The Ultrastructure of the Human Epidermis in Chronic Graft-Versus-Host Disease

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The epidermal ultrastructure of 11 allogeneic bone marrow recipients with chronic graft-versus-host disease (GVHD) was compared with that of 4 recipients without chronic GVHD. This electron microscope study revealed three patterns of epidermal injury typical of chronic GVHD. The first type was a nonacantholytic (nondissecting) injury with a prominent cellular infiltrate consisting primarily of lymphocytes accompanied by a few macrophages. The second type was an acantholytic (dissecting) injury with a prominent infiltrate, while the third was a nondissecting injury with a sparse infiltrate. Broad-zone contact was observed between lymphocytes and all epidermal cell types as well as between other lymphocytes and macrophages. Point contact was only observed between lymphocytes and epidermal cells. Lymphocytes appeared to detach desmosomes from adjacent keratinocytes by isolating them with cytoplasmic projections, a phenomenon not previously described. Typical damage to the epidermal cells in the basal and spinous layers consisted of either swelling of the organelles or condensation of the cytoplasm and nucleus. In the keratinocyte, the condensation reaction resulted in the formation of colloid bodies, some of which were phagocytized by macrophages. Besides the cytolytic events, a concurrent stimulatory reaction occurred in the epidermal cells. The number of melanosomes in melanocytes and of Langerhans cell granules and dense bodies in the Langerhans cells all increased. Extensive areas of replication and disruption of the basal lamina were subjacent to areas of necrosis in the basal layer. (Am J Pathol 95:643–662, 1979)

HUMAN CUTANEOUS graft-versus-host disease (GVHD) resulting from allogeneic bone marrow transplantation has two distinct clinicopathologic phases, acute and chronic. Previous ultrastructural studies of GVHD have focused only on the acute reaction. The first and most detailed studies by Woodruff et al [1] and Slavin and Woodruff [2] described the epidermal changes in the mucous membranes of irradiated chimeric monkeys. In these studies, acantholysis and autophagogenic degeneration of keratinocytes were associated with lymphocytes interpreted as invading aggressors. Another type of damage, satellite cell dyskeratosis (SCD), in which lymphocytes encircle and isolate target cells, was considered as a specific lymphocyte-target cell reaction. Two electron microscope studies

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Table 1—Type and Phase of Chronic GVHD, Patterns of Epidermal Damage Obtained from the Skin Biopsies

Unique patient number	Race*	Day†	Type and phase of chronic GVHD	Pattern of epidermal injury
262	C	339	Early generalized	3
274	C	946	Late generalized	3
294	AI	147	Early generalized	2
		384	Transitional generalized	3
		1420	Late generalized	‡
330	C	165	Early generalized	2
		239	Early generalized	3
366	C	535	Localized	3
387	C	395	Localized	1
		830	Localized	‡
512	C	210	Early generalized	3
		379	Late generalized	2
520	B	246	Early generalized	3
		355	Transitional generalized	1
585	C	137	Early generalized	2
		607	Late generalized	1
612	C	243	Early generalized	1
648	C	69	Early generalized	1

* C = caucasian; AI = american indian; B = black

† Day refers to time after transplant, with the day of transplant being day 0.

‡ Material preparation in these specimens was of poor quality, and no analysis of epidermal damage was performed.

on GVHD in humans [3,4] were each based on a single case. Neither patient was a bone marrow recipient. The results of the two human studies agreed in general with those reported by Woodruff et al,[1] except that DeDobbeleer et al [3] did not find dyskeratotic bodies associated with an SCD reaction. More recently, Sale et al,[5] using the light microscope, reported that SCD can also occur after transplantation as a dose-related reaction to chemotherapy and irradiation combined.

In our previous study we described in detail the pathologic and clinical features of chronic cutaneous GVHD, both the generalized and localized types.[6] Each type has some dermatologic features of the collagen-vascular diseases, generalized morphea and lupus erythematosus, and of lichen planus. The generalized type has a biphasic course, the early phase showing acanthosis, an extensive lichenoid reaction, often with a prominent infiltrate in the basal layer; the localized type has only mild basal vacuolization, inflammation, or cellular necrosis. The late phases of both types show poikilodermatous changes, with epidermal atrophy, hyperpigmentation, and little or no inflammation.

In this second paper on chronic GVHD we report on the ultrastructural alterations of the same epidermal specimens from 9 of the original plus 2 additional allogeneic bone marrow recipients. Our observations were not

Table 2—Non-GVHD Group

Unique patient number	Race	Day
171	C	1460†
324	AI	786
342	C	736
395	C	440

obstructed by the immediate pretransplant chemotherapy-radiation effects or by posttransplant methotrexate immunosuppression. The electron microscope findings suggest a sequence of reactions leading to both subcellular damage and cell death of all epidermal cell types and to a stimulatory reaction resulting in the activation of melanocytes and Langerhans cells. Several forms of lymphocyte-target cell interactions were seen in chronic GVHD which may be directly involved in cell damage.

Materials and Methods

Skin biopsy specimens from involved areas of generalized and localized chronic GVHD were obtained from 11 survivors of allogeneic bone marrow transplantation (8 with aplastic anemia, 3 with leukemia). The clinicopathologic features of 9 have been summarized elsewhere.[6] The other 2 [Patients (UPN) 612 and 648] had clinical and pathologic features of early generalized chronic GVHD. In all, 18 skin biopsies were performed on these patients at various stages of chronic GVHD (see Table 1). Biopsy specimens were also obtained from 4 long-term survivors of allogeneic bone marrow transplantation (2 with aplastic anemia, 2 with acute lymphocytic leukemia) without GVHD (Table 2), for purposes of comparison.

Specimen Preparation

The specimens (2–4) were removed under local anesthesia (1% lidocaine) and were initially placed into Millonig's buffered formalin [7] or into half-strength Karnovsky's fixative.[8] Half of the specimen (2 mm × 2 mm) was further processed for electron microscopy and the remainder prepared for light microscopy. The tissue for electron microscopy was fixed for an additional 2–6 hours in half-strength Karnovsky's fixative 4 C, washed in 0.1 M cacodylate buffer, and refrigerated in buffer until further processing. The blocks were postfixed in 1% OsO_4 in s-collidine buffer for 2 hours at room temperature, dehydrated in an ethanol, propylene oxide series, and embedded in Epon 812. Thin sections were stained with saturated aqueous uranyl acetate solution and Millonig's lead stain.[9] The sections were examined with an AEI Corinth 500 electron microscope operating at 60 kV.

Ultrastructural Criteria for Analysis of Epidermal Damage

The evaluation of each specimen focused on 4 factors: 1) Epidermal continuity based on the presence and amount of "spongiosis" and "acantholysis." In these specimens the range of damage was from none, a nondissecting lesion, to frank "acantholysis," a dissecting injury. 2) The degree of lymphocytic infiltration—a prominent infiltrate consisted of three or more lymphocytes per thin section, and a sparse infiltrate from a few lymphocytes per 20–30 sections to two lymphocytes per thin section. 3) The type and degree of subcellular damage sustained by an individual cell in the basal and spinous

Table 3—Comparison of the Three Patterns of Epidermal Injury

Feature	Pattern of epidermal injury		
	1	2	3
Epidermal continuity	Nondissecting	Dissecting	Nondissecting
Lymphocyte infiltration	Prominent	Prominent	Sparse
Subcellular damage	Tonofibrillar Disruption Prominent	Tonofibrillar Disruption Prominent	Organellar Vacuolation Prominent
Number of damaged cells	Few	Many	Many

layers. Minimal damage consisted of a change in a few organelles or subcellular structures, whereas a dyskeratotic or necrotic body was at the extreme end of the scale. 4) The overall impression of epidermal injury based on the subcellular damage to the individual cell (Factor 3) and on the number of cells affected in a thin section. Minimal injury to the epidermis as a whole included thin sections with a few cells with minor changes as well as those with one dyskeratotic body among normal cells. Damage in severe injury ranged from the disruption of the mitochondria to necrosis of most of the cells in the basal and spinous layers.

Characterization of each specimen, using the above criteria, then resulted in the recognition of a complex of changes which were grouped into three major categories of injury (Table 3). The first two factors, epidermal continuity and lymphocytic infiltrate, became the major identifying features for each type of injury. Table 1 lists the category of epidermal damage for each specimen.

