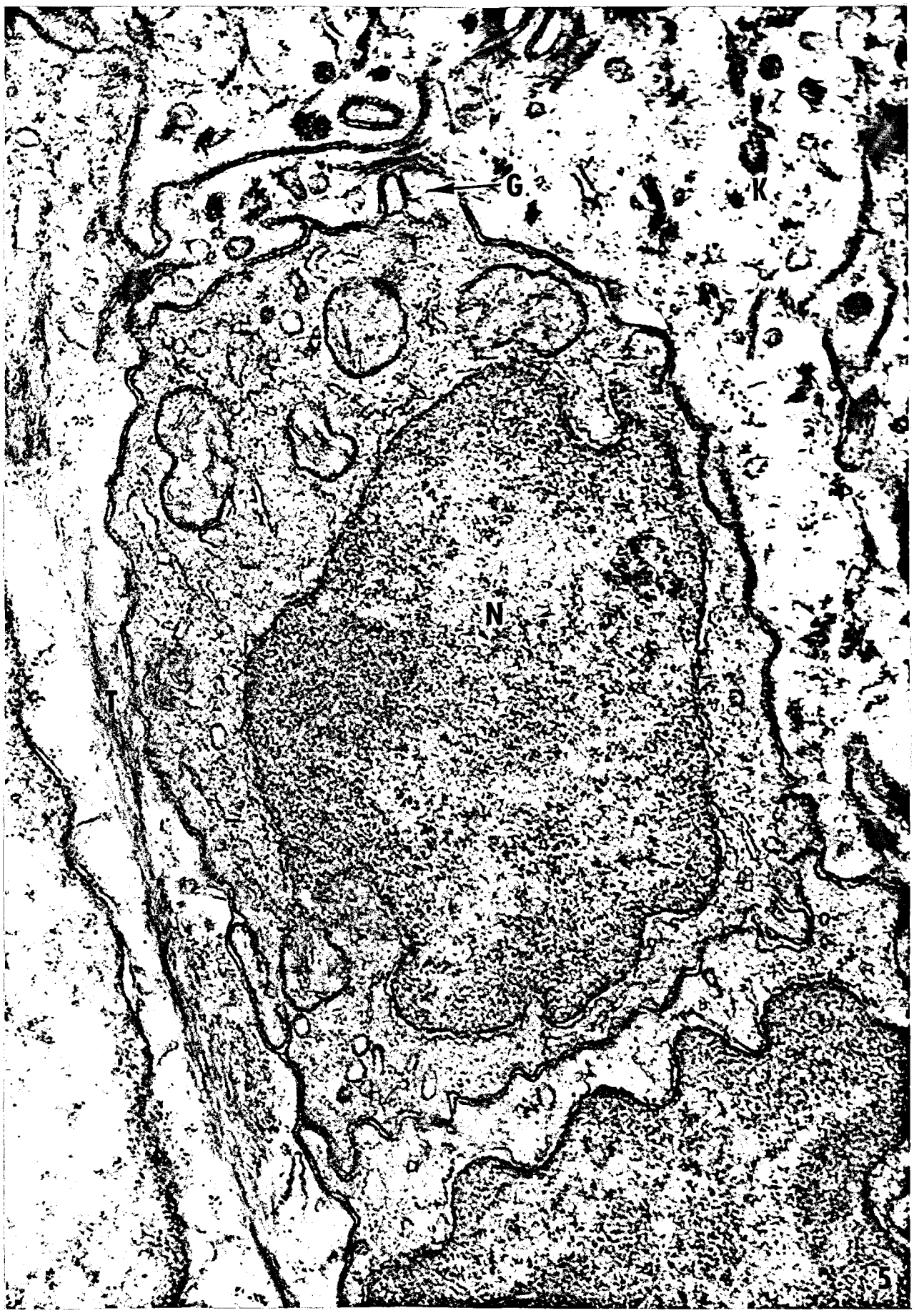

G
K
N
T

Figure 6 -- Electron micrograph of a basal nonkeratinocyte from 15-day-old PET mouse skin. This cell has characteristics of both epidermal Langerhans cells and basal melanocytes, except it lacks the characteristic granules. Nucleus, N; electron dense cytoplasm, c; keratinocyte, K; dermis, d. Magnification: 22,128x.

