STUDIES ON THE PATHOGENESIS OF EPIDERMOLYSIS BULLOSA*

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Clinical classification of the diseases in the epidermolysis bullosa group has led to little progress toward elucidation of the mechanisms of blister formation operative in these conditions. Histologic studies, although of considerable interest, have been the source of controversy, particularly as regards the role of the elastic tissue in these diseases. Two factors seem to be responsible for most of the variance in results between different workers. The first of these is use of unsuitable specimens. In most studies, naturally occurring blisters of uncertain age, or post mortem skin was examined. Secondly, there was the limitation in resolution imposed by use of the light microscope.

In the present study attempts were made to reduce these difficulties by the exclusive use of specimens obtained from non-blistered or experimentally blistered areas, and by supplementing light microscope examinations by electron microscope observations. This approach has given data which tends to localize to some extent the site of the defects responsible for blister formation. Some cautious speculations concerning the nature of these defects can also be made.

In other reports (1, 2) epidermolysis bullosa simplex blisters were shown to have developed as a result of disintegration of basal and suprabasal cells. For convenient reference an electron micrograph of a fully formed blister of epidermolysis bullosa simplex is included at the end of the *Results* section of this paper (Fig. 25), but primary concern is given to epidermolysis bullosa hereditaria letalis, epidermolysis bullosa dystrophica (recessive type), and porphyria cutanea tarda.

A working classification of the epidermolysis bullosa group of diseases is given in Table I.

Materials & Methods

Skin specimens were obtained by punch biopsy method from areas anesthetized by injection of a ring of procaine hydrochloride around the area. The method of blister production, where applicable, is noted in the *Results* Section. The specimens were divided with a razor blade into one mm^3 pieces, fixed for 1–2 hours at 4°C. in buffered osmium tetroxide, and embedded in epon (3) or Vestopal W. Thin sections were cut with glass or diamond knives on an LKB ultrotome, stained with 2–3% uranyl acetate or saturated lead hydroxide, and viewed in an RCA EMU 3-D electron microscope.

Formalin-fixed specimens were processed by routine methods.

TABLE I

Classification of The Epidermolysis Bullosa Group of Diseases

	Disease	Transmission
Non-scarring	Epidermolysis Bullosa Simplex	Dominant
	Recurrent Bullous Eruption of the Hands and Feet	Dominant
	Epidermolysis Bullosa Hereditaria Letalis	Recessive
	Atypical Cases	Variable
Scarring	Epidermolysis Bullosa Dystrophica	Recessive
	Epidermolysis Bullosa Dystrophica	Dominant
	"Acquired" Epidermolysis Bullosa Dystrophica	Uncertain
	Cutaneous Porphyria	Variable

Results

Normal Skin

Two electron micrographs of the general region of the dermal-epidermal junction (Figs. 1 & 2), where the blisters under consideration develop, are presented for orientation.

In this report the junction itself is defined as the three component structure consisting of the portion of the basal cell plasma membrane (or melanocyte plasma membrane) directly opposing the basement membrane (BM) on the dermal side, and the less dense area between the two membranes, the intermembrane space (IMS).