Results

Epidermal Damage and the Keratinocyte

Type 1 injury (Table 3) which was nondissecting occurred when there was a prominent lymphocytic infiltrate, and the ultrastructure of the majority of the cells appeared normal (Figure 1). Some keratinocytes, particularly those in the vicinity of mononuclear cells, revealed one of the early signs of subcellular injury, a disruption of the tonofibrillar system. This disruption resulted either in a ring of tonofibrils surrounding the nucleus or in a shortening or loss of the tonofibrils (Figures 1 and 12). The cytoplasm of these affected keratinocytes appeared less electron-dense than that of the surrounding keratinocytes, and cytolysosomes or autophagocytic vacuoles (Figure 12) were occasionally present. In the basal layer an infrequent dyskeratotic or necrotic keratinocyte consisted of a condensed nucleus, dense cytoplasmic material, remnants of the membraneous organelles, and tonofilaments. Occasionally, isolated desmosomes from the damaged keratinocytes were found in the intercellular space which itself was not widened.

Type 2 injury was characterized by "acantholysis" (dissection) and a prominent lymphocytic infiltrate (Figure 2). Acantholysis, ie, the presence

of free or detached keratinocytes, was preceded by spongiosis (extracellular edema) (Figure 2). The subcellular damage was similar to that in the Type 1 injury but always affected most of the cells of the basal and spinous layers.

In the severe stage of this injury, free keratinocytes were in microscopic bullae bounded below by the basal lamina and the remnants of basal keratinocytes and above by normal upper epidermal layers. Missing from the detached keratinocytes were both desmosomes and tonofibrils, while in the keratinocytes adjacent to the bullae, changes characteristic of spongiosis predominated. In these keratinocytes the desmosomes were present but inconspicuous, as a result of the shortening or partial loss of the tonofibrillar system (Figure 3); rare keratinocytes had intracellular desmosomes. In other areas, desmosomes were either lying free in the intercellular space, or one-half of the desmosome remained in the cell with the other half detached from the adjacent keratinocyte (Figure 3). On serial sectioning, both completely and partially detached desmosomes were surrounded by small amounts of cytoplasm and a plasma membrane (Figure 4). While the disruption of the tonofibrillar system was similar to Type 1 damage, in Type 2 injury there was also dilation and vesiculation of the mitochondria and endoplasmic reticulum (ER) (Figure 3).

The third pattern of injury was nondissecting with a sparse lymphocytic infiltrate, and the subcellular damage was characterized by dilation of the organelles in many keratinocytes. Disruption of the tonofibrillar system was a less conspicuous feature, especially when compared with Type 2 injury (Figure 5). In Type 3 injury the keratinocytes of the basal layer contained electron-lucent zones in which ribosomes, mitochondria, and tonofibrils were absent, although hemidesmosomes persisted. Where these electron-lucent areas in individual cells became extensive, the epidermis took on a checkerboard appearance with normal, electron-lucent and electron-dense keratinocytes alternating. With extensive Type 3 damage the dermal-epidermal junction was obscured by necrotic cells, cytoplasmic fragments, and the mononuclear-cell infiltrate in the upper dermis, and there were present some areas of replication and discontinuity of the basal lamina (Figure 5). Nevertheless, the hemidesmosomes of the remaining basal keratinocytes were still attached to the basal lamina, and the intercellular space was only minimally widened.

In Types 2 and 3 injury, in addition to the necrotic keratinocytes, colloid bodies [10] (apoptotic bodies) were also found. These bodies (Figure 6) lacked plasma membrane and contained few or no recognizable subcellular structures. Occasionally, amyloid bodies [10] were also found in the papillary dermis in close promimity to the basal lamina (not shown). In all

types of injury the granular and horny layers were normal, except in several cases where the horny layer was punctuated by lacunas (see Brody [11]), not bounded by any membranous structure (Figure 7).

Melanocytes—Pigment Formation and Distribution

The damage to the melanocytes was equal to or greater than that to the surrounding keratinocytes in all types of injury. Some of the damaged melanocytes were partly retracted from the keratinocytes, while others were in close apposition to the electron-lucent zones of the keratinocytes (Figure 8). Frequently, melanocytes and lymphocytes were found together. Sometimes lymphocytes would surround or replace the melanocyte in lacunas projecting into the dermis (Figure 9). The cytoplasm of the damaged melanocytes appeared either electron-dense or, more often, electron-lucent. The ER, the mitochondria, and, in severe injury, all organelles were dilated (Figure 8). In several cells with severe cytoplasmic injury, the nucleus was condensed and convoluted, resembling the nucleus of a Langerhans cell.

In most melanocytes all four stages of melanosome-granule formation, including the mature granules, were easily identifiable. In one case (UPN 294), the melanocytes appeared to be even more active than in the other cases; the cytoplasm was packed with granules in all four stages. Characteristic of this hyperactive state, one of the melanocytes appeared to have a flagellum projecting into what resembled a vacuole or the intercellular space (Figure 10). In the dermis immediately subjacent to this cell and to the basal laminas was a fibroblast with a similar structure.

Increased numbers of mature melanosomes correlated with increased amounts of pigment granules in the keratinocytes, except in Type 2 injury, where there was a disruption of normal melanocyte-keratinocyte contact. In cases where there was increased pigment in the keratinocytes of the lower strata, there was also an increased amount in the horny layer.

In the keratinocytes, vacuoles with single as well as multiple pigment granules were present, regardless of the patient's racial origins. As expected, the two biopsy specimens from the black patient contained vacuoles in which single granules predominated, but the length of the melanin granules overlapped, irrespective of the patient's race.

Macrophages, the third cell type which contained pigment granules, were occasionally present in the epidermis, and always in the papillary dermis. A distinguishing feature of the pigmented cell types was the larger number of pigment granules (10–15) in the phagocytic vacuoles of the macrophage (Figure 2); the keratinocyte had at most 4–5 pigment granules per vacuole and the melanocytes had single granules scattered throughout the cytoplasm.

Langerhans Cells

In all three patterns of injury, the subcellular damage to the Langerhans cells, as to the melanocytes, was always equal to or greater than that to the surrounding keratinocytes. There was dilation of the ER and mitochondria, with condensation of the cytoplasm in severe cases. Keratinocytes in close approximation to damaged Langerhans cells usually had an electron-lucent zone in the abutting area. This complex of the damaged Langerhans cell and the partly disrupted keratinocytes may be the "eosinophilic body" seen in the upper epidermal layers by light microscopy. Some Langerhans cells appeared activated, since their cytoplasm contained more Golgi bodies and Langerhans cell granules than did those in the non-GVHD group. In addition these cells contained lysosome-like structures, small dense bodies (Figure 11), but only rarely, phagocytic vacuoles. In the late chronic stage, Langerhans cells were typically found next to the basal lamina (Figure 11). However, this was also seen in a few of the non-GVHD specimens. Langerhans cells were also found associated with lymphocytes.

Lymphocytes and Macrophages

Lymphocytes were usually found in all three types of epithelial damage but were present in fewer numbers in Type 3 injury. An occasional lymphocyte was also seen in the specimens from patients without chronic GVHD. As described by Woodruff et al,[1] lymphocytes appeared to be of two sizes: The smaller more common oval profile had an average length of 6.9 μm $\pm$ 1.71 SD (n=268), and the larger more elongated profile had an average length of 13.7 μm $\pm$ 2.17 SD (n=24) and width of 4.6 μm $\pm$ 1.40 SD (n=24) (Figure 12). In all patterns of injury the small or oval lymphocyte outnumbered the large lymphocyte by 1.5 to 5 times. Most of the oval lymphocyte was taken up by the nucleus, which was filled with condensed chromatin and occasionally had a small nucleolus. The cytoplasm of both types of lymphocyte contained a few strands of rough ER, mitochondria, scattered single ribosomes, a few clumped ribosomes, and a Golgi apparatus (Figures 1, 2, and 12). Centrioles were also seen. The cytoplasm of the elongated lymphocyte generally contained more clumped ribosomes and strands of rough ER (Figure 12) than that of the small lymphocyte. Occasionally, other structures were present in both types: multivesicular bodies (mvb), dense bodies (probably primary lysosomes) (Figure 12), and an occasional Gall body.[12]

The irregular outlines of the oval lymphocytes suggest movement through the intercellular space and very active cell surfaces (Figures 2 and 12–15). Often fine single-point projections of the lymphocyte cytoplasm were seen to invaginate the plasma membrane and cytoplasm of an

adjacent epidermal cell (Figures 12 and 13). At the area of interdigitation, the glycocalyces but not the cytoplasmic membranes of the two cells were confluent (Figure 13). A second type of contact consisted of encirclement of whole desmosomes and small amounts of cytoplasm of two adjacent keratinocytes (Figures 14 and 15) by paired cytoplasmic projections of the lymphocyte. This did not appear to be a phagocytic process, since the plasma membranes of the keratinocytes were not breached; desmosomes were never seen in a phagocytic vacuole of a lymphocyte, and there was always a connection to the intercellular space or to the cytoplasm of one of the keratinocytes (Figures 14 and 15). This process could account for the ability of the lymphocytes to migrate through the epidermis and for the presence of detached desmosomes. Both the fine single and the encircling projections were peculiar to the lymphocytes.