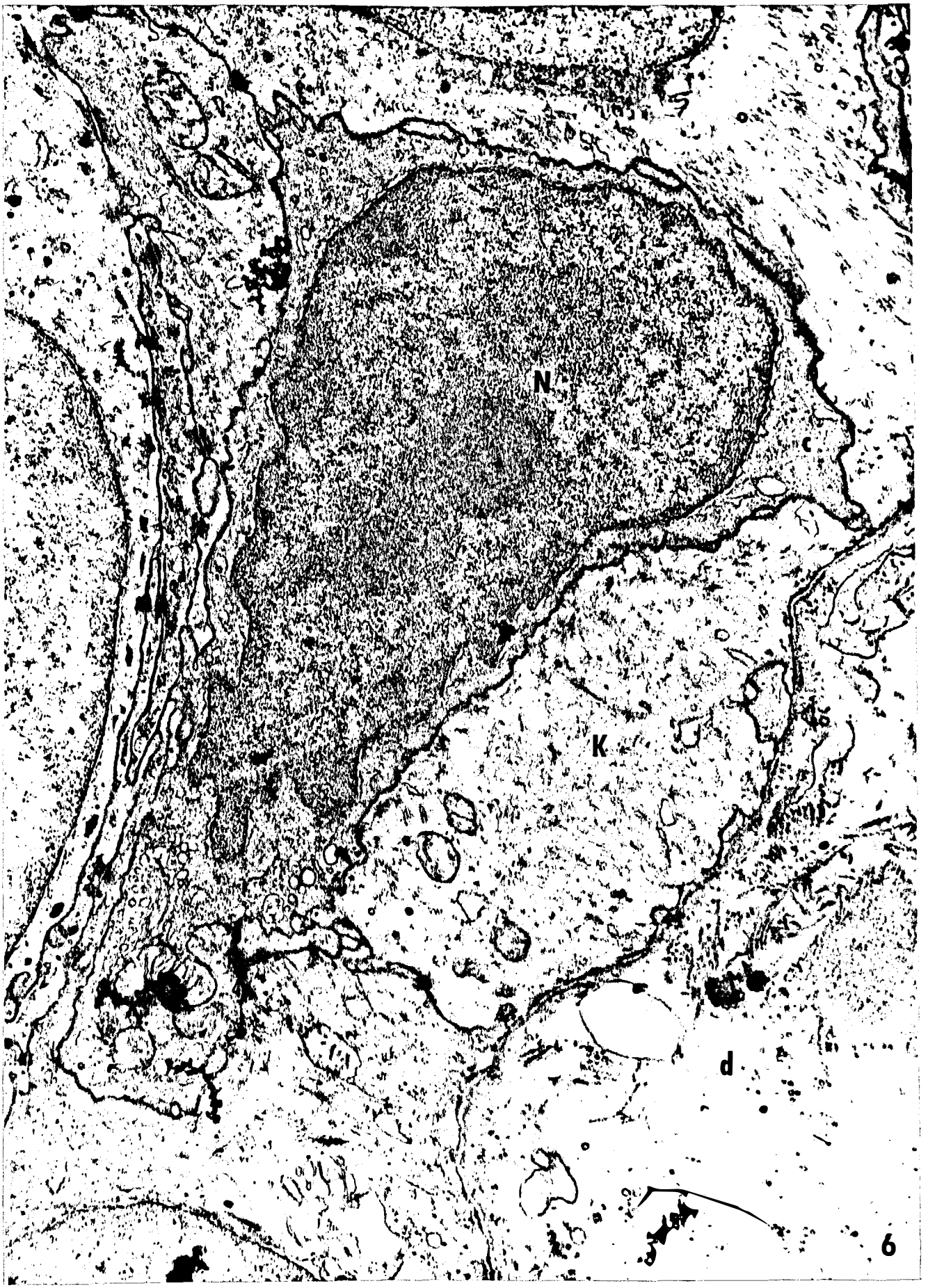

N
K
d
6

Figure 7 -- Electron micrograph of part of a suprabasal dendritic semikeratinocyte from 3-day-old normal PET mouse epidermis. The cytoplasm is relatively clear compared to the surrounding keratinocytes, and contains a reduced number of ribosomes. Minidesmosomes are seen along the plasma membrane. Ribosomes, r; minidesmosomes, md; semikeratinocyte, S; keratinocyte, K. Magnification: 16,110x.

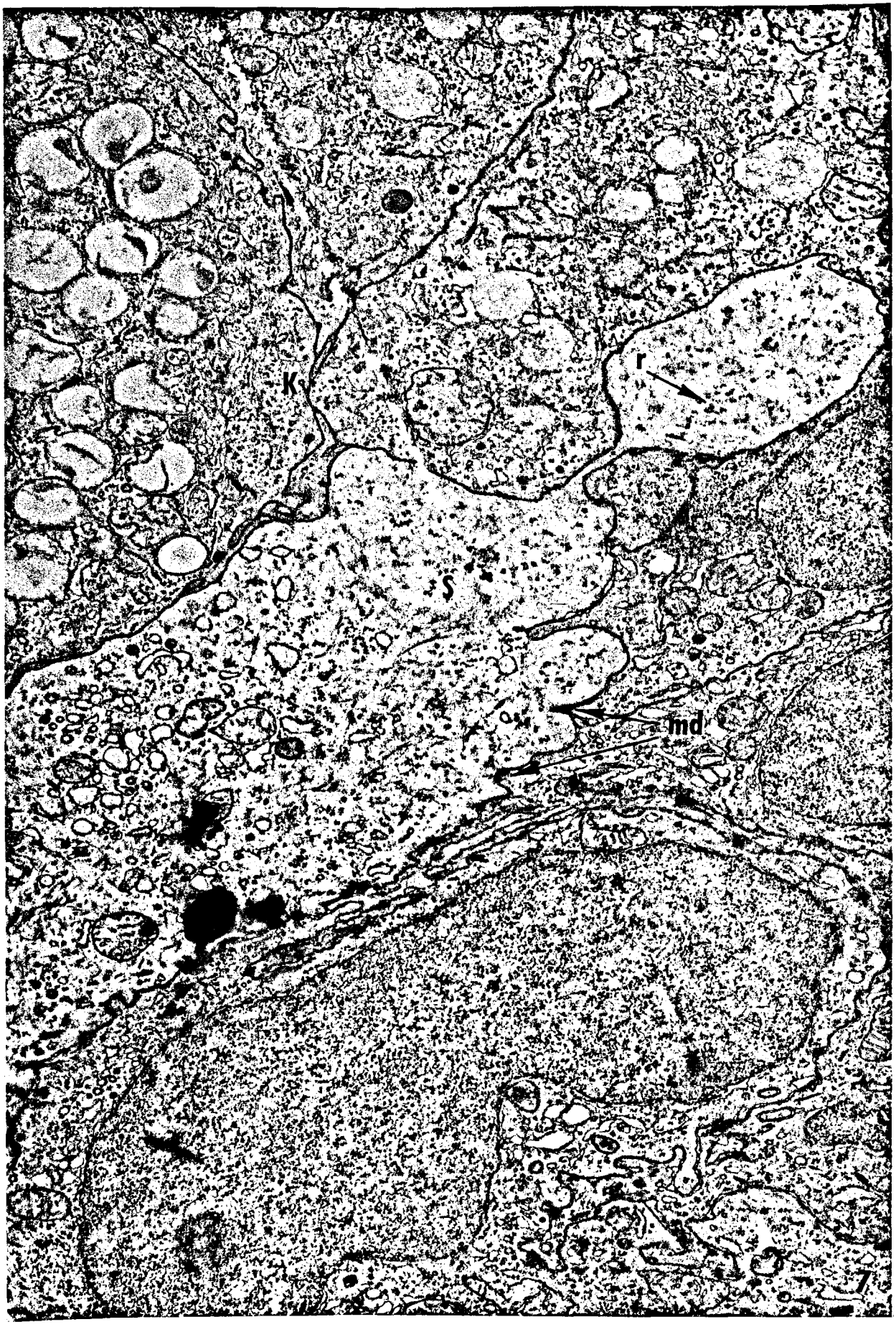

K
r
S
md

Figure 8 -- Electron micrograph of a basal semikeratinocyte (type A) from neural crest-free PET mouse epidermis. The nucleus is irregular and the cytoplasm is relatively clear compared to that of the adjacent keratinocytes. The cytoplasm contains a reduced number of ribosomes, and the plasma membrane possesses several minidesmosomes. Nucleus, N; ribosomes, r; minidesmosomes, md; semikeratinocyte, S; keratinocyte, K. Magnification: 15,280x.