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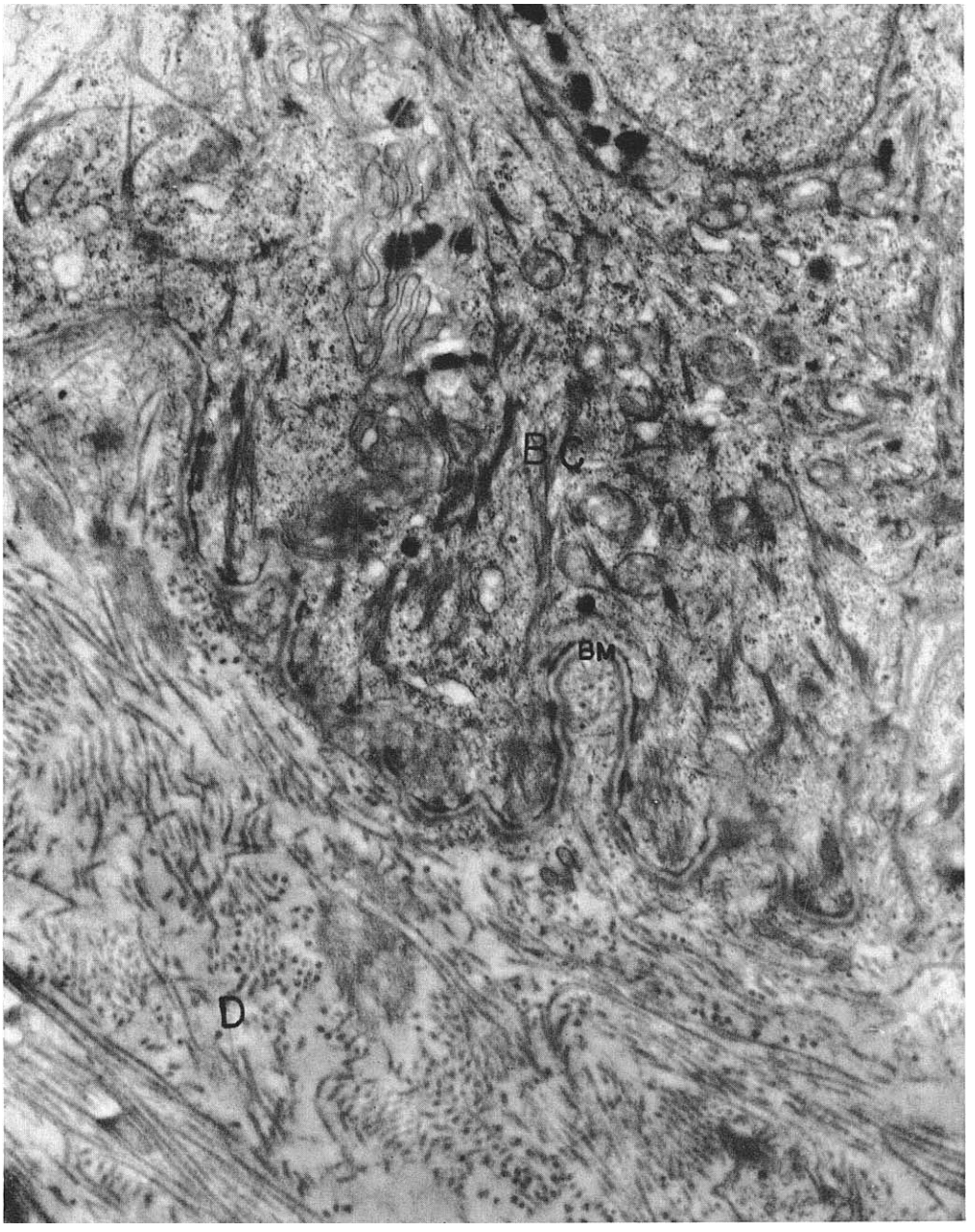

FIG. 1. *Normal skin.* Region of the D–E junction. Note the undulating contour of the basal cell (BC) plasma membrane opposing the basement membrane (BM), the loosely arranged collagen fibrils, and the smaller filaments. Approximately 14,600 ×.

Certain features of the region are of interest. Along the undulating plasma membrane of the basal cells at the junction are frequent dense thickenings. At these thickenings are also thin, less dense lines (arrows, Fig. 2) apparently just within the IMS. Between the thin line and the BM there is an increase in density, merging into thickened, dense areas of the basement membrane.

Fine, usually banded filaments (Fil) appear to merge into the dermal border of the BM. Beneath this there are similar filaments, collagen