Satellite dyskeratosis as described by Woodruff et al,[1] in which a single lymphocyte encircled a damaged keratinocyte, was not seen. However, a similar phenomenon was observed, ie, a lymphocyte encircling a melanocyte in one instance and a Langerhans cell in another (Figure 16). Possibly this is a type of broad-zone contact. The pseudopodia of the lymphocytes in this broad-zone contact contained only single dispersed ribosomes (Figure 16). Neither the melanocyte nor the Langerhans cell appeared necrotic.

When there were large numbers of lymphocytes in the epidermis, they were always accompanied by macrophages but in smaller numbers. The macrophages were distinguished from the lymphocytes by their greater amounts of rough and smooth ER, clear vacuoles, and less condensed nuclear chromatin (Figure 17). Besides the large number of pigment granules, colloid bodies were also present in the phagocytic vacuoles (Figure 17). Dense bodies or primary lysosomes were only present in the macrophages that contained a few phagocytic vacuoles, eg, in those breaching the basal lamina, lying in both the epidermis and dermis. Often, lymphocytes and macrophages were apposed in a broad-zone contact. Their membranes interdigitated over wide surfaces, but the continuity of the membranes always remained intact.

Basal Lamina

Alterations of the basal lamina (irregularity, breaks, and replication) paralleled the degree of injury to the basal layer. In the non-GVHD group and in the mildest forms of injury (Type 1), there were only occasional small areas of splitting (Figures 1 and 18). Breaks or discontinuities were rare and sometimes could be explained by the plane of section.

In severe injury, there were extensive areas of irregularity and of

replication (Figures 6 and 19). In areas where the replication consisted of two parallel lamellas, the associated fibrillar structures were present on the basal lamina farthest from the epidermis (Figure 19), suggesting that it was the older basal lamina. Cytoplasmic fragments, occasionally an amyloid body (not shown), or pigment granules surrounded by a small amount of cytoplasm were also found in the dermis next to and between the areas of basal lamina replication (Figure 6). By light microscopy the subepidermal basement zone of these specimens was irregularly thickened and stained PAS positive (see Shulman,[6] Figure 8).

Discussion

We described three characteristic patterns of epidermal injury in chronic GVHD based on the presence of a lymphocytic infiltrate, dissection or acantholysis, and subcellular damage (Table 3). The term dissection was preferred to acantholysis, since the acantholytic process was not complete, detached desmosomes were found in the intercellular spaces, and hemidesmosomes persisted. None of the three patterns of epidermal injury, as seen by electron microscopy, was associated with any type or with the early or late phases of chronic cutaneous GVHD (Table 1) as seen with light microscopy. Furthermore, it does not appear that one pattern of injury was unique to an individual since, in 4 of the 5 patients with two specimens, each specimen was associated with a different epidermal injury (Table 1). Comparable changes in the epidermis were not seen in the group of asymptomatic bone marrow recipients.

The damage in the basal and spinous layers, especially in Type 1 and Type 2 epidermal injury, resembled the acantholytic lesions in acute GVHD and in cutaneous [13-17] and oral [18-21] lichen planus. Since most papers emphasize only one aspect of the ultrastructural changes, it is difficult to make exact comparisons. However, in all these conditions acantholysis was accompanied by an inflammatory infiltrate in the epidermis, a widening of the intercellular spaces, and degenerative changes in the basal cells. In chronic GVHD, lymphocytes were present in the epidermis prior to the widening of intercellular spaces. In oral lichen planus, the widening of the intercellular spaces may occur prior to the migration of the inflammatory infiltrate.[21] Nevertheless, the changes in the basal lamina were similar in chronic GVHD and in lichen planus, which was a possible indication of basal cell destruction.[22] No special mention of changes in the basal lamina or in the melanocytes and Langerhans cells occurred in the earlier reports of acute GVHD.[1,3,4]

Our observation of two types of lymphocyte-epidermal cell contact in chronic GVHD indicates that some of the epidermal changes are due to

cytotoxic lymphocytes. Similar types of contact were previously observed during cell-mediated cytotoxic reactions.[23,24] Point contact (PC) was mediated by small processes or microvilli (of lymphocytes) and involved small (0.2 μm) areas of membrane apposition. Broad-zone contact (BC) involved larger areas of membrane contact, often several microns in length.[23] BC does not alter the shape of the cells, whereas in PC, the lymphocyte pseudopodia tend to project into the cells.[24] In acute GVHD, these two types of contact can also be seen in micrographs 7, 8, and 11 in the paper by Woodruff et al.[1]

The satellite cell dyskeratosis (SCD) which Woodruff et al [1] described and the similar phenomenon in our material (Figure 16) is possibly a variant of BC. Woodruff et al [1] and Grogan [4] considered SCD a specific target-cell reaction. In chronic GVHD, satellitosis was not associated with necrosis, and BC was observed between lymphocytes, between lymphocytes and macrophages, and between lymphocytes and epidermal cells. Hence, cytotoxicity and BC may be independent of each other in chronic GVHD. Since PC in chronic GVHD was only observed between lymphocytes and epidermal cells, the presumed target cells, it may be the initiating cytotoxic event. Sanderson [24] also suggested PC was associated with cytolysis. Biberfield,[23] however, doubted that either type of contact can be proven morphologically as a specific expression of cytotoxicity.

The possible involvement of the humoral immune system in GVHD, as suggested by the presence of IgM and complement at the dermal-epidermal junction in some of these same patients,[25] suggests alternative methods of injury. Most of the damage in chronic GVHD is limited to the basal and spinous layers. Saurat et al [26-28] demonstrated that epidermal antibodies produced by allogeneic bone marrow recipients distinguished the basal cell layer from the upper cell layers. They suggested that different surface molecules are present at the various stages of keratinocyte differentiation and may be the possible target antigens.[27] Alternatively, the granular layer or, more specifically, the extracellular membrane coat of these cells may serve as a barrier, preventing the interaction of cytotoxic compounds or of lymphocytes with the target cells. In both keratinized and nonkeratinized epithelium, this coat serves as a permeability barrier to intercellular tracers, such as horseradish peroxidase and lanthanum, that have been introduced subepithelially.[29-32] Indeed, one of our findings was the presence of a complex of a damaged Langerhans cell and the partly disrupted keratinocytes surrounded by normal keratinocytes. The Langerhans cell may be susceptible to a specific cytotoxic reaction since it is unprotected by an extracellular membrane coat, whereas the keratinocyte in the granular layer would not be susceptible.

The ultrastructural findings in the epidermis in chronic GVHD are consistent with the findings by light and immunofluorescence microscopy,

suggesting a complex interplay of lymphocytes with other types of cells, as well as the possibility of damage mediated by the humoral immune system. Damage in Types 1 and 2 injury appears to be predominately due to the lymphocytic infiltrate. Type 2 injury would be a progression of Type 1 injury, with dissection resulting from the continued presence of a prominent lymphocytic infiltrate. The nondissecting or Type 3 injury may represent the aftermath of Type 1 injury, or an injury mediated by cytotoxic antibodies. Additional studies are needed to test these hypotheses.

Since the submission of this paper, Sanderson et al have further described the lymphocyte–target cell reaction,[33] and Searle et al [34] and Don et al [35] have described the association of apoptotic bodies and cell-mediated cytotoxicity. This supports our evidence for cell-mediated damage in chronic GVHD.