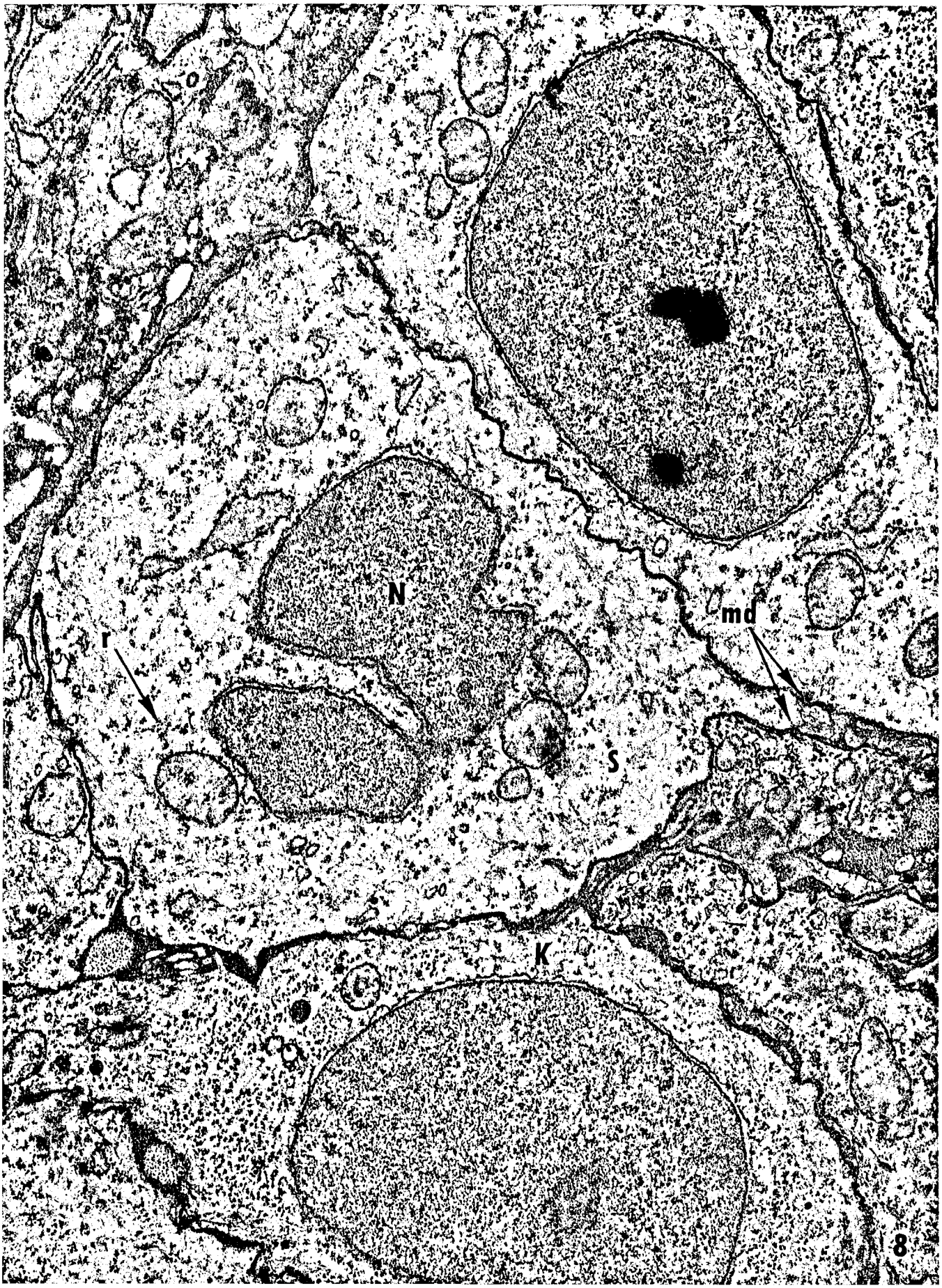

N
r
md
S
K

Figure 9 -- Electron micrograph of a basal semikeratinocyte (type A) from neural crest-free epidermis. The nucleus appears to be acquiring a convoluted shape. Nucleus, N; semikeratinocyte, S; minidesmosome, md; dermis, d. Magnification: 36,900x.

