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Bullous Pemphigoid, an Ultrastructural Study of the Inflammatory Response: Eosinophil, Basophil and Mast Cell Granule Changes in Multiple Biopsies from One Patient

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We have studied by electron and light microscopy the inflammatory reaction in lesions at various stages of clinical development from a patient with bullous pemphigoid. The evolution of clinical lesions was associated with a sequence of histopathologic events which began with alterations of mast cells and proceeded to infiltration, first with lymphocytes and later with eosinophils and basophils. Mast cells in the papillary and reticular dermis demonstrated a unique, focal, irregular loss of granule contents. Intact eosinophils demonstrated intracytoplasmic losses of granule contents and karyorrhectic and karyolytic eosinophils had released membrane-bound granules. Partially and completely degranulated basophils were present within a fibrin gel which formed in the dermis. Thus, the sequence of histopathologic events in the pathogenesis of bullous pemphigoid includes mast cell granule alterations and release of granule contents from eosinophils which are undergoing nuclear and cytoplasmic damage.

Bullous pemphigoid is a chronic, frequently self-limited, blistering skin eruption which occurs in elderly people. Routine pathologic study has revealed dermal-epidermal junction separation and a mononuclear and eosinophilic cellular infiltrate [1]. Immunopathologic abnormalities include serum antibody directed against basement membrane antigen [2,3], basement membrane linear deposits composed of the third complement component (C3), immunoglobulins [4–7] and low bullous fluid complement levels [8]. Ultrastructural studies have shown basement membrane discontinuities in fully developed blisters [9].

Clinical cutaneous lesions are present in varying stages of development as erythematous macules, erythematous plaques, and blisters [10]. Light microscopic examination of 1 μm thick Epon embedded sections from lesions at each stage of clinical development has disclosed an orderly sequence of pathologic events that culminate in the development of bullae [10]. The pathologic process appears to begin with deposition of immunoreactants and subtle mast cell alterations. Lymphocytes are the initial infiltrating cell and are followed by an influx of eosinophils, basophils, and macrophages, with the eosinophil being the most prominent. That the release of mast cell contents may contribute to the eosinophil infiltration is suggested by the demonstration of acidic, low molecular weight eosinophil chemotactic activity in bullous fluids [10,11]. In the present study, which was undertaken to define the ultrastructural alterations characterizing the inflammatory reaction associated with the development of clinical lesions, a unique mast cell granule lesion, cytotoxic eosinophil degranulation, and piecemeal basophil degranulation were detected.

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MATERIALS AND METHODS

A patient with characteristic clinical lesions of bullous pemphigoid and linear deposits of C3 at the dermal-epidermal junction was hospitalized and treated only with saline compresses during a study period before initiation of systemic steroid therapy. After the periphery of each biopsy site had been injected with 2% lidocaine without epinephrine, a method we have frequently utilized without inducing alterations in the ultrastructure of mast cells [12–16], a 4-mm trephine skin biopsy specimen was obtained from clinically uninvolved skin 12 cm distant from lesions, clinically uninvolved skin adjacent to lesions, erythematous macules, erythematous plaques, and bullae less than 2 mm in diameter. Biopsy specimens were bisected and one-half of each was fixed by immersion in a mixture of 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.025% CaCl in 0.1 M sodium cacodylate buffer pH 7.4, at room temperature for 5 hr [17]. Tissue blocks were then washed overnight in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C, and postfixed for 2 hr in 1.5% collidine-buffered osmium tetroxide at 20°C. The specimens were washed 3 times in 0.05 M sodium maleate buffer, pH 5.2, stained *en bloc* for 2 hr with 2% uranyl acetate, washed 3 more times in the sodium maleate buffer, dehydrated in a graded series of alcohol [17] and embedded in a low viscosity medium, as described by Spurr [18]. One-μm, alkaline Giemsa stained, Spurr-embedded sections were examined by light microscopy [17]. Thin sections were cut with diamond knives and stained with lead citrate for ultrastructural analysis; sections from 20 blocks from a total of 5 biopsies from 5 areas of skin were examined in a Philips 301 electron microscope. The second half of each specimen was fixed in the same manner, embedded in Epon and stained with Giemsa's reagent, and examined by light microscopy as previously described [17]. Four sections of each specimen were examined, and the extent of inflammatory cell infiltration was determined by a quantitative method that described the absolute number of cell per linear millimeter and the relative frequency of cells per square millimeter in 1-μm thick tissue sections [17].

RESULTS

Single biopsy specimens obtained from bullous pemphigoid lesions at different stages of clinical development were evaluated by the 1-μ thick plastic section technique [17] in order to confirm and extend in this patient the sequential histopathologic events (Table) reported earlier for a series of 5 patients [10]. Distant clinically normal skin was histopathologically abnormal; it contained a sparse dermal perivenular infiltrate composed of lymphocytes, a finding which was absent from skin biopsies of normal skin in a number of previous studies which we have completed [12–16,19,20]. In normal skin adjacent to a clinical lesion a sparse perivenular dermal infiltrate was also observed and was composed of lymphocytes, monocyte-macrophages, basophils, neutrophils, and eosinophils. In the earliest detectable clinical lesions, the erythematous macule, the extent and composition of the infiltrate were unaltered. As the disease progressed to the erythematous plaque stage, the extent and composition of the infiltrate changed; the most prominent infiltrating cell was now the eosinophil. Basophils,

TABLE I. *Light microscopic histopathologic changes of clinical lesions at different stages of development*[a]

Dermis	Normal skin distant from lesion		Normal skin adjacent to lesion		Erythematous macule lesion		Erythematous plaque lesion		Bulla	
	P	R	P	R	P	R	P	R	P	R
Edema	–	–	±	–	1 – 2+	±	4+	3+	4+	1 – 2+
Compaction	–	–	–	–	1+ (focal)	–	–	–	3+	± – 1+
Red blood cell extravasation	–	–	±	–	±	–	–	–	±	–
Cellular infiltrate										
Extent	± (25)*	± (21)	± (30)	± (27)	± (24)	± (7)	1+ (88)	1+ (62)	2+ (168)	2+ (179)
Distribution										
Perivascular	±	±	±	1+	±	±	±	1+	1+	1 – 2+
Intervascular	–	±	±	±	±	±	± – 1+	±	1+	± – 1+
Composition										
Lymphocytes	± (14)	± (14)	± (7)	± (10)	± (9)	–	± (28)	± (15)	± (40)	± (97)
Activated lymphocytes	–	–	–	–	–	–	–	–	–	–
Monocyte-macrophages	–	± (7)	± (5)	± (11)	± (2)	± (1)	± (8)	–	± (2)	± (5)
Neutrophils	–	–	± (2)	–	± (1)	± (2)	± (3)	± (6)	± (7)	± (4)
Basophils	–	–	± (3)	–	± (1)	± (2)	± (4)	± (4)	± (4)	± (5)
Eosinophils	± (1)	–	1+ (13)	± (3)	1+ (12)	± (2)	1 – 2+ (55)	1 – 2+ (37)	3+ (115)	2+ (60)
Plasma cells	–	–	–	–	–	–	–	–	–	–
Activated histiocytes	± (11)	± (12)	± (24)	± (29)	± (11)	± (17)	± (30)	± (17)	± (10)	± (9)
Mast cell granule alterations	–	–	+	+	+	+	+	+	+	+
Fibrin										
Intravascular	–	–	–	–	–	–	–	1+	–	–
Perivascular	–	–	–	–	–	–	–	1+	–	–
Interstitial	–	–	–	–	±	2+ (Focal)	±	2 – 3+	3+	2+
Peri-elastica	–	–	–	–	–	–	±	2+	2+	2+
Vessel changes										
Venous	–	–	±	1+	3+	3 – 4+	3 – 4+	4+	2 – 3+	2 – 3+
Arterial	–	–	–	–	–	–	–	1 – 2+	–	–
Epidermis										
Epithelial changes										
Hyperkeratosis	–		–		–		–		–	
Parakeratosis	–		–		–		±		–	
Necrosis (extent)	–		–		±		1 – 2+		1+ (Focal)	
Edema										
Intercellular	± (3)		1+		2+		1 – 2+		2+ (Focal)	
Intracellular	–		–		–		–		–	
Vesicles	–		–		–		–		–	
Bullae	–		–		–		1+		4+	
Cellular infiltrate										
Extent	±		±		±		±		± – 1+	
Composition										
Lymphocytes	± (3)		± (7)		± (5)		± (2)		± (5)	
Monocyte-macrophages	–		–		–		–		–	
Neutrophils	–		–		–		–		1 – 2+ (5)	
Basophils	–		–		–		1+ (1)		1+ (1)	
Eosinophils	–		–		–		1+ (2)		3+ (7)	
Mast cells	–		–		–		1+ (1)		–	
Fibrin	–		–		–		–		–	