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[*Illustrations follow*]

Figure 1—UPN 648. Type 1 epidermal damage with prominent lymphocytic (*L*) infiltrate. Keratinocyte (*K*) with disruption of the tonofibrillar system. Areas of splitting of basal lamina (BL) (*arrows*). One area of BL breached by macrophage (*M*). (× 5600)

Figure 2—UPN 512. Type 2 epidermal damage, prominent lymphocyte infiltration, and widening of intercellular space. Disruption of the tonofibrillar system and autophagosome (*arrow*) in the keratinocyte. A convoluted replicated BL. A macrophage with a vacuole containing pigment granules is present in the epidermis. (× 5600)

Figure 3—UPN 585. Type 2 damage, dissecting injury. Keratinocytes with shortened tonofibrils, inconspicuous desmosomes (*circle*), and vacuolated mitochondria (*m*). *ER*, endoplasmic reticulum. Lymphocyte (*L*) in widened intercellular space. (× 5600)

Figure 4—UPN 585. Type 2 damage. Desmosomes lying free in the intercellular space, each surrounded by a small amount of cytoplasm and a plasma membrane. *K*, keratinocyte. (× 8100)

Figure 5—UPN 512. Type 3 damage. Extensive injury to the basal layer with vacuolation of membranous organelles of the keratinocytes. Damaged peripheral nerve (*PN*) in the dermis. *K*, keratinocyte. (× 6600)

Figure 6—UPN 330. Type 2 damage. Colloid body (*C*) near an irregular replicated basal lamina (*BL*). A lymphocyte (*L*) is migrating through a break in the BL. (× 5200)

Figure 7—UPN 585. Type 2 damage. Granular layer normal. Cells of the horny layer punctuated by lacunae (*arrow*). (× 6400)

Figure 8—UPN 387. Type 1 damage. Melanocyte (*Me*) with vacuolated organelles adjacent to keratinocytes (*K*) with a partial clearing of the cytoplasm (*arrows*) and to a lymphocyte (*L*). (× 9900)

Figure 9—UPN 366. Type 3 damage. Epidermal lacunae projecting into the dermis and containing a melanocyte (*Me*) surrounded by several lymphocytes (*L*). (× 4600)

Figure 10—UPN 294. Melanocyte with a flagellum (*f*) and numerous melanosomes. (× 30,300)

Figure 11—UPN 387. Langerhans' cell (*LC*) near basal lamina (*BL*) containing Langerhans granules and dense bodies. (× 11,500)

Figure 12—UPN 648. One elongate lymphocyte (*L*) and several oval lymphocytes (*L*) near basal lamina. The elongate lymphocyte contains a Golgi apparatus (*g*), dense bodies (*db*), multivesicular body (*mvb*), and a microvillus which extends into an adjacent keratinocyte (*double arrow*). Adjacent keratinocytes (*K*) with disrupted tonofibrillar systems and cytolysosome (*arrow*). Basal lamina is split and replicated. (× 4600)

Figure 13—UPN 512. The glycocalyces of lymphocyte (*L*) and keratinocyte (*K*) meet at apex of the microvillus (*arrow*). (× 42,000)

Figure 14—UPN 262. "Pinching off" of desmosomes (*arrow*) by a lymphocyte (*L*). *K*, keratinocyte. (× 17,400)

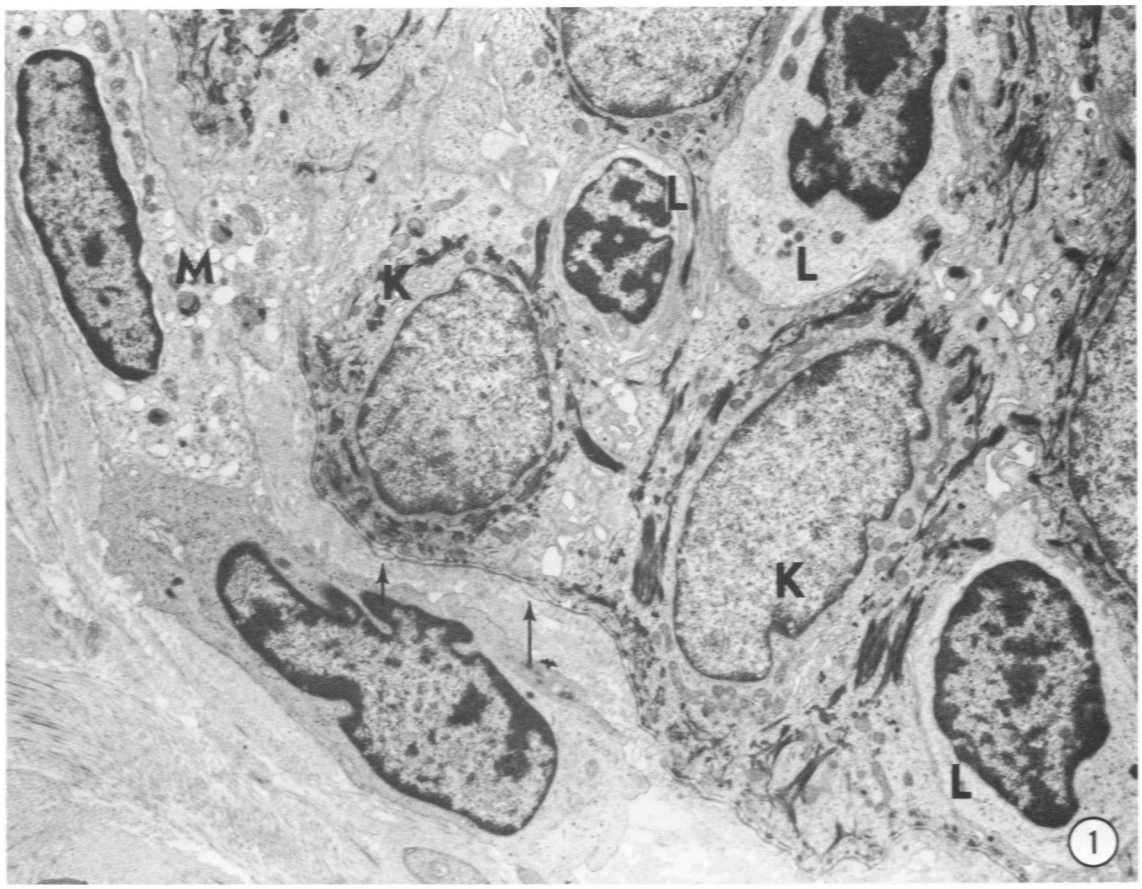
Figure 15—Several pinched-off desmosomes between the pseudopods of a lymphocyte (*L*). *K*, keratinocyte. (× 14,000)

Figure 16—UPN 648. "Satellite dyskeratosis" or broad-zone contact between a lymphocyte (*L*) and a Langerhans cell (*LC*). *Arrow* points to Langerhans cell granule. (× 9000)

Figure 17—UPN 520. Macrophage (*M*) containing colloid body (*C*). Dense bodies also present in cytoplasm. (× 8100)

Figure 18—UPN 171. Epidermis of asymptomatic bone-marrow recipient. Normal basal keratinocytes (*K*). Splitting of the basal lamina (*arrow*). (× 5600)