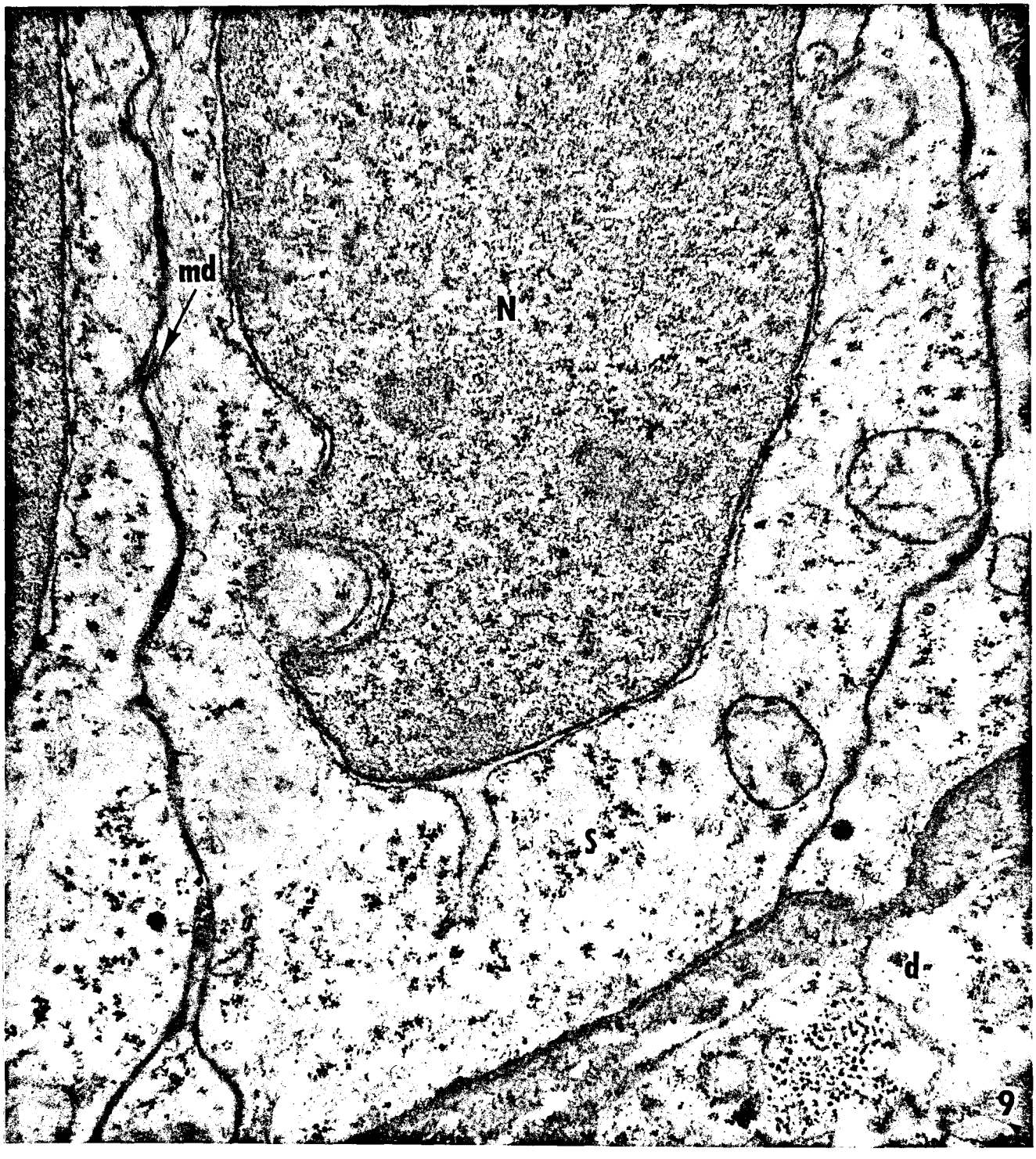

md
N
S
d
9

Figure 10 -- Electron micrograph of a semikeratinocyte (type A) migrating out of the basal layer into the suprabasal layers. The nucleus appears to be acquiring a convoluted shape. Nucleus, N; semikeratinocyte, S; dermis, d. Magnification: 19,680x.

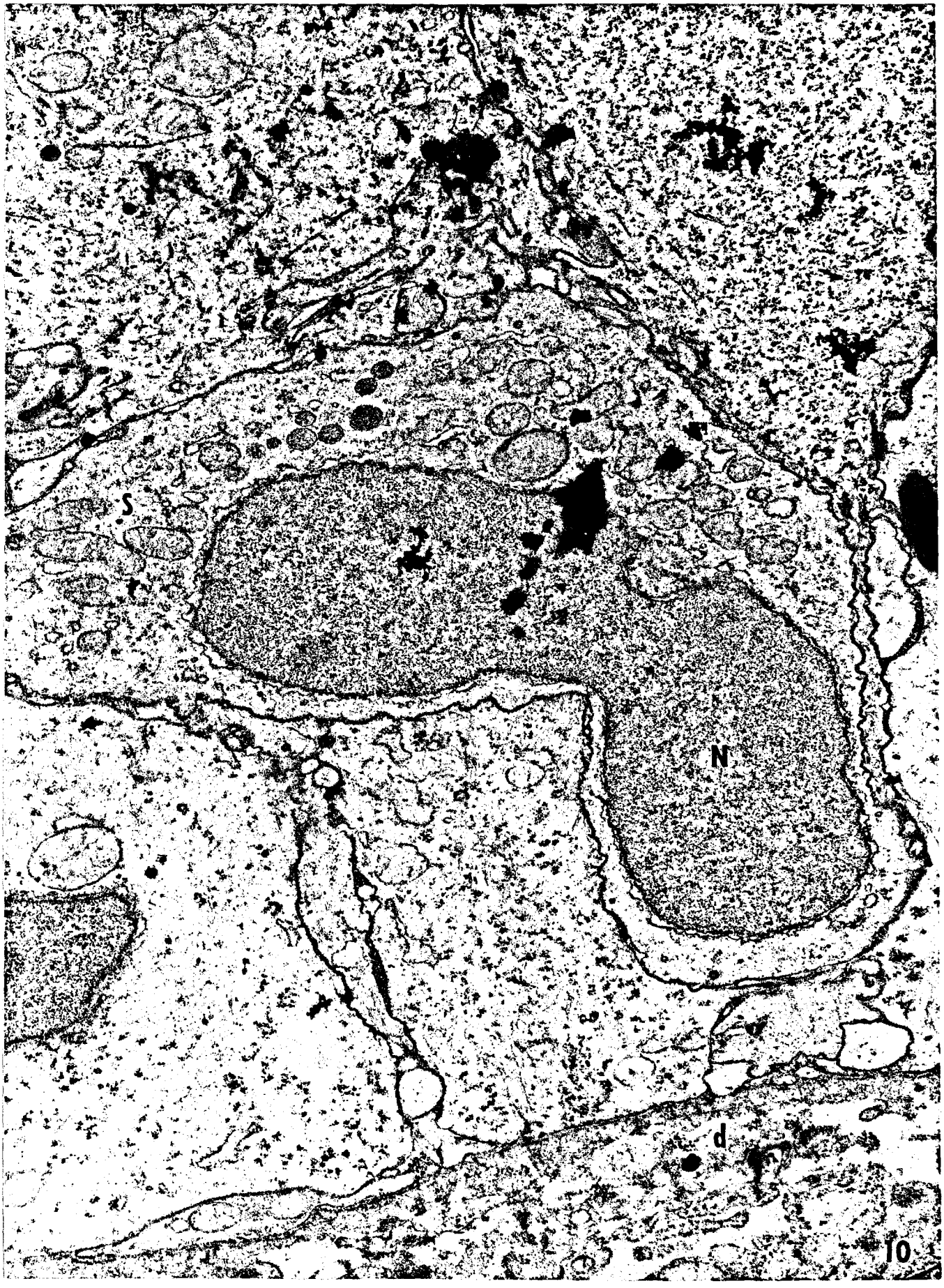

S
N
d

Figure 11 -- Electron micrograph of a dendritic suprabasal semikeratinocyte (type A). The nucleus is irregular and the cytoplasm is relatively clear compared to that of the adjacent keratinocytes. The cytoplasm contains fewer ribosomes than the adjacent keratinocytes. Nucleus, N; semikeratinocyte, S; ribosomes, r; keratinocyte, K. Magnifiaction: 22,960x.