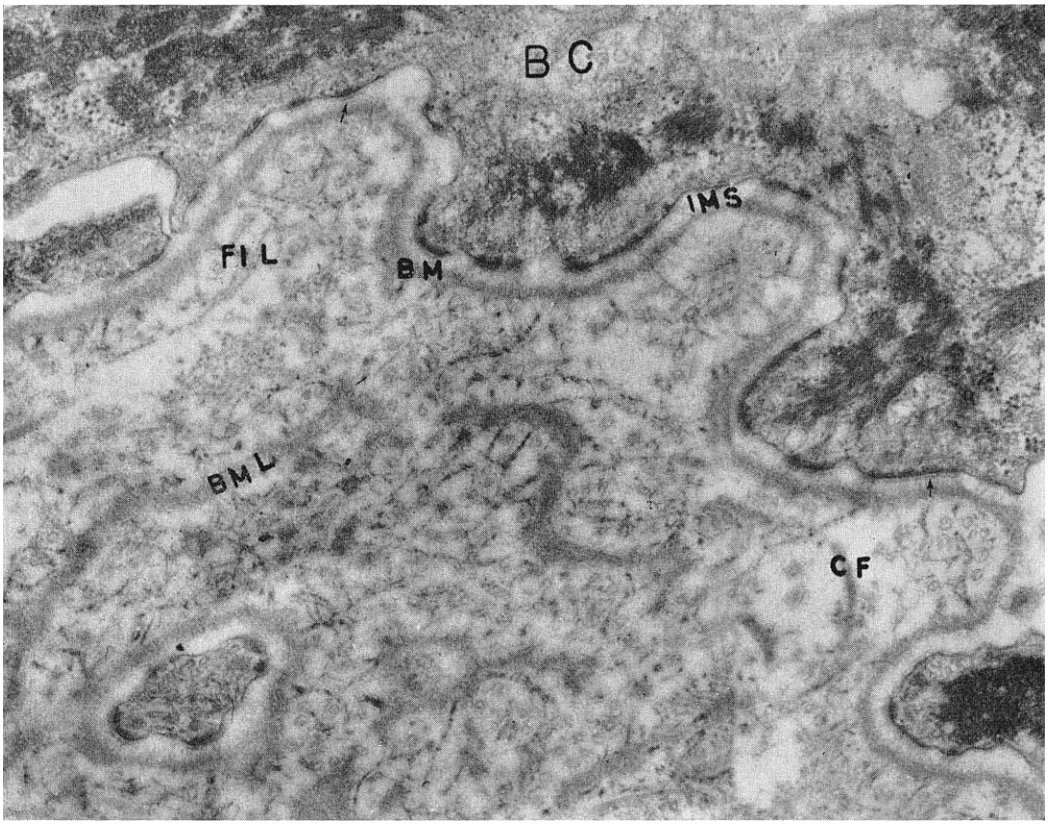

FIG. 2. *Normal Skin.* Region of the dermal-epidermal junction. Dense thickenings are present along the basal cell plasma membrane, opposite thickenings in the basement membrane (BM). The intermembrane "space" (IMS) in these areas shows increased density. Near the plasma membrane are fine lines parallel to the membranes (arrows). Note the fine dermal filaments (Fil) merging into the basement membrane, the collagen fibrils (CF), and the basement membrane like structure (BML). Approximately 35,500 ×.

fibrils (CF), and occasionally, basement membrane-like structures (Fig. 2, BML), irregularly arranged, separated by low density, relatively amorphous areas. On the average this type of structure extends a few microns into the dermis. At this level the collagen fibrills begin to show organization into distinct groups, and the filaments are fewer in number. In some areas the more organized groups of collagen fibrils are present higher in the dermis, even approaching the BM. This normal variability compels caution in interpretation of suspected pathologic alterations.

When referring to light microscope observations the term dermalepidermal junction is used as a gross approximation, and the term basement membrane is used to designate the PAS positive structure at the upper limit of the dermis.

Epidermolysis Bullosa Hereditaria Letalis

Only one patient with this disease was available for study. Minimal frictional trauma with a cotton swab produced rapid blistering. Such blistered sites were difficult to remove for biopsy study. Specimens from normal appearing skin provided more useful material.

Light microscope examination showed a nearly complete dermal-epidermal separation. The dermal side was lined by a PAS positive basement membrane. (Fig. 3). In areas where the skin was intact a number of basal cells were vacuolated (Fig. 4), in accord with the findings of Roberts and associates (4), who regarded such vacuolization as the earliest stage of blister formation.

Electron microscope observations were some-

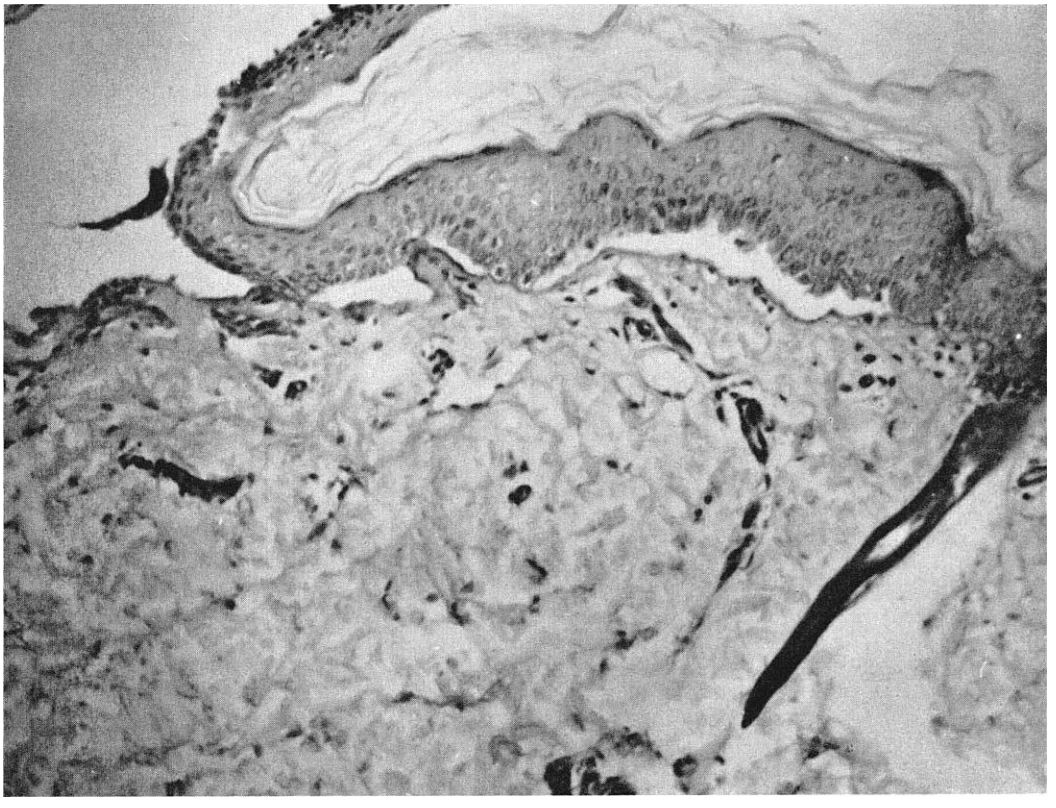

FIG. 3. *Epidermolysis bullosa hereditaria letalis.* Biopsy of apparently normal skin. Light micrograph. PAS stain. The basement membrane remains intact on the dermal side of the separation.