Figure 19—UPN 274. Areas of replicated basal lamina (*BL*). BL next to epidermis associated with fewer fibrillar structures. *Arrow* points to the anchoring fibril complex. (× 9500)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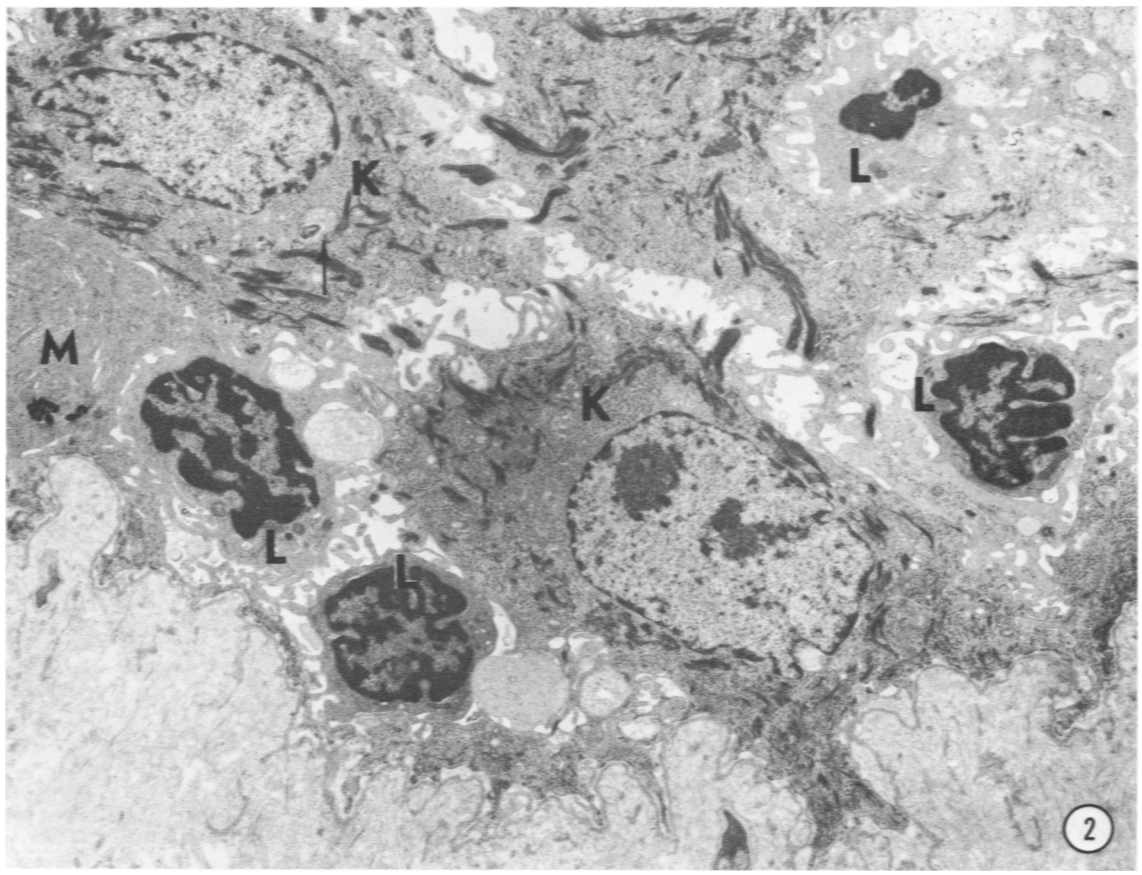
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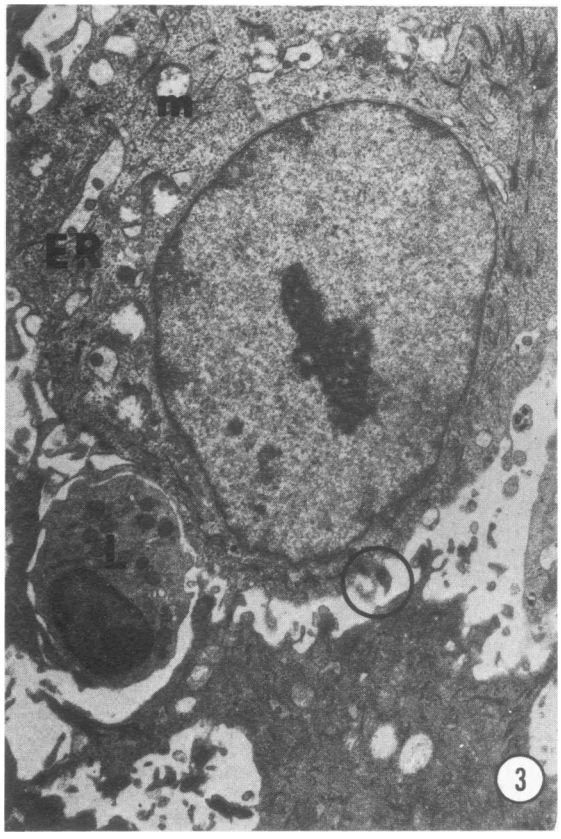
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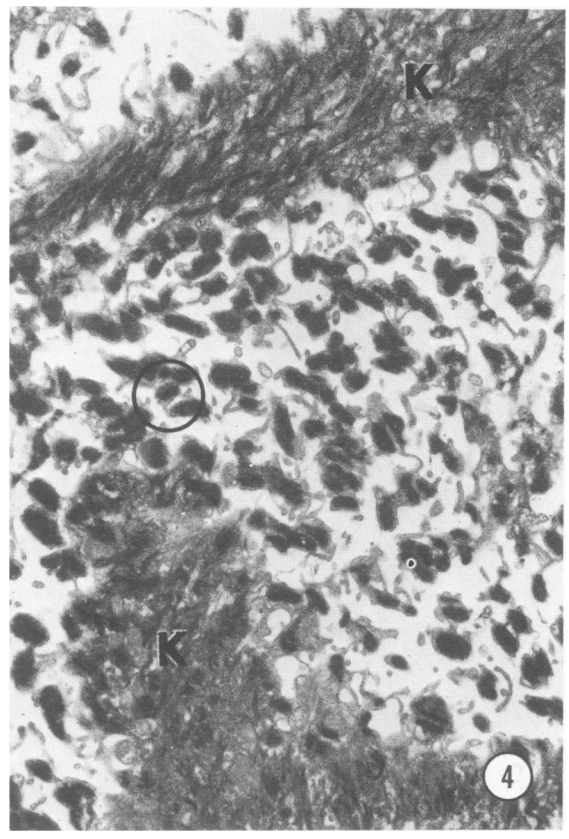