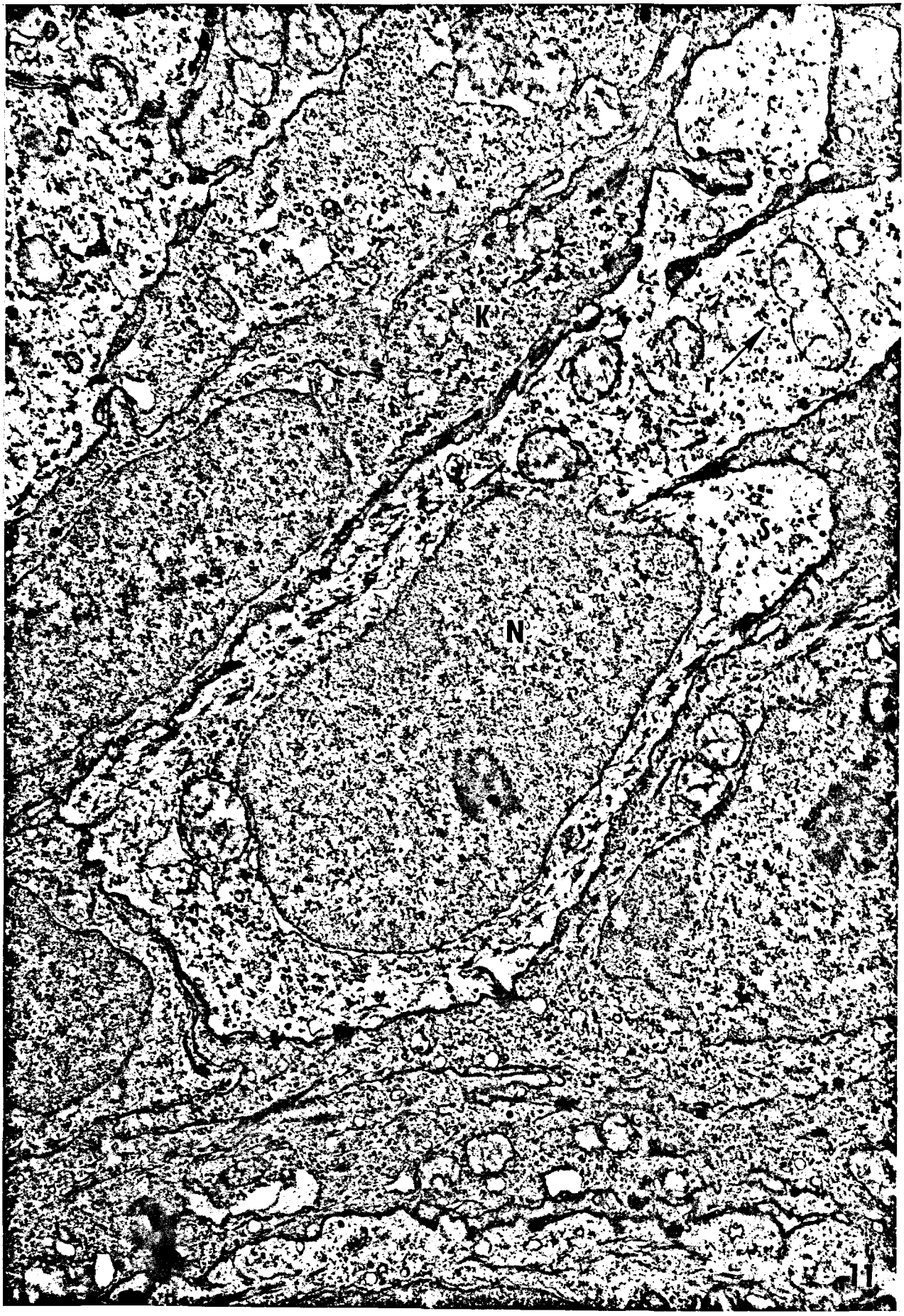

K
r
S
N

Figure 12 -- Electron micrograph of suprabasal dendritic semikeratinocytes (type A). Semikeratinocyte, S. Magnification: 12,606x.