what impaired by the lack of good cellular detail.* Positional relationships between adjacent cells were relatively normal but plasma membranes were visualized poorly in uranyl stained sections (Fig. 5, 7, 8, 9). Only the desmosomes and to a lesser extent the thickened plates at the dermal-epidermal junction were better visualized. Lead staining improved plasma membrane visualization somewhat (Fig. 6). Round or oval, circumscribed, moderately dense structures without internal detail, mitochondrial in size and location, were present in most cells. In a few micrographs structures suggestive of cristae were noted (Fig. 8).

At the dermal-epidermal junction separation occurred in the plane of the IMS. It is of interest to note that where separation has not taken place the basal cells rest directly against the basement membrane, indicating absence or alteration of the material normally occupying the IMS.

Below the basement membrane the dermis did not appear to be directly involved in blister formation, although there was edema and minor degenerative alteration.

Epidermolysis Bullosa Dystrophica, Recessive Type

Biopsy specimens were obtained from five patients with this disease. Two of the five patients blistered so readily that the trauma of biopsy was sufficient to produce gross dermal-epidermal separation. In the other three patients mild frictional trauma for a few seconds resulted in immediate separation.

Light microscope examination showed dermal-epidermal separation in all instances. In most areas of "normal" or separated skin the PAS positive "basement membrane" was poorly developed but there was rather diffuse positive

* Fixation and processing artifacts were initially considered as possibly responsible for the apparently poor tissue preservation, but other material fixed and processed at the same time showed good tissue preservation.

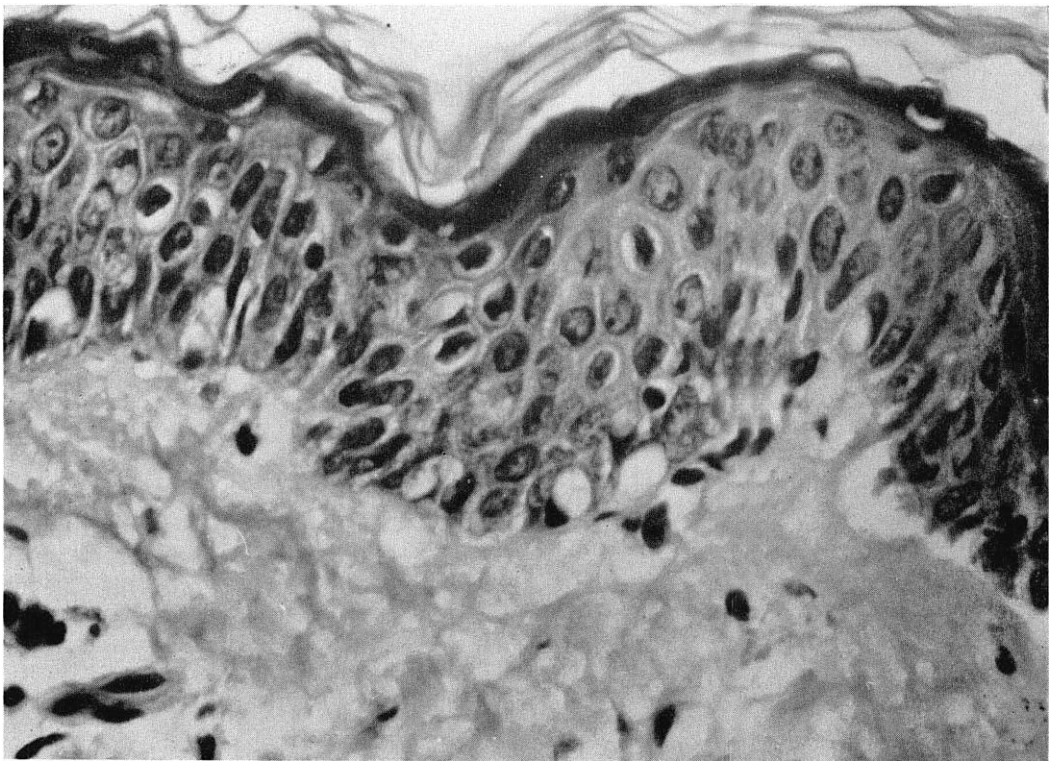

FIG. 4. *Epidermolysis bullosa hereditaria letalis*. Light micrograph. H & E stain. Note the vacuolar degeneration of basal cells and other epidermal cells and also small vesicles apparently on the dermal side of the junction. At one edge frank dermal-epidermal separation is present.

staining of the papillary dermis. In blisters, fragmentation of the papillary dermis was often apparent and debris collected within the separation (Fig. 10). At the edges of blisters or in very early separations microscopic vesiculation was apparent (Fig. 11).