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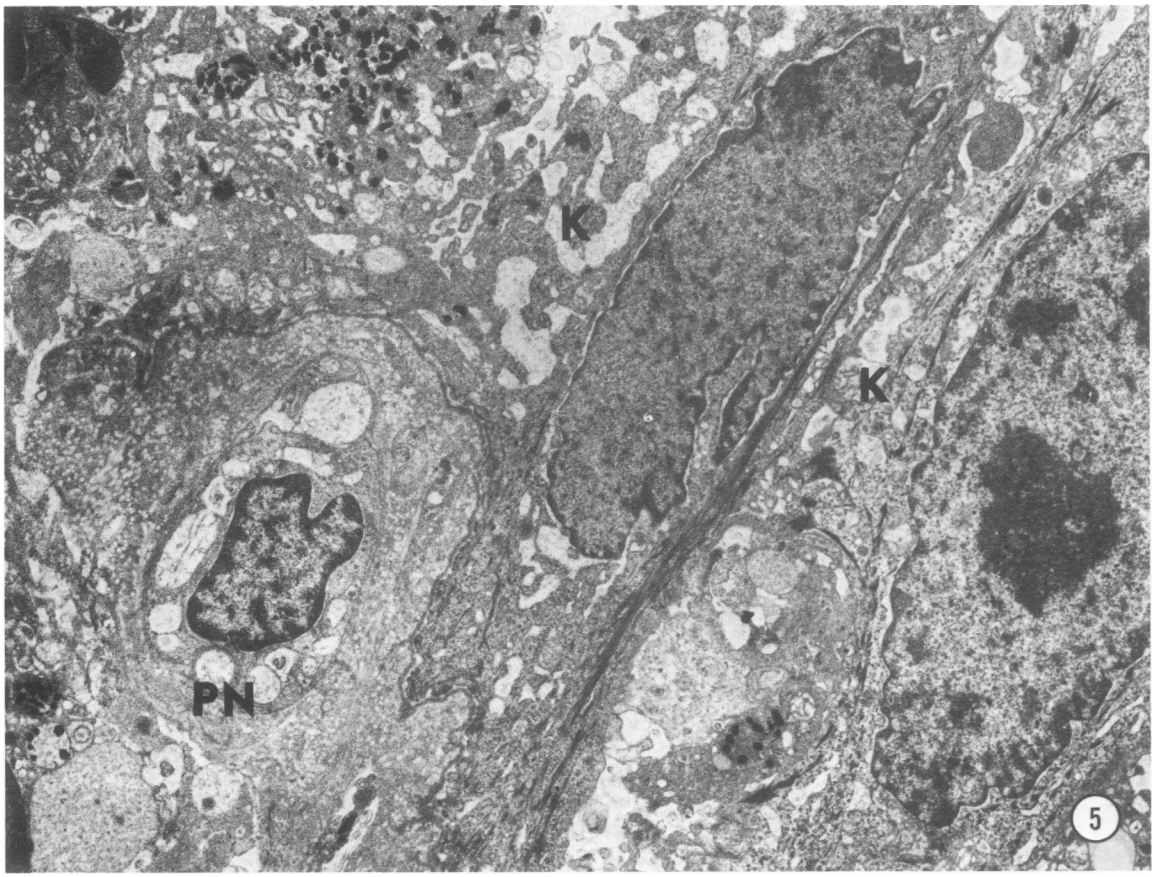
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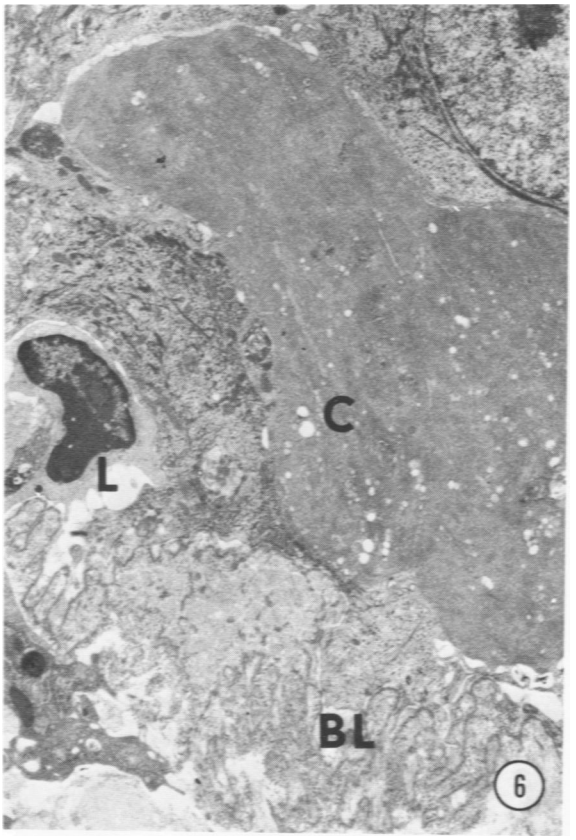
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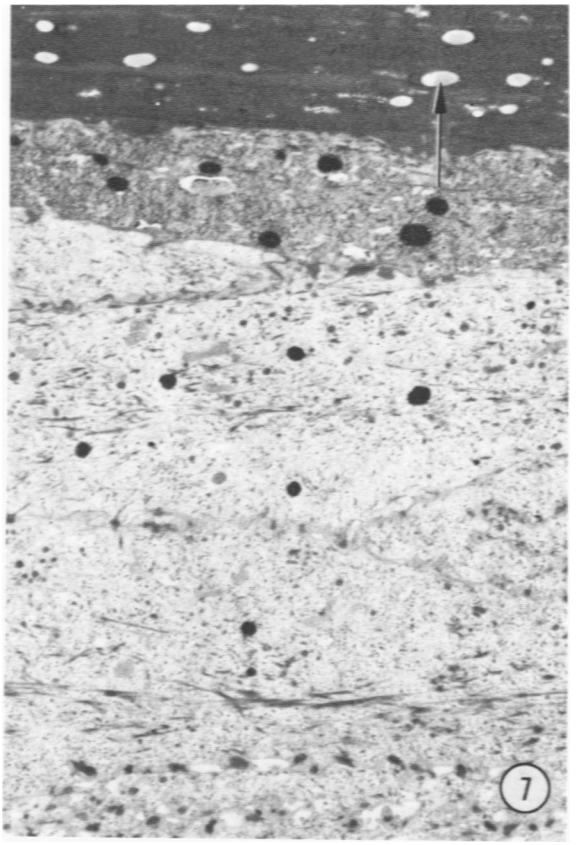
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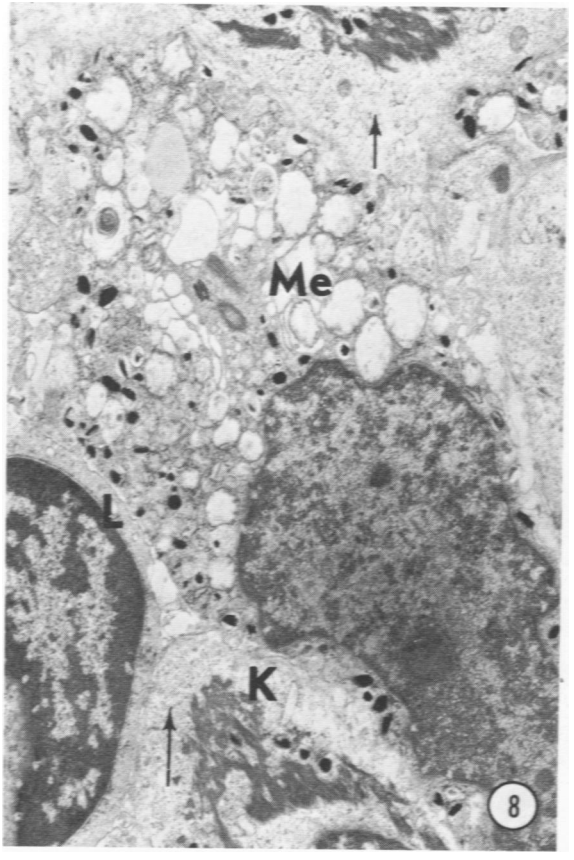
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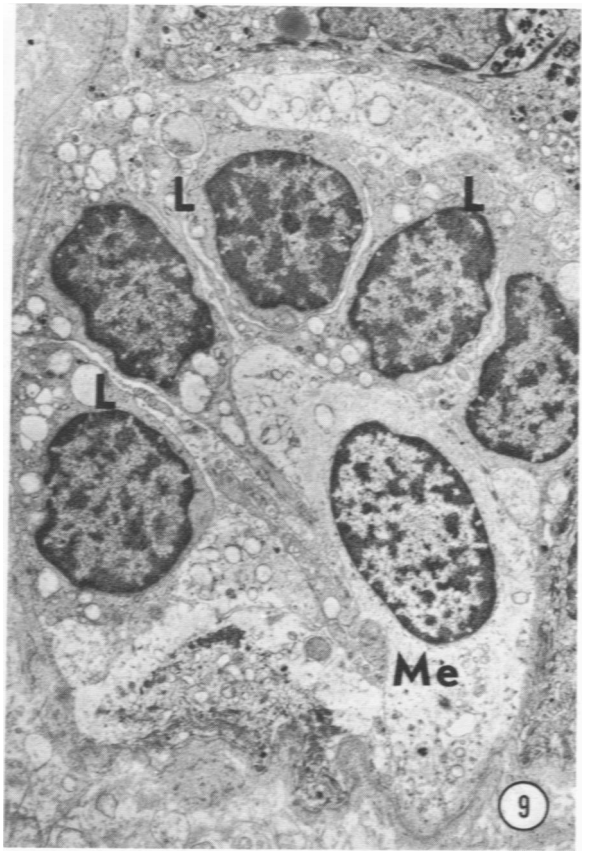