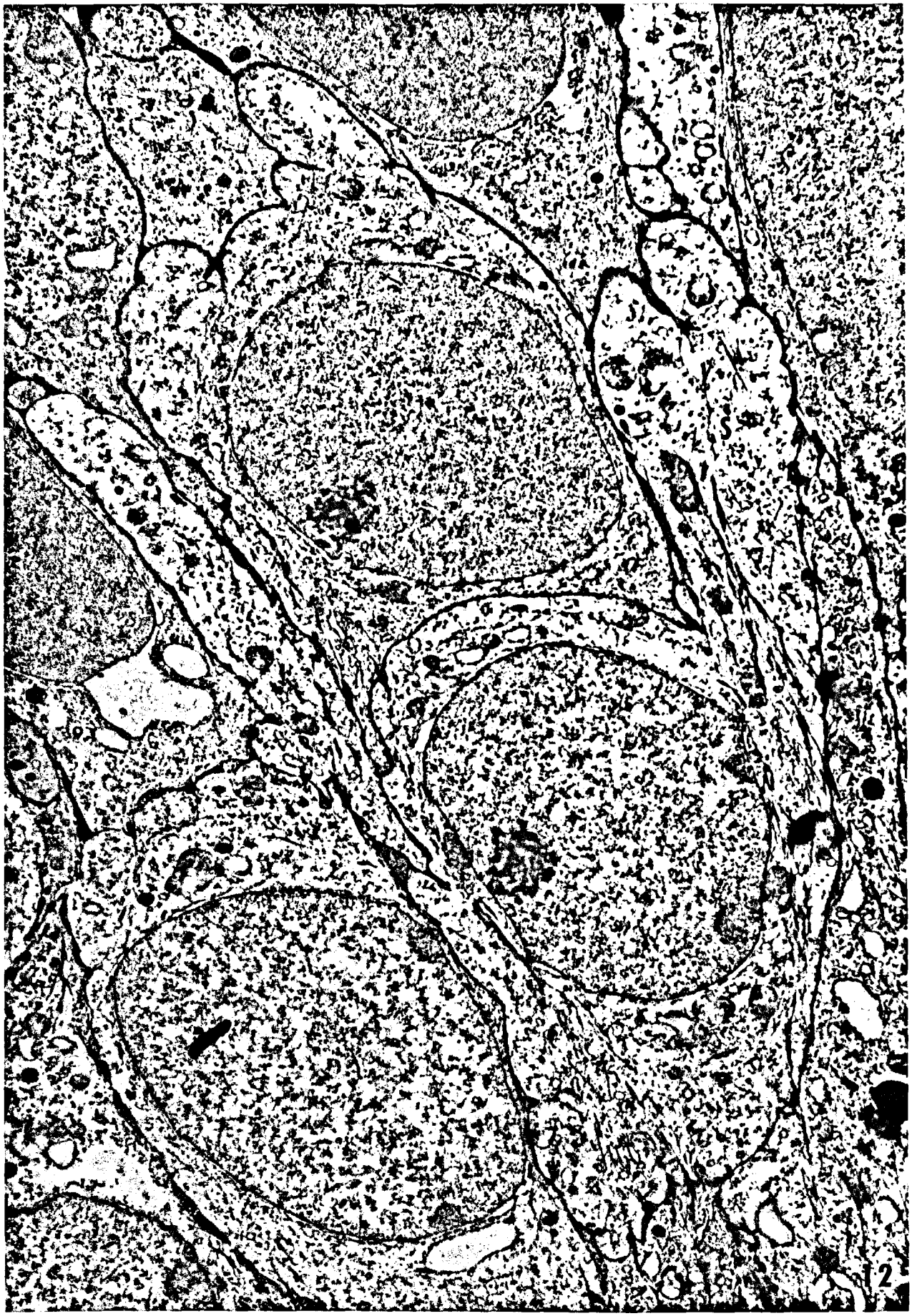


Figure 13 -- Electron micrograph of a suprabasal semikeratinocyte (type A). The nucleus is irregular and the cytoplasm is relatively clear compared to that of the surrounding keratinocytes. The cytoplasm contains few ribosomes, and a