[a] The microscopic abnormalities were evaluated semiquantitatively for multiple variables with a score of 0 to 4+. For constituent structures of the skin a score of ± indicated trace pathology, 1+ or 2+ intermediate, and 3+ or 4+ extensive changes.

The extent of inflammatory cell infiltration was also evaluated semiquantitatively from 0 to 4+ in relation to the normal frequencies for the presence of each cell type expressed as cells per mm^2 in 1μm-thick sections. A score of ± represented < 100 mononuclear cells or < 10 granulocytes, 1+ to 2+ represented 100 to 400 mononuclear cells or 10 to 100 granulocytes, and 3+ to 4+ represented > 400 mononuclear cells or > 100 granulocytes.

The absolute number of infiltrating cells(*) was determined by counting each cell type per linear mm in four sections from each lesion and is expressed as the average number per linear mm per section.

Mast cell granule alterations were evaluated nonquantitatively; in the table + represents presence of alterations and – represents absence.

P = papillary dermis. R = reticular dermis.

neutrophils, and monocyte-macrophages did not increase in number as compared to earlier lesions. The formation of a blister was associated with even more marked lymphocyte and eosinophil infiltration, again without similar increases in the other infiltrating cell types. Mast cell alterations, fibrin deposition, and venular changes could also be correlated with different stages of lesion development (Table). Because light microscopic study of 1-μm thick sections from this patient revealed the identical sequence of histopathologic events previously reported in 5 patients [10], specimens from lesions at each stage of development were further evaluated by electron and light microscopy for detailed morphologic alterations of infiltrating cells, mast cells, fibrin and venules.

Infiltrating Cells

Eosinophils: Light microscopic examinations showed that eosinophils changed in number (Table) and appearance as lesions developed. When seen in adjacent normal skin and the erythematous macule, eosinophils appeared normal, although occasional free eosinophil granules were observed in the papillary dermis. At later stages, numerous free eosinophil granules were detected throughout the dermis of the erythematous plaque and bullous lesions.

Ultrastructural examination of the erythematous plaque lesion showed that the majority of eosinophils were intact and fully granulated (Fig 1–3), although some cells were undergoing

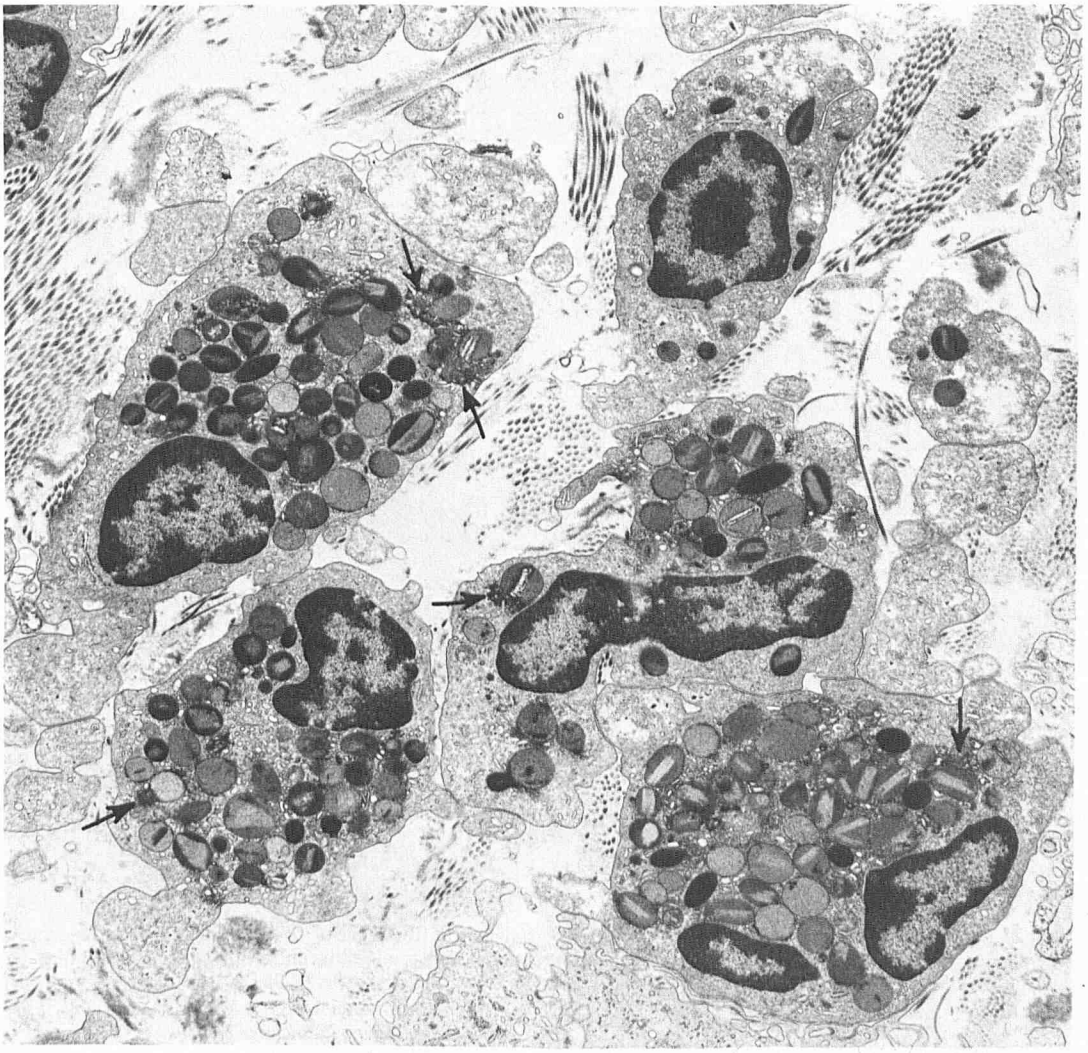

FIG 1. A nest of viable, mature eosinophils in the reticular dermis of an erythematous plaque lesion shows leakage of granule contents and loss of granule density which is particularly striking in the centrally located granule crystal area (*arrows*). No granule extrusion is present (× 7000).

karyorrhexis and karyolysis with subsequent cellular and nuclear breakup (Fig 4). Nuclear chromatin particles and recognizable membrane-bound eosinophil granules which displayed partial or complete lucent areas in the crystalline core were dispersed throughout the adjacent extracellular spaces (Fig 4,5). The free granules often had a layer of moderately dense particulate matter coating their surfaces (Fig 4,5). Viable eosinophils were never observed to extrude granules—a process requiring membrane fusion and relese of membrane-free granules. Individual eosinophils often contained 1 to 3 round membrane-free lipid structures, which were slightly larger than the granule size of eosinophils (Fig 3*A*). Although granule extrusion was never seen in eosinophils, granule changes were frequent (Fig 1–3), especially in eosinophils present in plaques and bullae. These ranged from loss of density of the normally dense central crystal to marked loss of density of the entire granule. These changes were associated with clouds of cytoplasmic dense granule material adjacent to such altered granules and sometimes in continuity with the plasma membrane (Figs. 1, 2, 3*B*). Some eosinophils were virtually devoid of granules.