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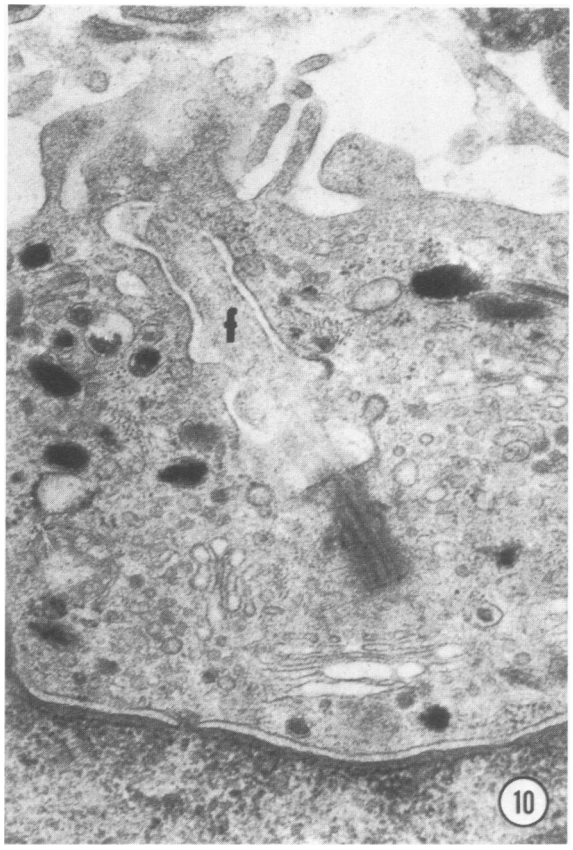
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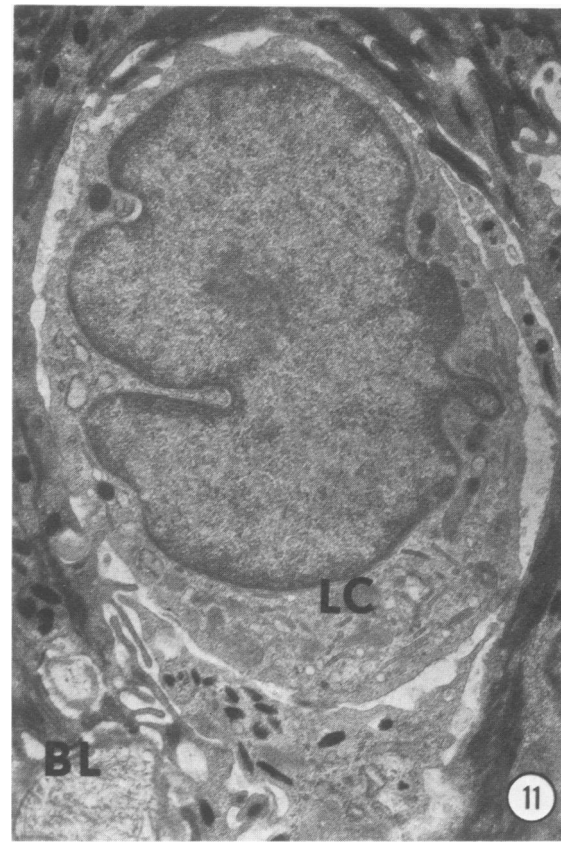
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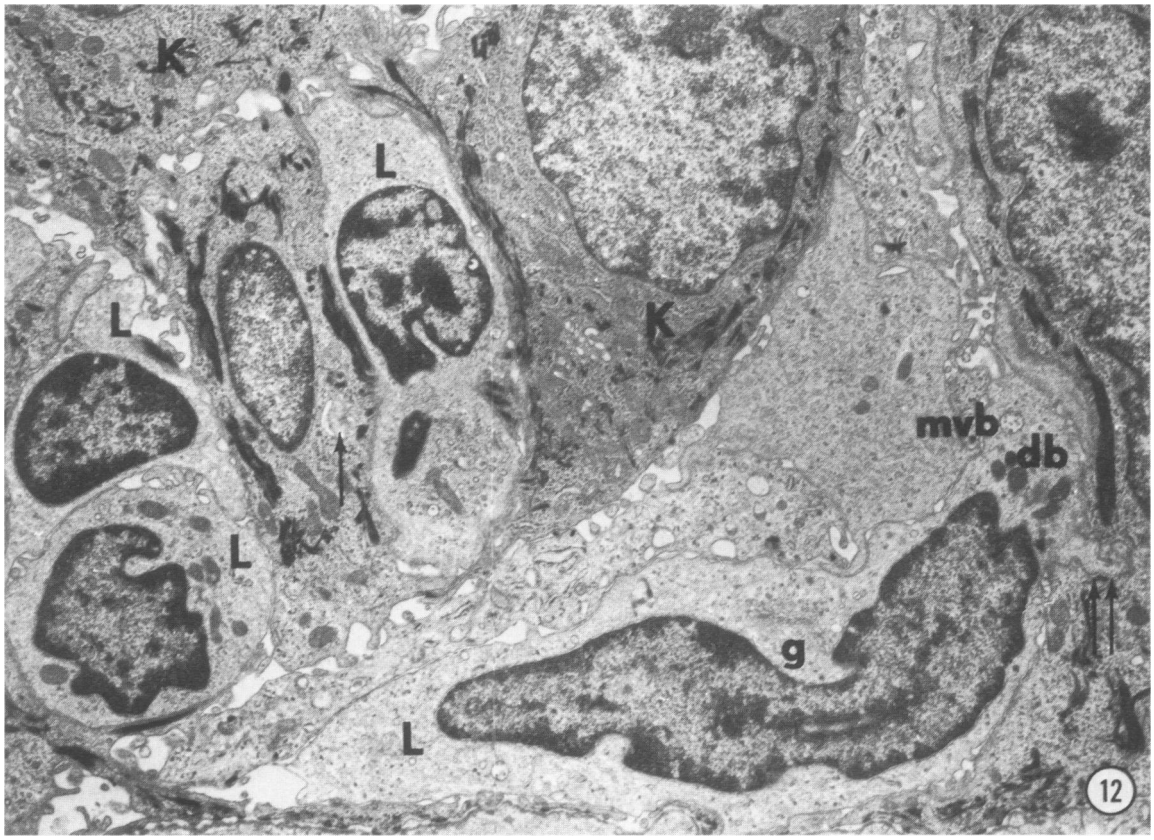
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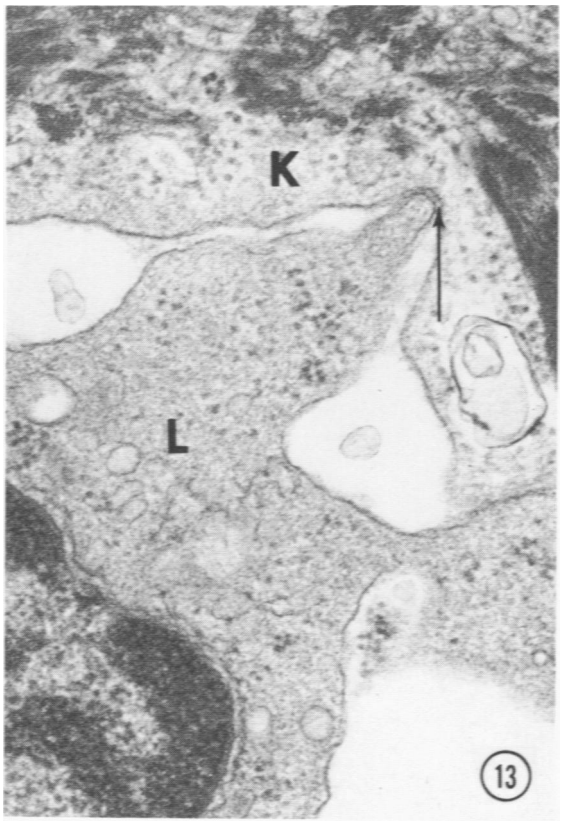
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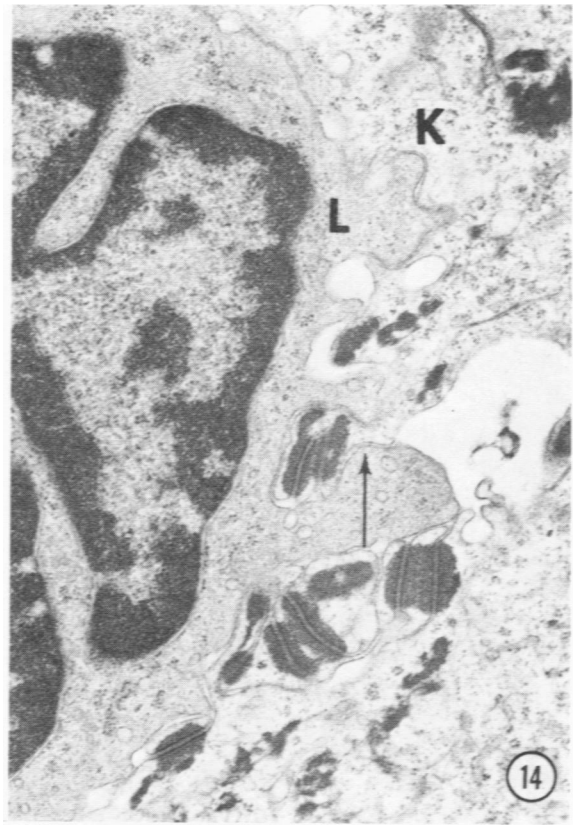