vacuole is lined with the remnant of an autophagocytized desmosome. Nucleus, N; ribosomes, r; remnant of an autophagocytized desmosome, A; semikeratinocyte, S; keratinocyte, K. Magnification: 29,910x.

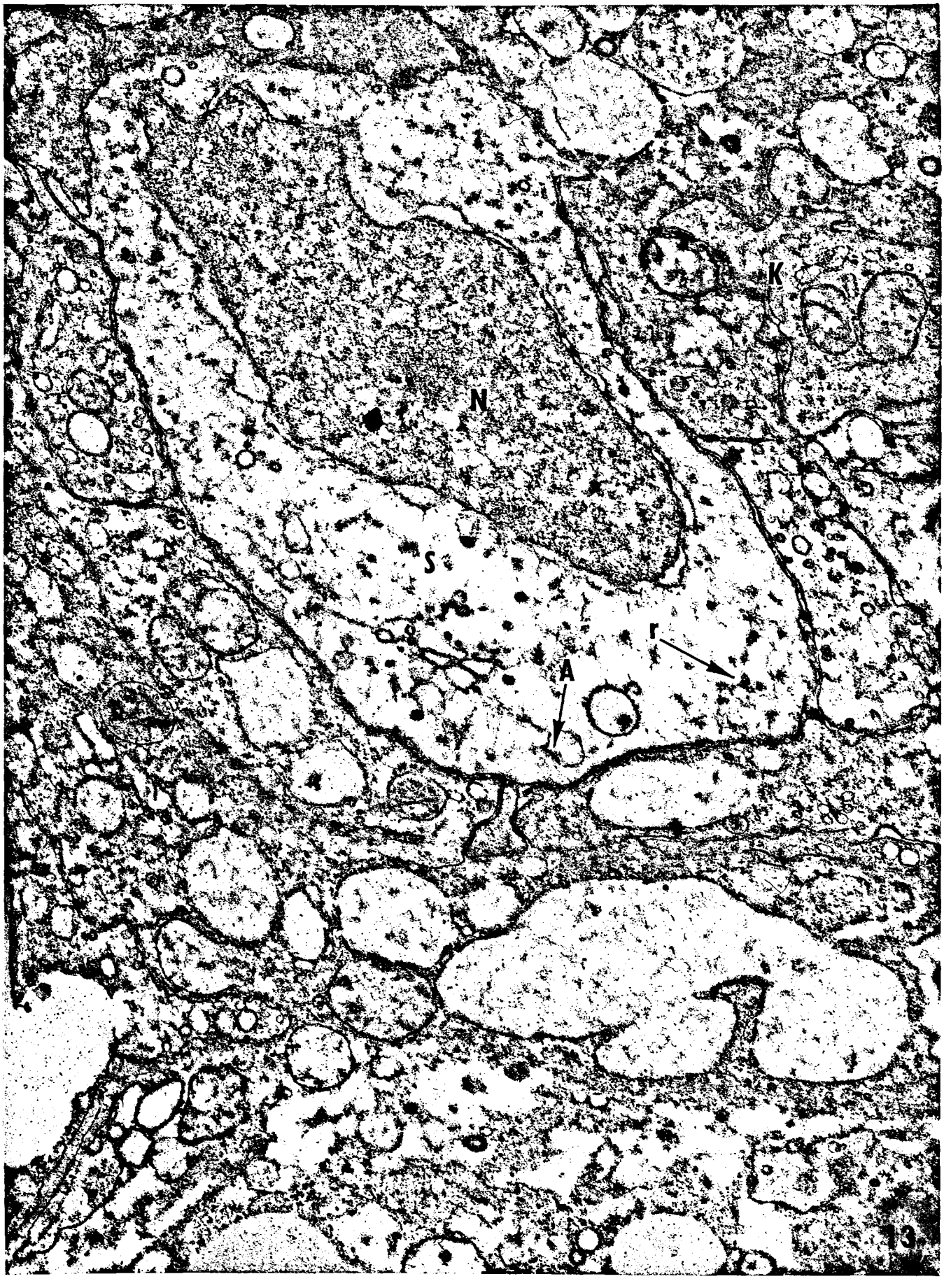
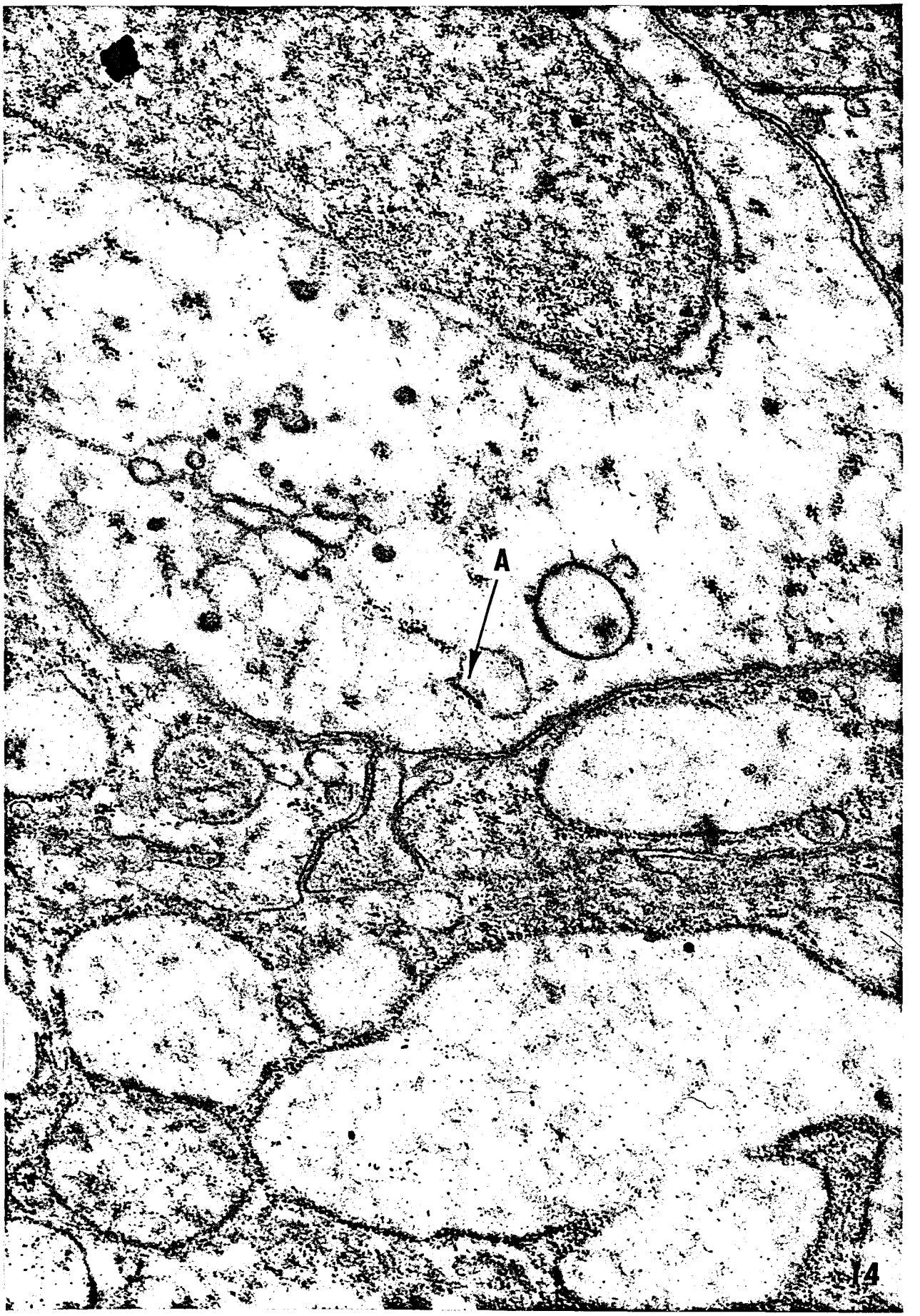