Basophils: Basophils were detectable by light microscopy in trace quantities in adjacent normal skin and in clinically appreciable lesions and did not increase in number as the lesions developed (Table). The cytoplasm of basophils contained discrete lucent areas. Ultrastructural study of an erythematous plaque lesion showed that the basophils were primarily within a fibrin gel [21,22] which had formed in the papillary dermis (Fig 6) and that they exhibited a loss of granule dense particles and contained numerous cytoplasmic vesicles (Fig 7). Neither karyorrhexis of basophils nor extrusion of individual basophil granules was seen; further, the active cell surface changes known to accompany antigen-induced exocytosis of basophil granules in the human were absent [23]. Rather, basophils were fully granulated, partially granulated (Fig 7), or filled entirely with membrane-bound empty granules [12,13,23]. Eosinophils were sometimes found closely associated with these degranulated basophils.

Macrophages: Macrophages were detected by light microscopy in all lesions and did not increase in number as lesions developed (Table). In erythematous plaque lesions, activated macrophages were large cells with active surface folds, a large lobular nucleus with dispersed chromatin, numerous cytoplasmic vesicles and vacuoles, and large numbers of lysosomes (Fig 8). These cells contained a mixture of large, moderately dense lysosomes and slightly smaller, intact, phagocytized eosinophil granules.

Mast Cells

Light microscopic study of sections from each stage of lesion development disclosed mast cell alterations which included changes in distribution, the appearance of mast cell motile forms, and the presence of granule abnormalities. In distant and adjacent clinically normal skin, mast cells were observed high in the papillary dermis and they were most frequently

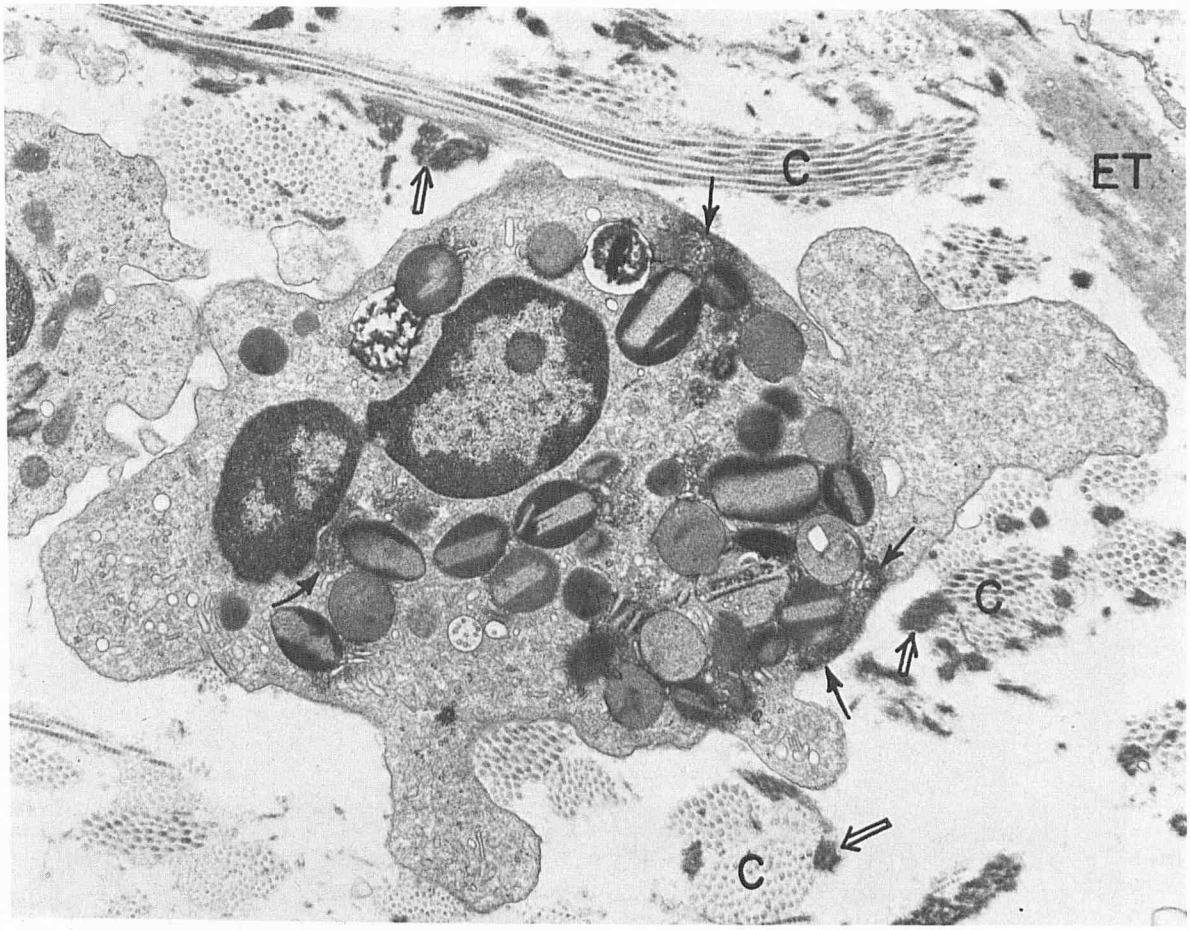

FIG 2. Higher magnification micrograph of a viable eosinophil located in the fibrin-gel of the papillary dermis of an erythematous plaque lesion. Numerous granules show granule-content losses with clouds of dense material filling adjacent cytoplasmic areas (*arrows*). Increased vesicles are present at one cytoplasmic pole. *C*, collagen, *ET*, elastic tissue, *open arrow*, fibrin (× 12,000).

observed at this location in the erythematous macule. In the upper reticular dermis of the erythematous plaque lesion many mast cells exhibited eccentric nuclei and tail-like cytoplasmic projections (motile forms [24–26]). Young mast cells were seen in the dermis of the plaque and bullous lesions. Hypogranulation of young mast cells and variably metachromatic mature granules occurred in dermal mast cells of adjacent normal skin, erythematous macules, plaques, and bullous lesions.

Ultrastructural study of sections from the erythematous plaque lesion showed that mast cells were a mixture of mature and immature cells as judged by their nuclei and cytoplasmic contents. Chromatin condensation was dispersed and the nucleus was larger in immature cells; these cells were also hypogranulated, mature granules being present only in the peripheral cytoplasm. Mature cells were a mixture of cells with a nearly complete complement of typical dense granules (Fig 10 and 12*A*), cells with portions of many of their granules in an altered state (Fig 11 and 12*B*), and a few with nearly all granules showing density changes. Granule extrusion was not present in this case [10]. Nor did we find focal, extensive cytoplasmic, membrane-bound, granule-size lacunae resulting from loss of granules such as we have seen in human mast cells in Crohn's disease [27]. Rather, mast cells showed a moth-eaten appearance of individual granules (Fig 11, 12*B* and *C*). Such altered granules were slightly larger than their more dense, unaltered counterparts. The focal irregular losses in granule density were interrupted by the preservation of small granule areas displaying the usual density and crystalline array of normal granules. Mast cell surfaces showed numerous short villous processes which sometimes were aggregated into irregular areas of increased numbers of such structures (Fig 12*C*) as well as elongated tail-like structures (Fig 10) [24,25]. Numerous close contacts of mast cells with elastic fibers were noted (Fig 12*C*).