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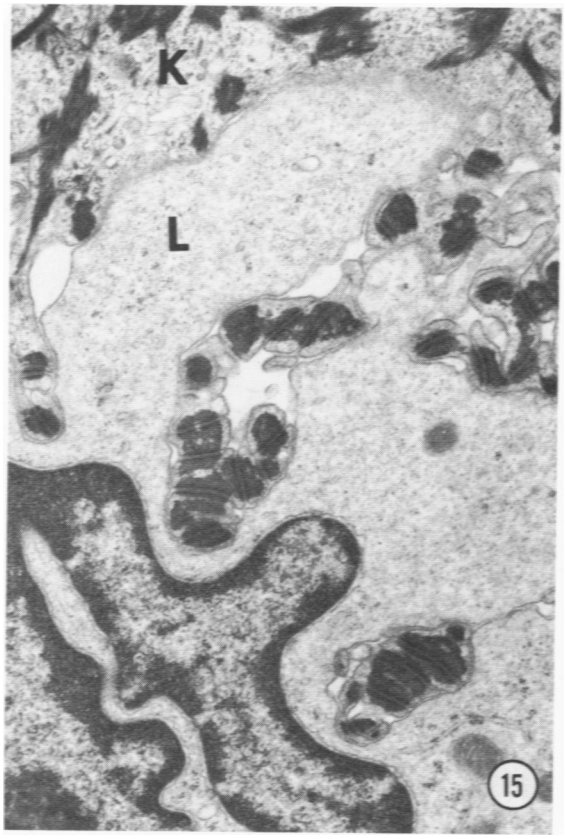
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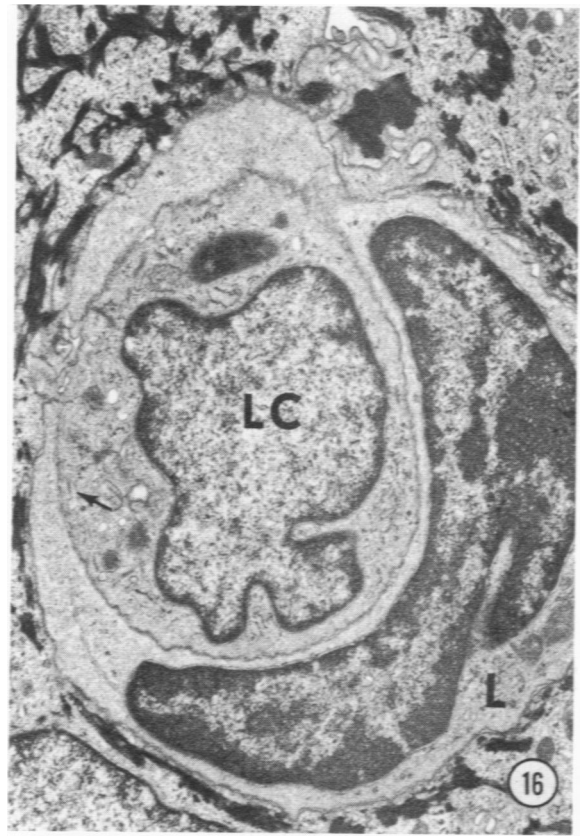
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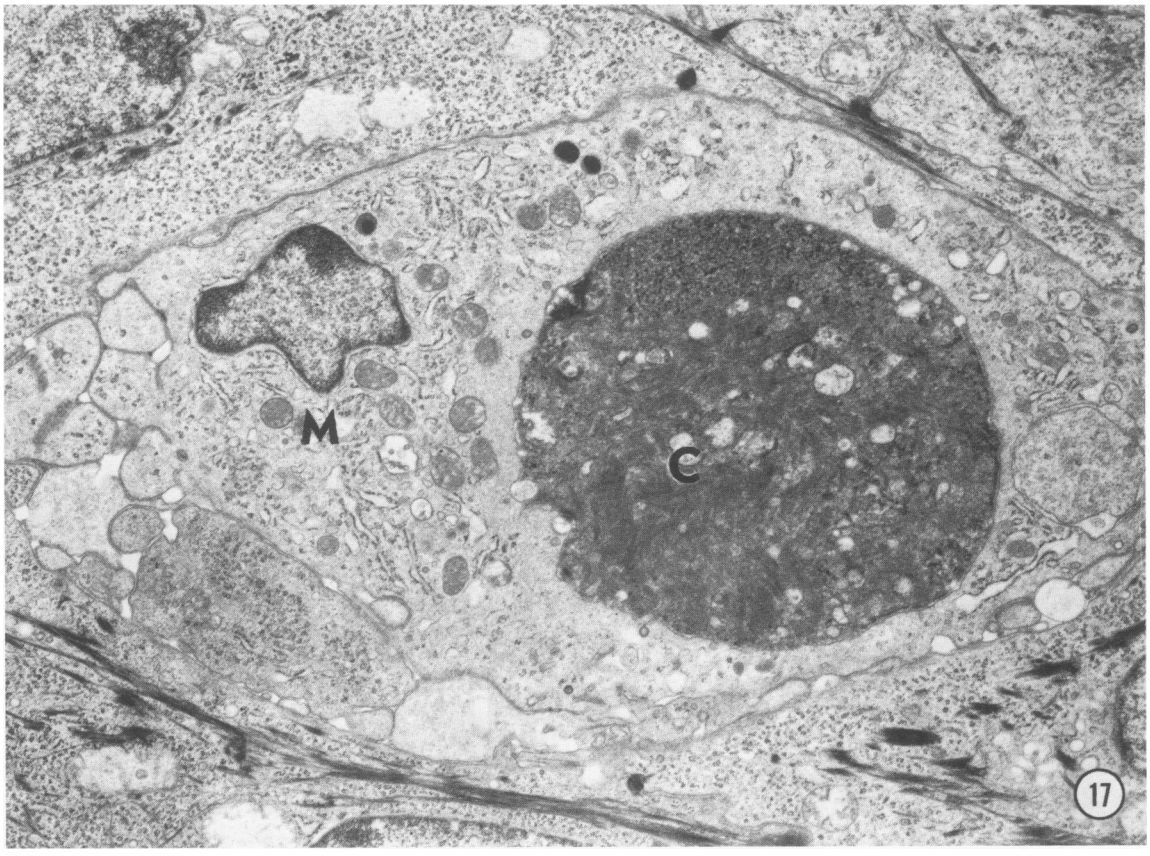
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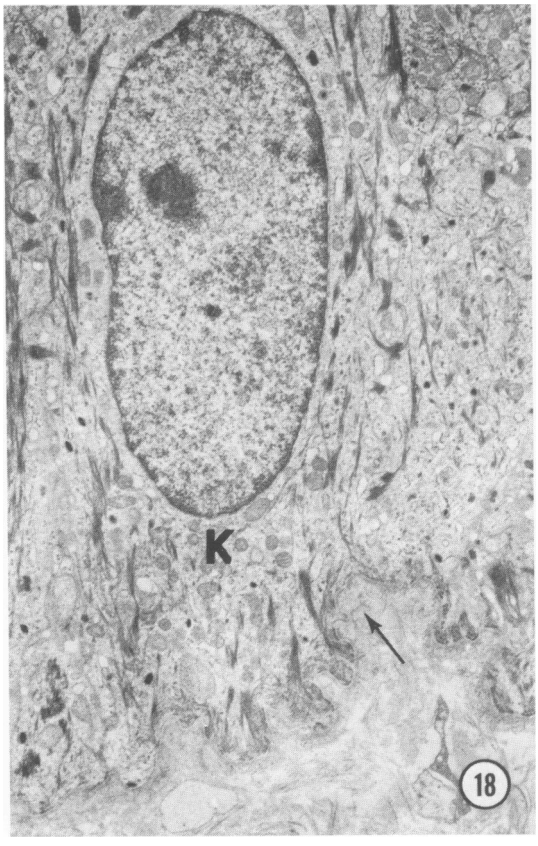
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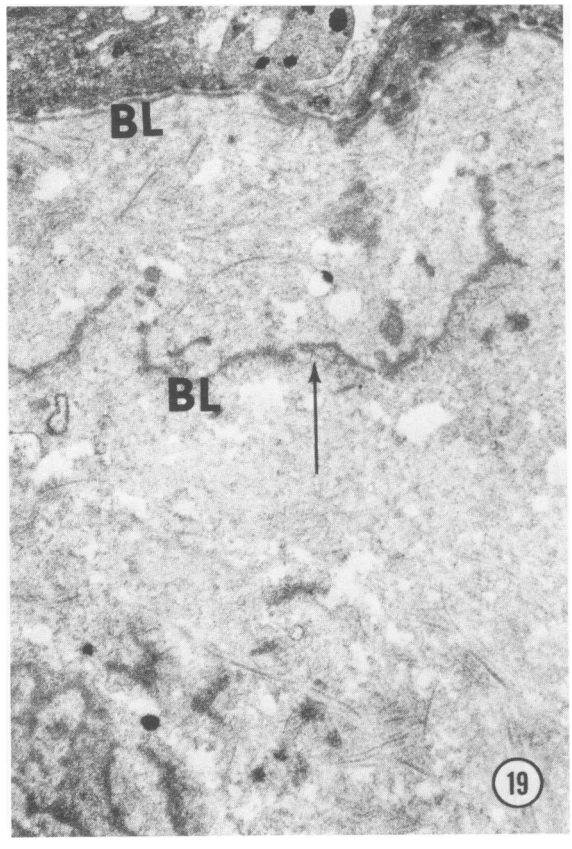
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