K
N
S
r
A

Figure 14 -- Electron micrograph of a high magnification of Figure 13 showing the vacuole containing an autophagocytized desmosome (A). Magnification: 44,850x.



14

Figure 15 -- Electron micrograph of suprabasal dendritic semi-keratinocyte (type B). The nucleus is irregular and the cytoplasm is more electron dense than that of the adjacent keratinocytes. The plasma membrane appears to lack desmosomes. Nucleus of the semi-keratinocyte, N; keratinocyte, K. Magnification: 34,114x.

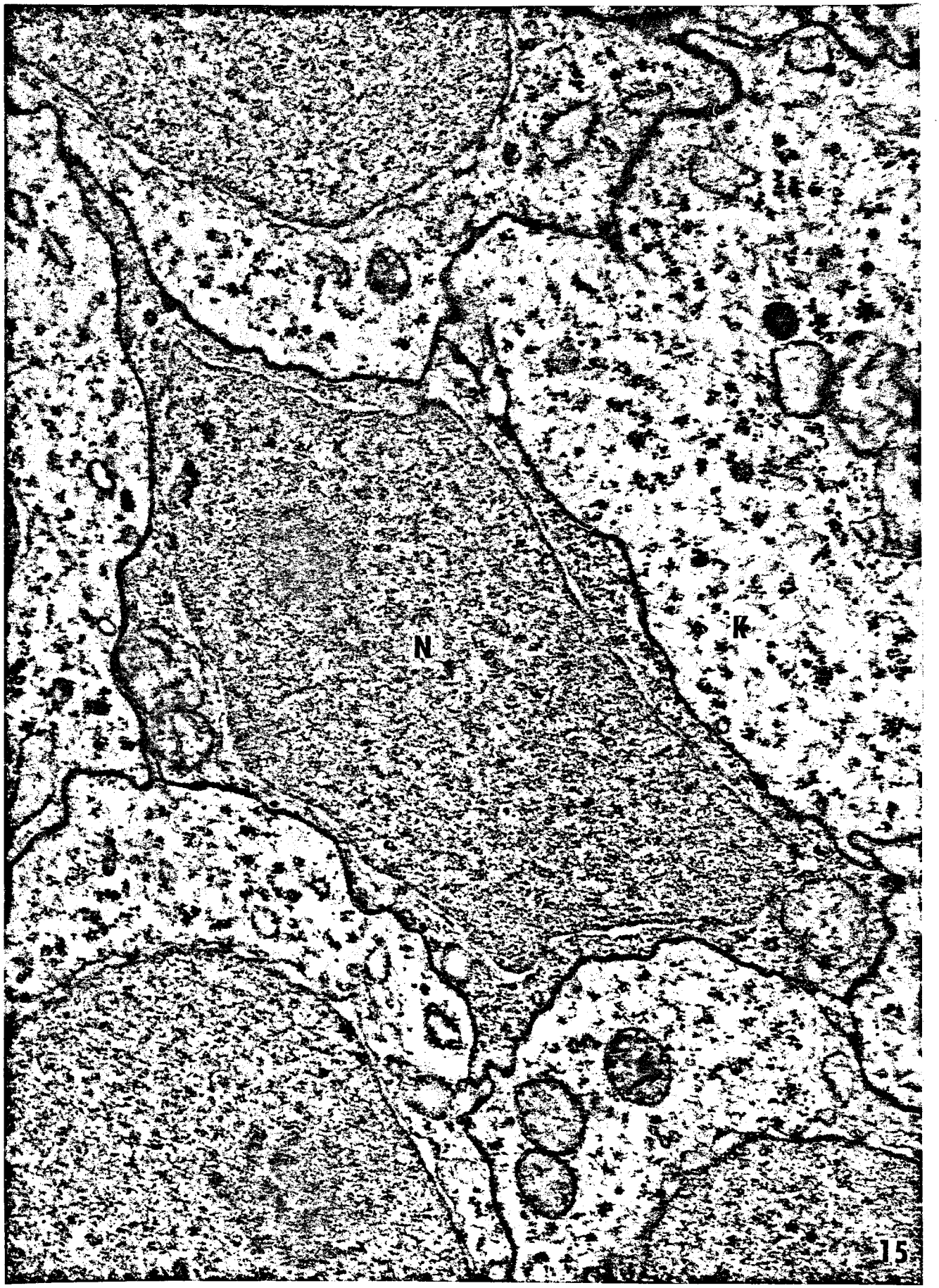
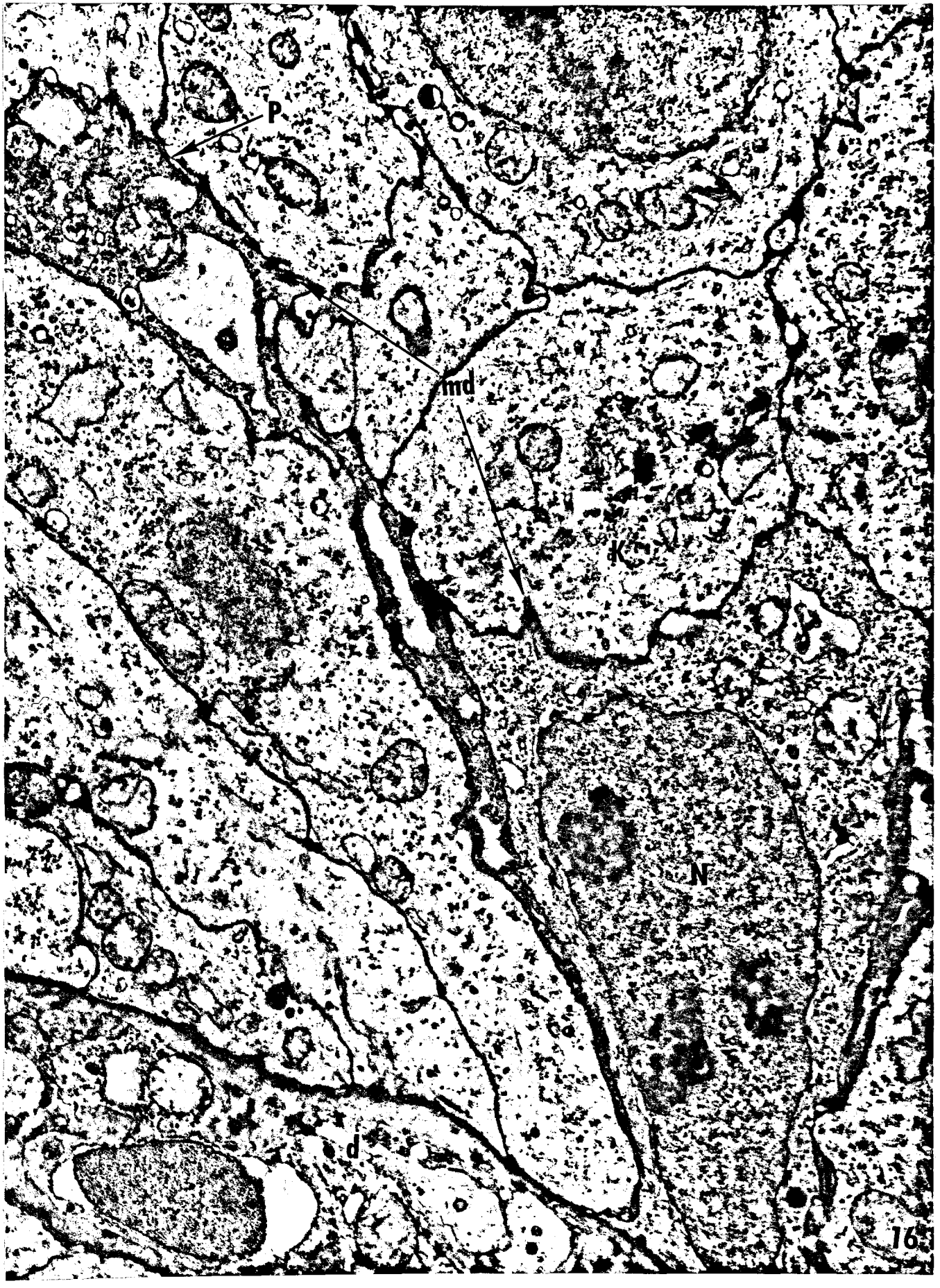

N
K
15

Figure 16 -- Electron micrograph of a basal dendritic semikeratinocyte (type B). The nucleus is irregular and the cytoplasm is more electron dense than that of the adjacent keratinocytes. A dendritic process is extending into the superficial cell layers. The plasma membrane possesses a few minidesmosomes. Nucleus of the semikeratinocyte, N; minidesmosomes, md; dendritic process, P; keratinocyte, K; dermis, d. Magnification: 17,056x.


P
md
N
d
16

VITA

Stanley Powell Tompkins was born in Portsmouth, Virginia, on April 18, 1943. He received his elementary and secondary education through the Norfolk County public schools and was graduated from Churchland High School in June, 1961. He began his undergraduate studies at Old Dominion College in September, 1961. After attending Virginia Polytechnic Institute, he returned to Old Dominion College where he received a Bachelor of Science degree in biology in June, 1967.

In September, 1967, he entered the Graduate School at the University of Richmond to pursue studies leading to a Master of Science degree in biology. During his graduate studies, he was elected to membership in the Beta Beta Beta Biological Honor Society and the Atlantic Estuarine Research Society. He assisted in the General Biology laboratories, and was a research assistant in electron microscopy in the Department of Dermatology at the Medical College of Virginia, under the supervision of Dr. W. M. Reams, Jr. While engaged in this research, he was a coauthor on the following paper:

Reams, W. M., Jr. and S. P. Tompkins (1969) An EM study of the dendritic keratinocytes of the PET mouse. The ASB Bulletin, 16(2) : 64.

Upon receiving a Master of Science degree in biology in August, 1969, he plans to pursue a professional career through studies in the School of Dentistry at the Medical College of Virginia.

He is married to the former Patricia Virginia Tynes.