Electron microscope findings in "normal" skin of patients with relatively mild disease showed marked increase in macrophage activity near the dermal-epidermal junction (Fig. 12). Red blood cells were frequently noted in the same subjunctional area. Another finding of interest was the apparent phagocytosis of rather large diameter collagen fibrils (Fig. 13, 14). These larger diameter fibrils were often found in bundles where the majority of fibrils were of ordinary diameter (Fig. 14). In addition, varying degrees of collagen degeneration or necrosis were found in "normal" skin, correlating quite well with the clinical severity of the disease.

Blisters appeared to occur as a result of disintegration of the collagen of the upper dermis, somewhat less severe alteration extending to mid or lower dermis (Fig. 15, 16). At the blister edge, collagen disintegration is much less striking (Fig. 17). The basement membrane seemed to be relatively unaffected, remaining with the epidermis (Fig. 18, 19). This could even be discerned in the low magnification micrograph (Fig. 19). In some specimens the basement membrane was studded with small, dense particles similar in appearance to ribosomes (Fig. 18).

Porphyria Cutanea Tarda

Specimens were obtained from three patients in exacerbation. All showed essentially the same pathologic findings. Blisters were produced by pressure with a glass syringe plunger, pinching, or cellophane tape stripping. Somewhat unexpectedly the stripping method was most reliable. It was often necessary to strip only a few layers to produce blisters. In some instances the entire epidermis would be dislodged onto the tape. Definite evidence of separation usually would

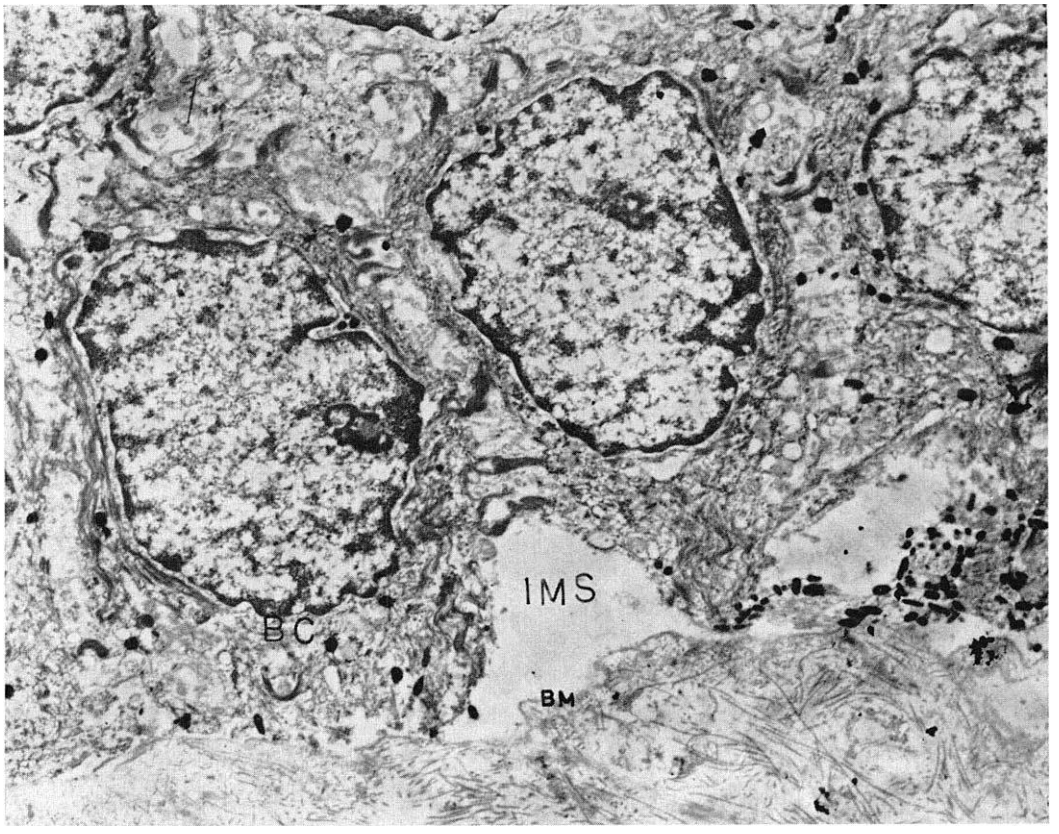

FIG. 5. *Epidermolysis bullosa hereditaria letalis*. Region of the dermal-epidermal junction. The basal cells (BC) retain good positional relationship to one another for the most part. Plasma membranes are poorly visualized. Note widening of the intermembrane space (IMS) and the intact basement membrane (BM). Approximately 8,400 ×.

not occur until 10–45 minutes after trauma, creating the problem of marked secondary alterations, which complicated interpretation of micrographs. The specimens chosen for study were from the extensor surface of the forearm where blisters had not previously occurred. It was sometimes possible to produce blisters or the phenomenon of the entire epidermis being stripped off on the relatively lightly sun-exposed ventral forearm surface.