Fibrin Deposition

Light microscopic evidence of interstitial fibrin deposition was first noted in the dermis of the erythematous macule but increased in amount to 3+ quantities in the reticular dermis beneath the erythematous plaque and the bulla. Beneath bullous lesions 1+ perivenular fibrin was noted (Table). Ultrastructural study of an erythematous plaque lesion showed that edema of the papillary dermis was characterized by the presence of a fibrin gel [21,22]. This consisted of a loosely woven meshwork of solid fibrin strands oriented in multiple directions with intervening collections of protein-rich, moderately dense edema fluid (Fig 13). Irregular extra fibers of elastic tissue were also prominent in this meshwork and activated macrophages often were closely adherent to irregular portions of elastic fibers (Fig 8 and 9). Thicker masses of more densely aggregated fibrin with very little intervening collections of edema fluid were closely interwoven between dermal collagen fibers in the reticular dermis. Although a number of other inflammatory cells could be found in the edematous dermis, basophils with fibrin strands opposed to their surfaces were most often found in the midst of the fibrin mesh (Fig 6).

Vascular Alterations

Light microscopic evidence of mild vascular changes were present in normal adjacent but not normal distant skin; these changes progressed to 3+ to 4+ alterations beneath the plaque and persisted beneath the bulla. The vascular alterations in-

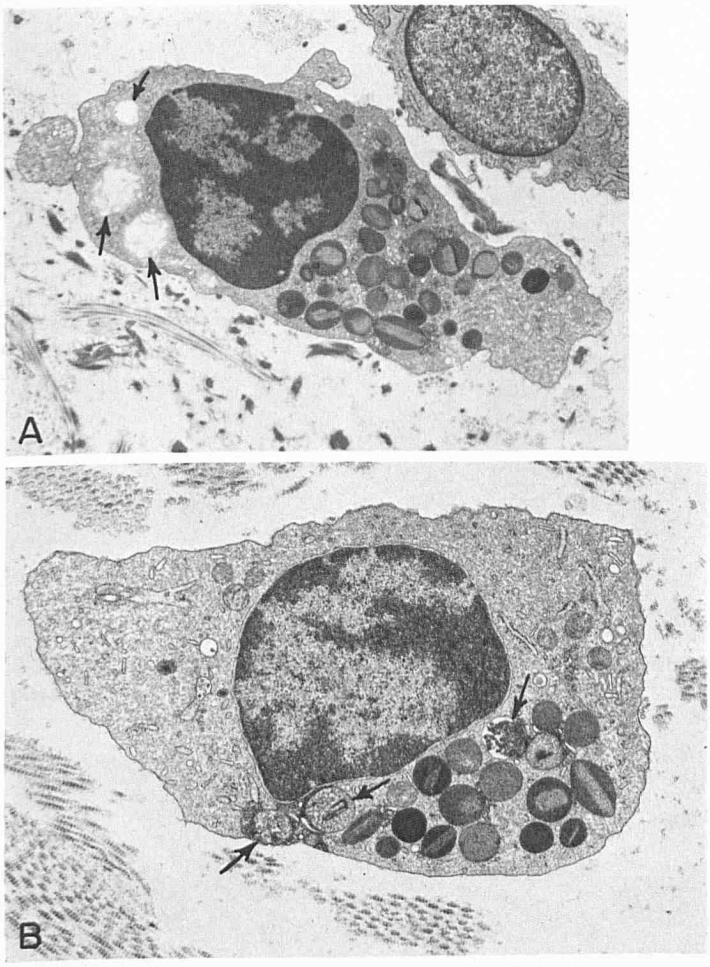

FIG 3. In *A*, an eosinophil within the fibrin-gel of an erythematous plaque lesion shows granule changes and contains 3 large membrane-bound lipid cytoplasmic inclusions (*arrows*). The eosinophil in *B* with prominent granule changes (*arrows*) also contains several strands of cytoplasmic rough endoplasmic reticulum (reduced from × 11,500).

cluded endothelial cell hypertrophy with focal luminal obliteration in adjacent normal skin and in the erythematous macule. More marked endothelial cell hypertrophy with evidence of focal endothelial cell death, appreciated as vacuolization of cytoplasm, pyknosis of nuclei and apparent sloughing of endothelial cells, was noted in maximally involved skin (Table). Progressive basement membrane thickening and edema were most noticeable in the erythematous plaque and beneath the bulla. Ultrastructural examination of a bullous lesion confirmed that the vessel changes consisted of focal endothelial cell necrosis, individual endothelial cell hypertrophy, and basal lamina reduplication (Fig 14) [14]. Platelets were present in vascular lumens, were unaltered and were not attached to damaged endothelium. Intravascular thrombi were not present. Perivascular cuffs of small lymphocytes were present (Fig 14).

DISCUSSION

The development of bullous pemphigoid lesions was studied in 5 patients [10]. Characteristically, an orderly sequence of immunopathologic events began with deposition of C3 at the epidermal basement membrane, mast cell hypogranulation and maldistribution and venular alterations of endothelial cell hypertrophy. Lymphocytic infiltration followed by an infiltration of many eosinophils and fewer basophils was also noted. Activated macrophages appeared as the lesions developed further. Dermal venular alterations increased in severity with progression of the lesions until endothelial cell necrosis occurred. Because the sequence of histopathologic events observed in a 6th patient (Table) was typical of the initial group [10], sections from lesions at each stage of development from this patient were examined with the electron microscope to determine the nature of the cellular abnormalities occurring in specific cell types.

In agreement with others [9,28], eosinophils, the predominant

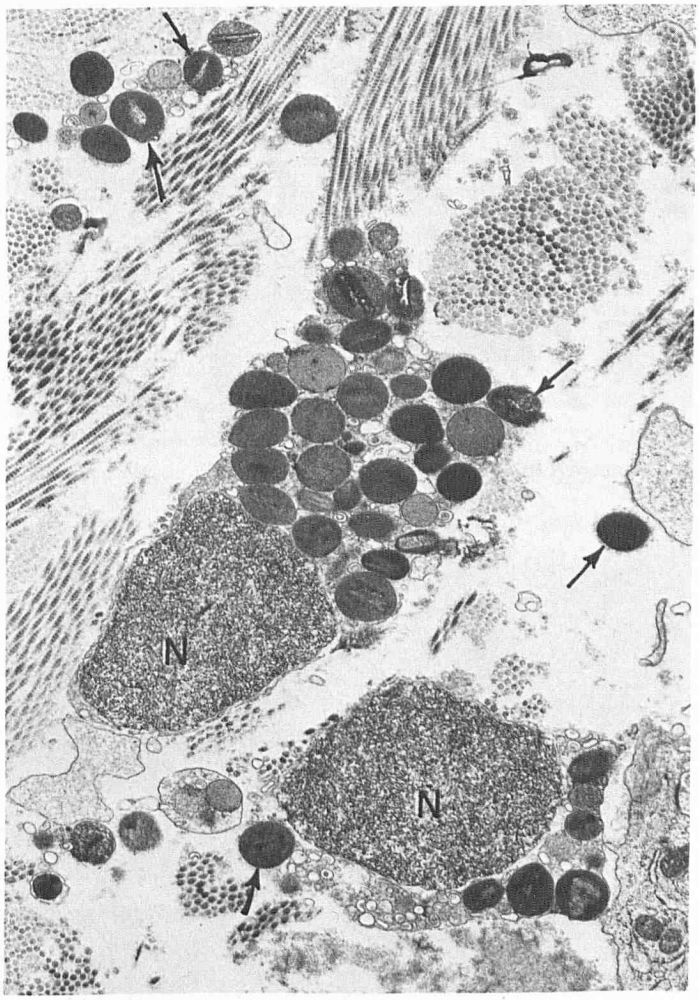

FIG 4. Karyorrhexis of 2 eosinophils in the reticular dermis of an erythematous plaque lesion. Dense pyknotic nuclei (*N*), ruptured plasma membranes, and extracellular membrane-bound eosinophil granules (*arrows*) characterize this process (reduced from × 11,000).

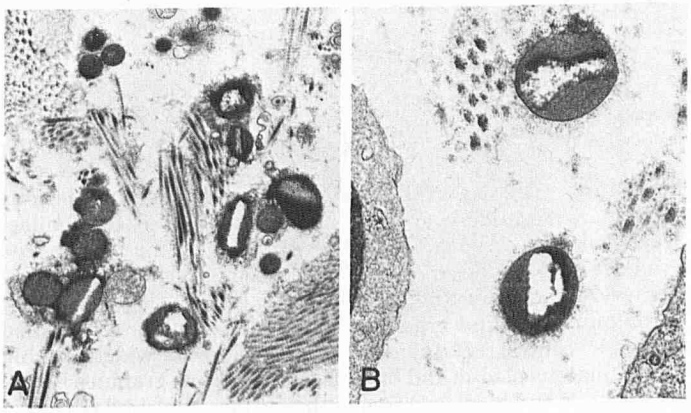

FIG 5. Higher magnification electron micrographs showing extracellular membrane-bound eosinophil granules which result from eosinophil karyorrhexis and not from degranulation of viable eosinophils. Note losses of density of central cores (*A*, reduced from × 10,500; *B*, reduced from × 23,000).

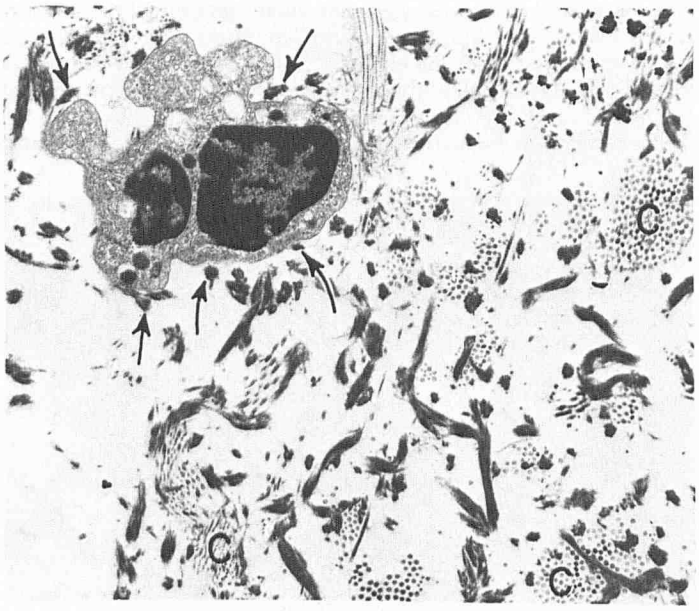

FIG 6. A partially degranulated basophil within the fibrin-gel of the papillary dermis of an erythematous plaque lesion. Note close association of fragments of fibrin with basophil cell surface (*arrows*) and with collagen (*C*) (reduced from × 8,500).

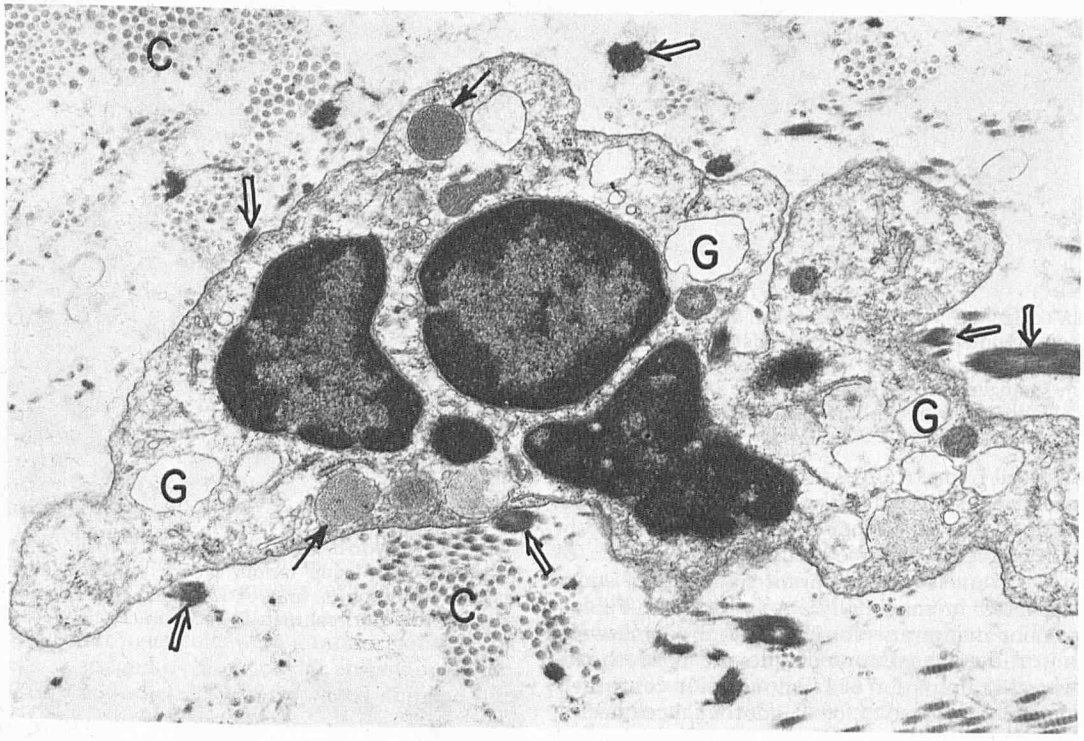

FIG 7. This partially degranulated basophil present in the papillary dermis of an erythematous plaque lesion has a polylobed nucleus with dense aggregates of chromatin, normal granules with particulate content (*arrows*), and membrane-bound empty granule spaces (*G*). Note closely associated fibrin fragments and plasma membrane (*open arrows*). Cytoplasmic small vesicles are lucent. *C*, collagen. (× 14,000).

infiltrating cell in erythematous plaque and bullous lesions (Table), were widespread in the dermis and within bullae. Degranulation of eosinophils by extrusion of membrane-free granules was not observed. Instead, eosinophils in large numbers were undergoing karyorrhexis and karyolysis with release of membrane-bound granules. Eosinophil granules, identified by their central crystal core, were scattered widely within erythematous plaque and bullous lesions. Some granules within eosinophils and scattered free in the dermis displayed partial or complete electron-lucent areas in the crystalline core. Such granule changes may represent release of some granule materials. Similar eosinophil granule changes have recently been described in Crohn's disease [27,29].