Light microscope examination demonstrated dermal-epidermal separation. PAS positive material remained attached to the epidermal cells. (Fig. 20, 21). A marked inflammatory reaction was present about the small dermal vessels (Fig. 20), but this feature was quite variable, in accord with the findings of Feldaker *et al* (5).

From the electron micrographs it was evident that blisters developed within the papillary dermis. (Fig. 22, 23, 24). There was an apparent increase in basement membrane-like material and a decrease in the number of collagen fibrils present on both the epidermal and dermal sides of the blisters. The changes extended on the average to the mid dermis where the dermis was near normal in appearance (Fig. 24).

DISCUSSION

On the basis of the data presented, it is possible to suggest that quite distinct mechanisms of blister formation operate in each disease studied, although all show grossly dermal-epidermal separation by light microscope examination.

Speculations concerning the nature of the defect in epidermolysis bullosa hereditaria letalis have recently been reviewed by Roberts (4). Except for those of Roberts herself, these speculations, attributing blister formation to defects

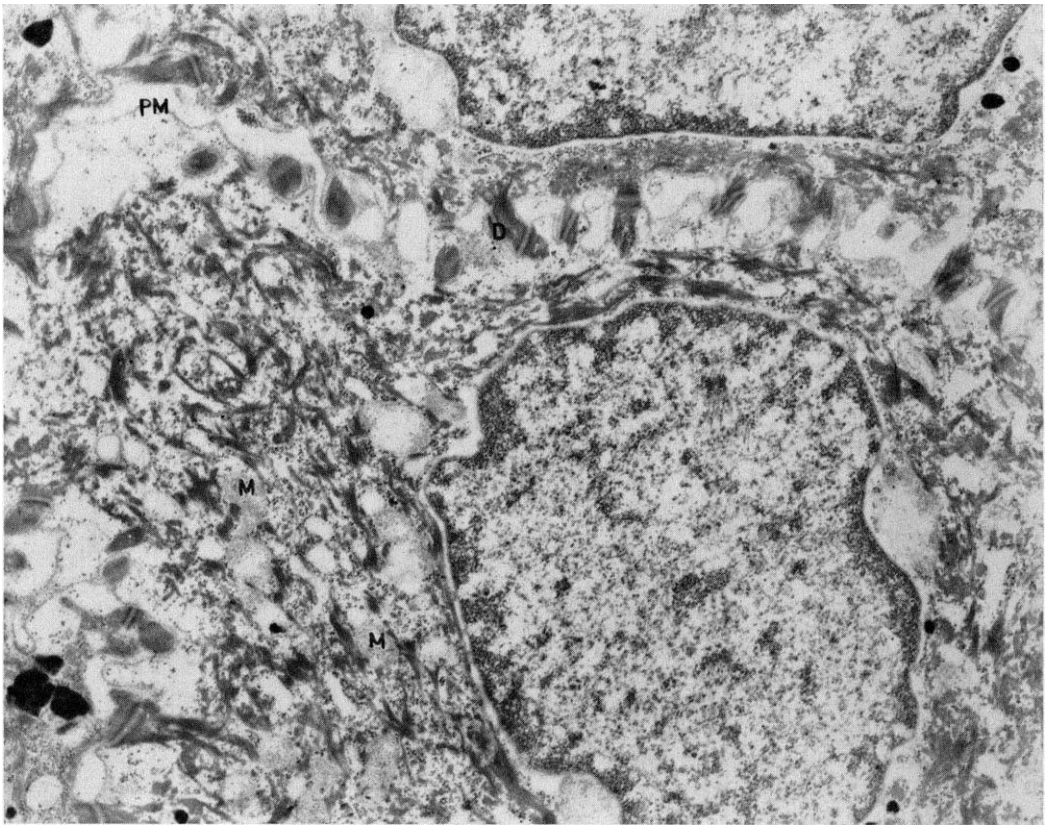

FIG. 6. *Epidermolysis bullosa hereditaria letalis.* Mid malpighian layer. Uranyl acetate plus lead hydroxide stain. The plasma membranes (PM) are seen as wide fuzzy lines. The desmosones (D) are quite well preserved. Small oval structures without membranes are presumed to be mitochondria (M). Approximately 30,000 ×.