Basophils were identified primarily in the fibrin-gel which formed in the papillary dermis and within epidermal bullae. Fragments of fibrin were often attached to the surfaces of basophils and many basophils displayed degranulation changes of the type described as piecemeal degranulation of human basophils in contact allergy [12,13] and skin graft rejection [15,16]. This type of release of granule materials from basophils may be mediated by vesicular transport [30–37], occurs slowly, and results in the progressive increase in membrane-bound intracytoplasmic empty granules [12,13]. Extrusion of membrane-free granules from viable cells, such as is seen in antigen-induced, IgE-mediated [23] in C5a-induced [38] or in mannitol-induced [39] degranulation of human basophils was not seen in bullous pemphigoid.

As lesions progressed, light microscopy revealed mast cells to

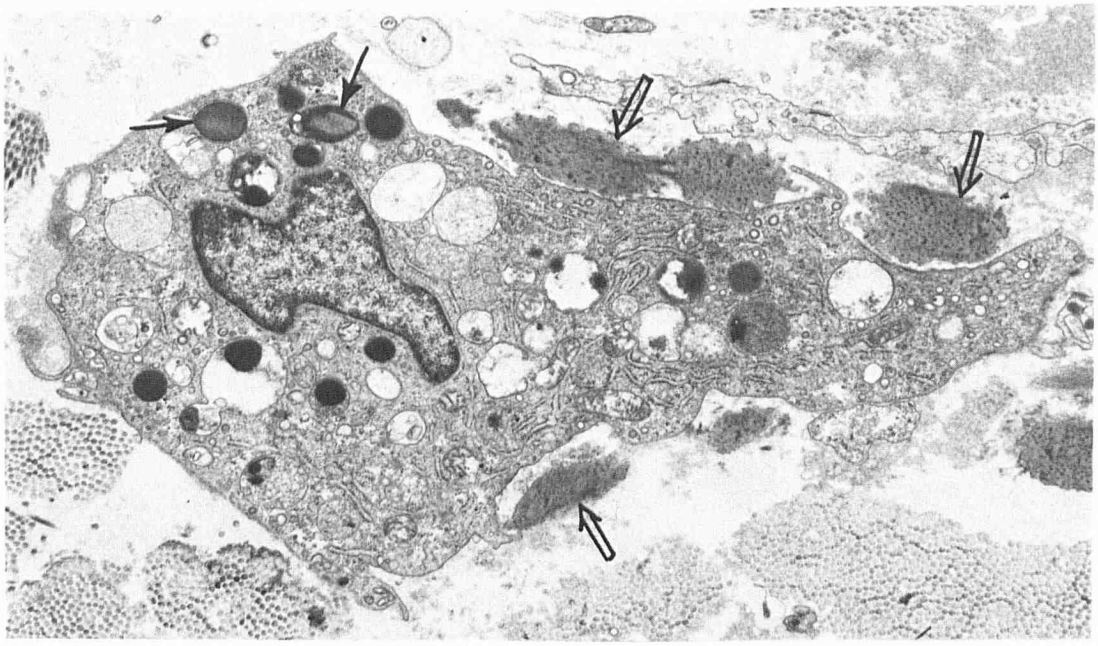

FIG 8. This activated macrophage in the reticular dermis with closely attached elastic fibers (*open arrows*) is filled with lysosomes and phagocytized eosinophil granules (*arrows*) (× 7,500).

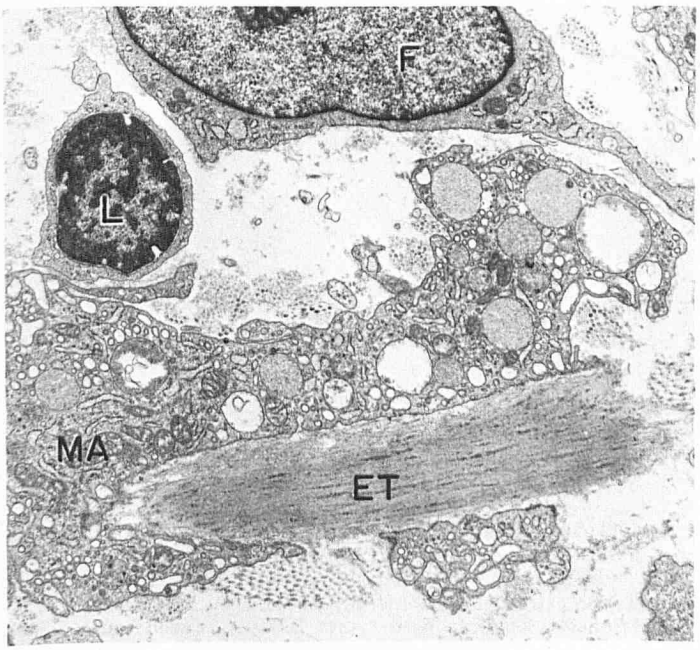

FIG 9. An activated macrophage (*MA*) with numerous lysosomes encircles a large mass of elastica (*ET*) in the reticular dermis. A small lymphocyte (*L*) and fibroblast (*F*) are also shown (reduced from × 8,500).

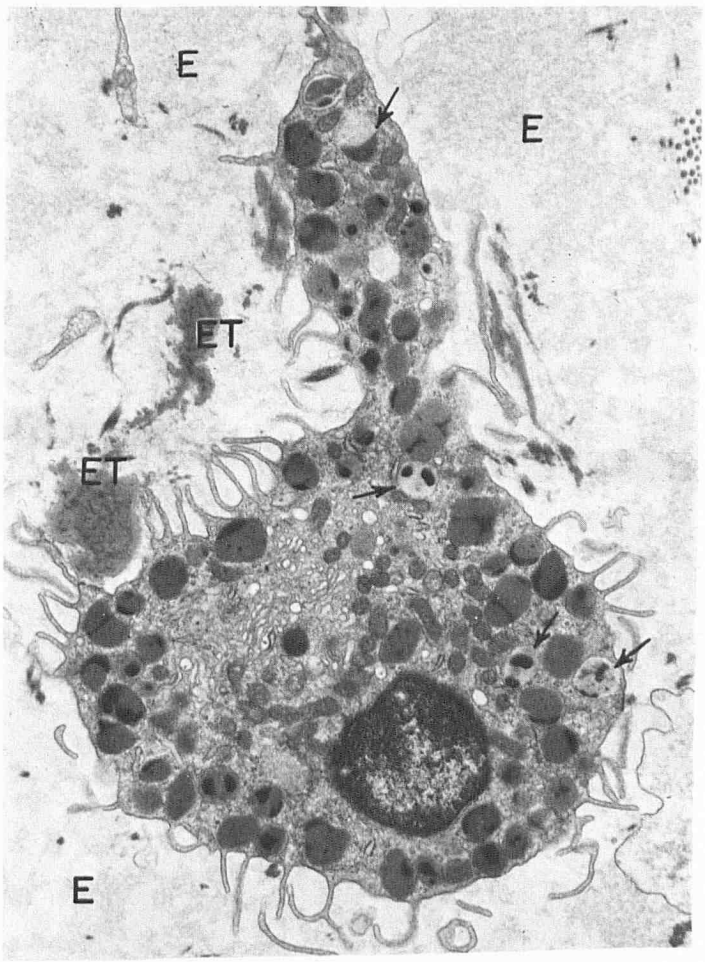

FIG 10. This mast cell within the edema fluid (*E*) of the papillary dermis of an erythematous plaque lesion shows a motile configuration. This granule-filled tail has formed at one pole and the remainder of the surface displays numerous villi, some of which are in contact with fragments of elastica (*ET*). Some granules show focal losses of granule density (*arrows*). No granule extrusion is seen (reduced from × 12,500).

be present high in the papillary dermis adjacent to the dermal-epidermal junction. Mast cells displaying granules with variable metachromasia were present in this location, even in adjacent normal skin and in all lesions. That mast cell migration may be occurring is suggested by the detection of mast cells in the epidermis, in the bullous lesion and the identification of motile forms in the reticular dermis of the erythematous plaque lesion [24–26]. Changes in dermal mast cells were characterized by focal, piece-meal, vesicle-sized, ragged, and irregular losses of density and the underlying crystalline structure of individual granules. Vesicular transport of granule materials [13,30–37] could not be identified and extrusion of membrane-free granules

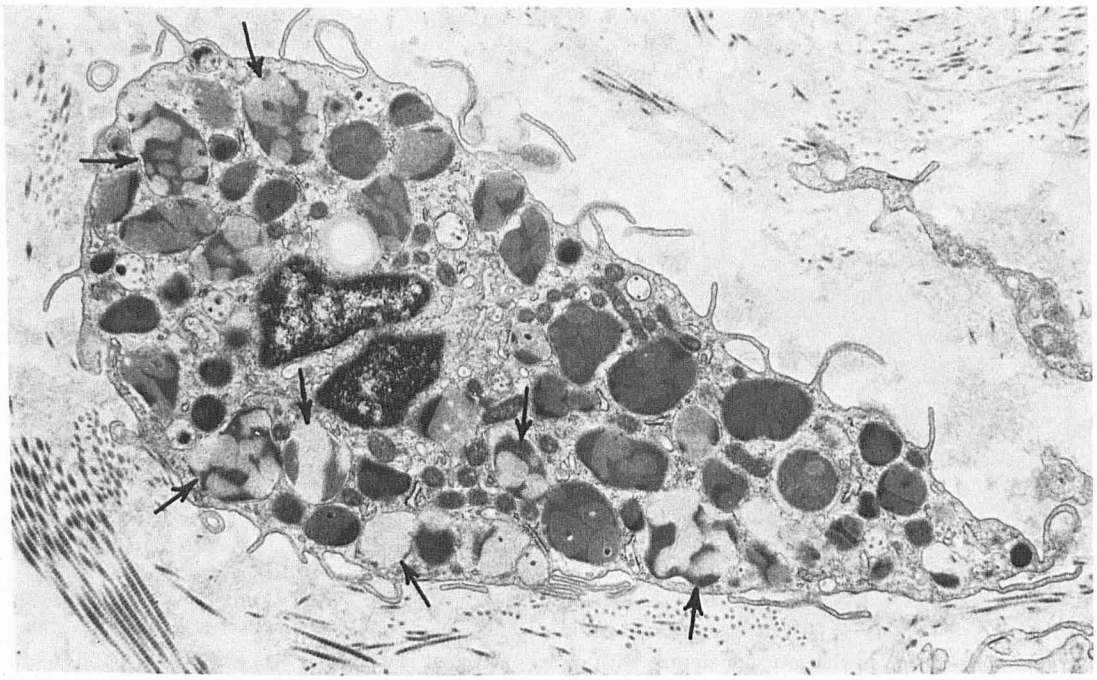

FIG 11. Mast cell from the reticular dermis of an erythematous plaque area showing the focal and extensive irregular losses of granule density (*arrows*). Granule extrusion is not present (× 10,000).

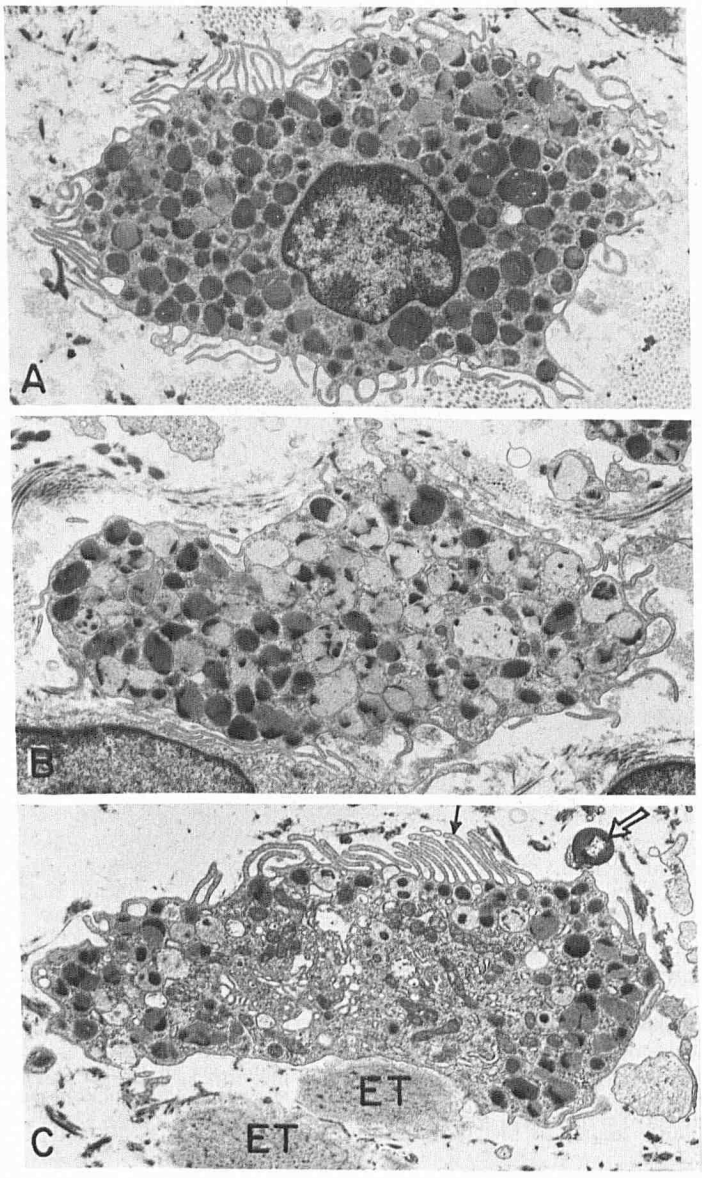

FIG 12. Three mast cells from erythematus plaque lesions illustrating varying degrees of granule changes (*A–C*). Closely associated elastic (*ET*) and focal elongated villi (*arrow*) are shown in *C*. The *open arrow* indicates an extracellular membrane-bound eosinophil granule (*A*, reduced from × 6,500; *B*, reduced from × 7,500; *C*, reduced from × 7,500).

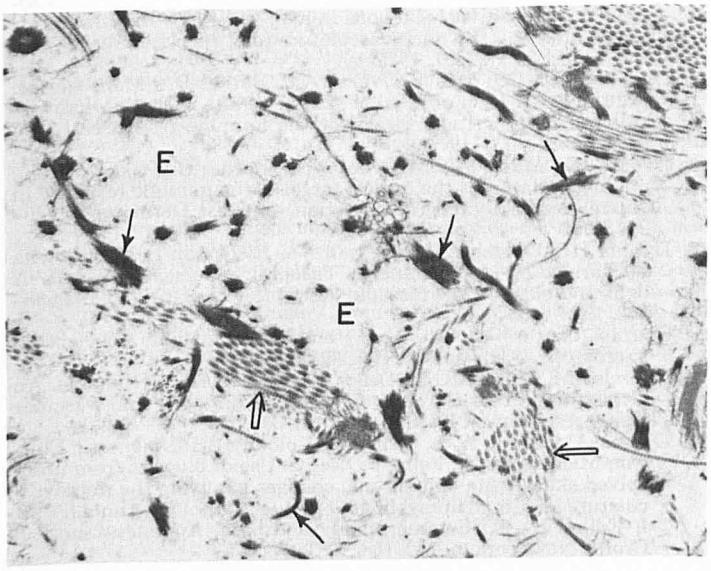

FIG 13. Fibrin-gel, a prominent feature of the papillary dermis in an erythematous plaque lesion, is composed of a loose interlacing mesh of fibrin (*arrows*) and entrapped edema fluid (*E*). *Open arrows* indicate collagen (reduced from, × 12,000).

was not present. Moreover, free membrane-bound mast cell granules from dead or dying mast cells were not seen. Mast cells, in bullous pemphigoid, also did not show intracytoplasmic vacuoles typical of the *in situ* loss of granules similar to those in mast cells of Crohn's disease [27], in contact allergy [14], graft rejection [15,16], and when human C3a was injected intradermally in humans [40]. Exocytosis of membrane-free granules from mast cells was not present in this case. Two previous studies have mentioned the presence of free granule-like structures which may represent abnormally fragmented collagen [10,27]. Mast cells, rather than undergoing changes of injury and death, usually displayed the nuclear and cytoplasmic changes of replicating young cells. Such immature mast cells were invariably hypogranulated.