of collagen, elastic tissue, or mucopolysaccharides, can be ignored. Roberts considered the vesicular changes she observed within basal cells to be the first step in blister formation. This view is objectionable because apparent vesicular changes in basal cells are commonly found in developing blisters and at the edges of formed blisters in diseases where basal cells are clearly not involved in blister formation. The light microscope cannot distinguish between vesiculation in the intermembrane "space" and adjacent portions of basal cells. Moreover, vesicular changes in epidermal cells (and perhaps especially of basal cells) are commonly observed as a nonspecific response to injury of many kinds. This matter of vesicular changes in basal cells is of some importance to resolve because there is a type of epidermolysis bullosa, epidermolysis bullosa simplex, in which the basal cell itself participates in blister formation in a specific manner. The electron micrographs indicate that the blisters in epidermolysis bullosa hereditaria letalis form in the plane of the IMS. The usual contents of the IMS appear to be absent. The nature of the normal contents of the IMS, its source, and mode of attachment to basal cells and to the basement membrane are not known, so that there is no basis for judging whether its absence is primary or secondary. Likewise, the poor visulization of membranes could be a secondary phenomenon. It is also possible that both apparent abnormalities could be related directly.

The marked degenerative changes of the dermal collagen found in epidermolysis bullosa dystrophica, recessive type, which are accentuated by trauma, resulting in blister or erosion formation, suggest that abnormal collagen is present or that a collagenase-like substance may

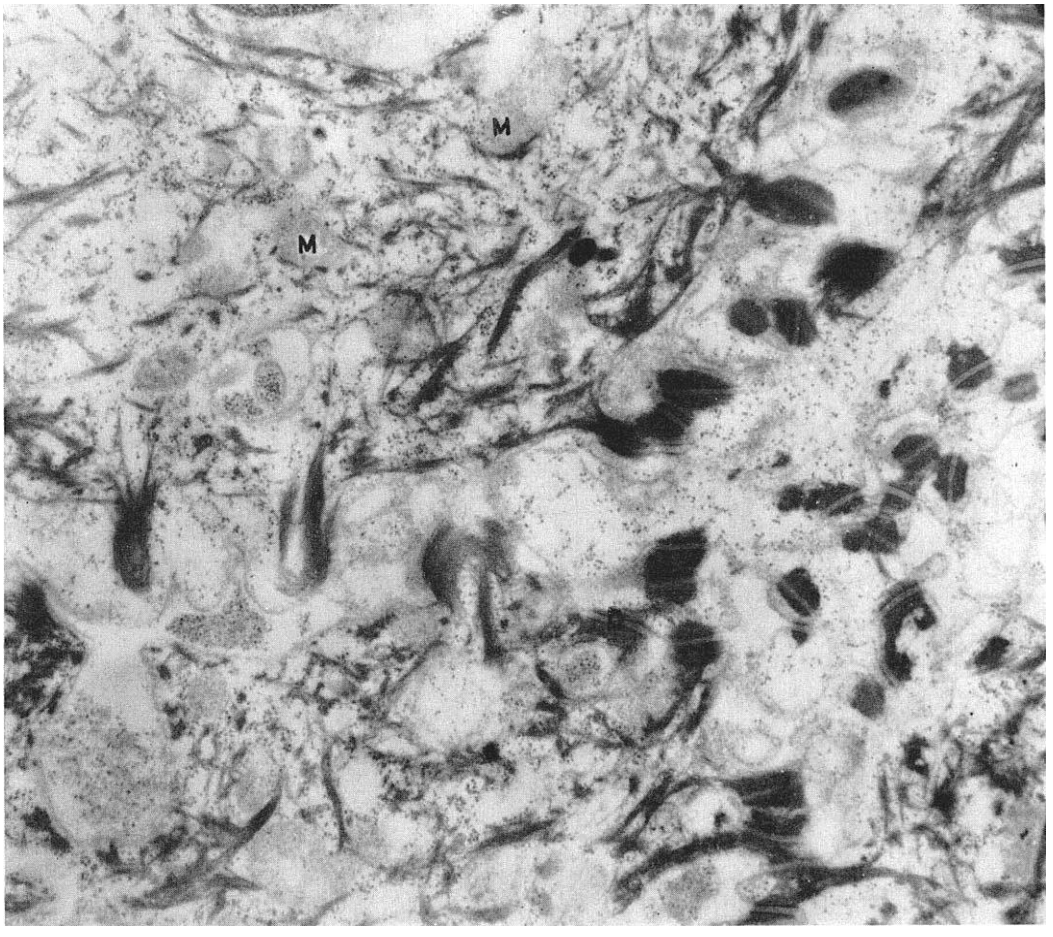

FIG. 7. *Epidermolysis bullosa hereditaria letalis.* Malpighian layer. Uranyl stain. Desmosomes (D) are well visualized but plasma membranes and presumed mitochondria (M) are poorly defined. Approximately 25,180 ×.

be active. The observation of large diameter bundles, which seem to be selectively attacked by macrophages would also seem to indicate that the collagen is abnormal. However, it is possible to induce phagocytosis of collagen fibrils in guinea-pig skin by intradermal injection of collagenase (Dermal-epidermal blisters also result).