The papillary and reticular dermis of erythematous plaque and bullous lesions were characterized by the presence of irregular, disorganized collagen in increased amounts and by associated and similarly disorganized elastic fibers. Activated macrophages and hypogranulated mast cells were found in close contact with these elastic fibers. Closely packed fibrin strands were often interwoven within the abnormal collagen and elastica. Fibrin was conspicuously absent within vessels and in perivascular spaces. At other points the fibrin strands formed a loosely knit meshwork with pockets of edema fluid, characteristic of a gel as recently described in relationship to the host response in syngeneic tumor rejection [21,22]. The fibrin-water

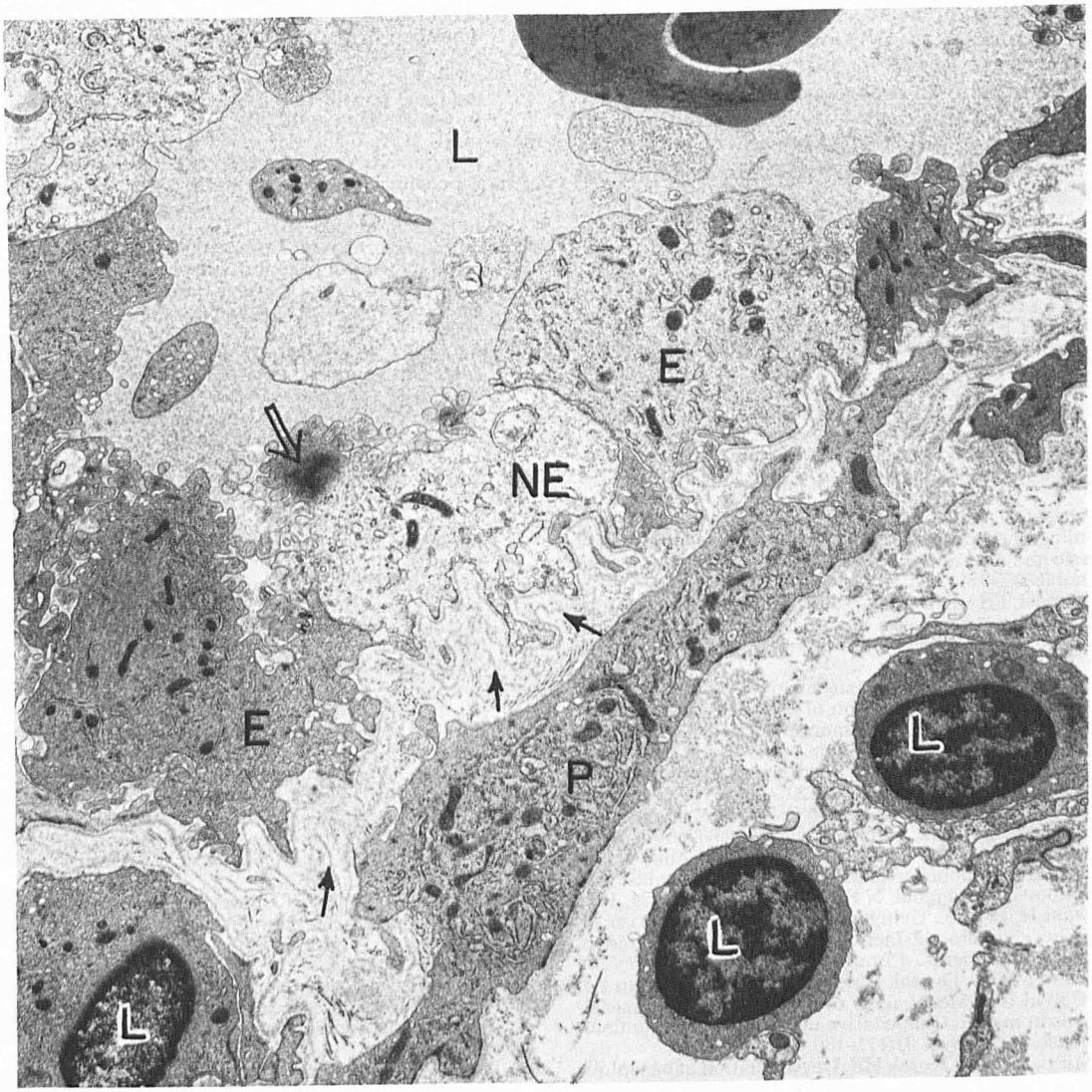

FIG 14. A venule in the reticular dermis underlying a bullous lesion shows focal endothelial necrosis (*NE*) with a dense cytoplasmic filament tangle (*open arrow*) and a reduplicated basal lamina (*arrows*). A perivascular cuff of small lymphocytes is present (*L*). *P*, pericyte; *E*, endothelium, *L*, lumen (× 7,000).

mesh (gel) in the dermis is probably responsible for the clinically apparent induration since in studies of contact allergy the induration was correlated with the presence of dermal fibrin and not with the number of inflammatory cells [41].

Vessels changes were confined to venules and included endothelial cell hypertrophy and necrosis and basal lamina reduplication with entrapped cellular debris. Similar changes have been described in contact allergies [14], during skin graft rejection in humans [15,16] and in association with skin melanomas [19] and they reflect repeated episodes of endothelial cell injury and repair [42,43]. Intravascular fibrin thrombi were not seen. Individual endothelial cells were often packed with Weibel-Palade bodies, membrane-bound granules whose function is as yet unknown.

An attractive but unproven pathobiologic sequence can be offered which would encompass the pathologic alterations and the immunopathologic abnormalities described in bullous pemphigoid. Because of the presence of an auto-antibody or undefined biochemical alterations of the basement membrane zone, an enzyme capable of cleaving C3 may present at the cutaneous dermal-epidermal junction. The major cleavage product, C3b, is deposited at the site of activation and the minor fragment, C3a, may activate dermal mast cells, causing loss of granule contents and release of chemotactic factors for eosinophils [10,11]. Complement cleavage products could also account for the early influx of lymphocytes [44]. The subsequent release of lymphocyte [45] or even basophil [46] derived eosinophil chemotactic activities might contribute further to the influx of eosinophils. Eosinophil karyorrhexis and karyolysis and release of free eosinophil granules with electron-lucent central cores could provide cytotoxic eosinophil major basic protein (MBP) [47] which might contribute to progression of the lesion to a bullous form. In support of this theory, we have recently found markedly elevated levels of MBP of eosinophil granule core origin in the bullous fluid in patients with bullous pemphigoid [48].

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Correction: The list of Reviewers that appeared in the December, 1981 journal (78: 489, 1981) should have been Reviewers for 1980 not 1981 as printed.