Methods are available for analysis of many properties of collagen*. Application of such technics to skin specimens from patients with this disease should provide useful information. Of particular interest would be studies using methods developed by Keech (8, 9) to assess the effects of mucopolysaccharides and other substances on collagen formation. Keech was able to produce collagen susceptible to degradation induced by mechanical stress. Estimation of collagenase activity in the skin of epidermolysis bullosa dystrophica should be attempted but the present methods are difficult to control (9).

Blisters in porphyria cutanea tarda appear to develop by cleavage of a mechanically weak dermis. This weakening is presumably the result of an ultraviolet light-porphyrin reaction in the dermis, but the substrate of this reaction is presently obscure. In the usual clinical situation the dermal damage is apparently cumulative, but reversible, since blistering ordinarily lags increased porphyrin excretion and/or UVL exposure by several weeks, and subsides slowly

* References to methods of collagen analysis are to be found in the comprehensive recent review of Harkness (6).

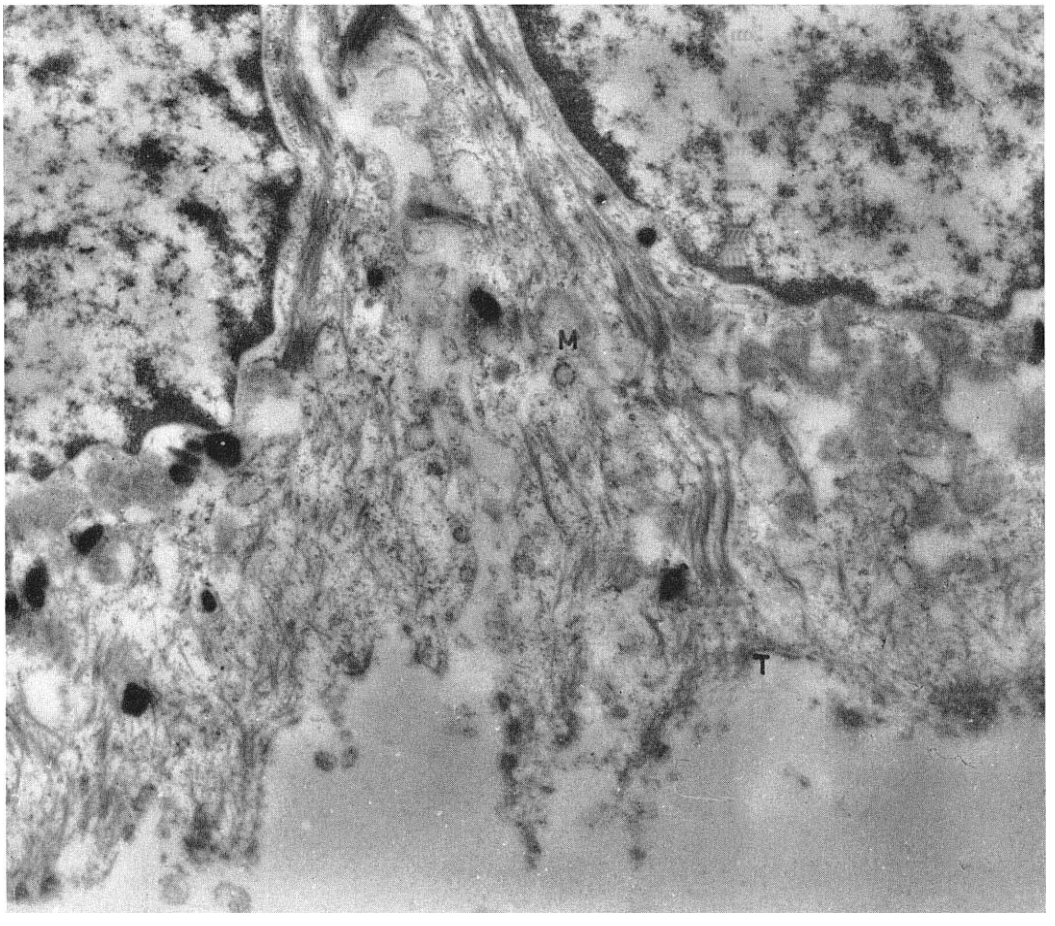

FIG. 8. *Epidermolysis bullosa hereditaria letalis*. Epidermal side of blister. Uranyl stain. Membranes in this area were better visualized than elsewhere. Note the possible cristae within presumed mitochondria (M), and the relatively intact thickenings (T) along the dermal side of the plasma membranes. Approximately 17,000 ×.

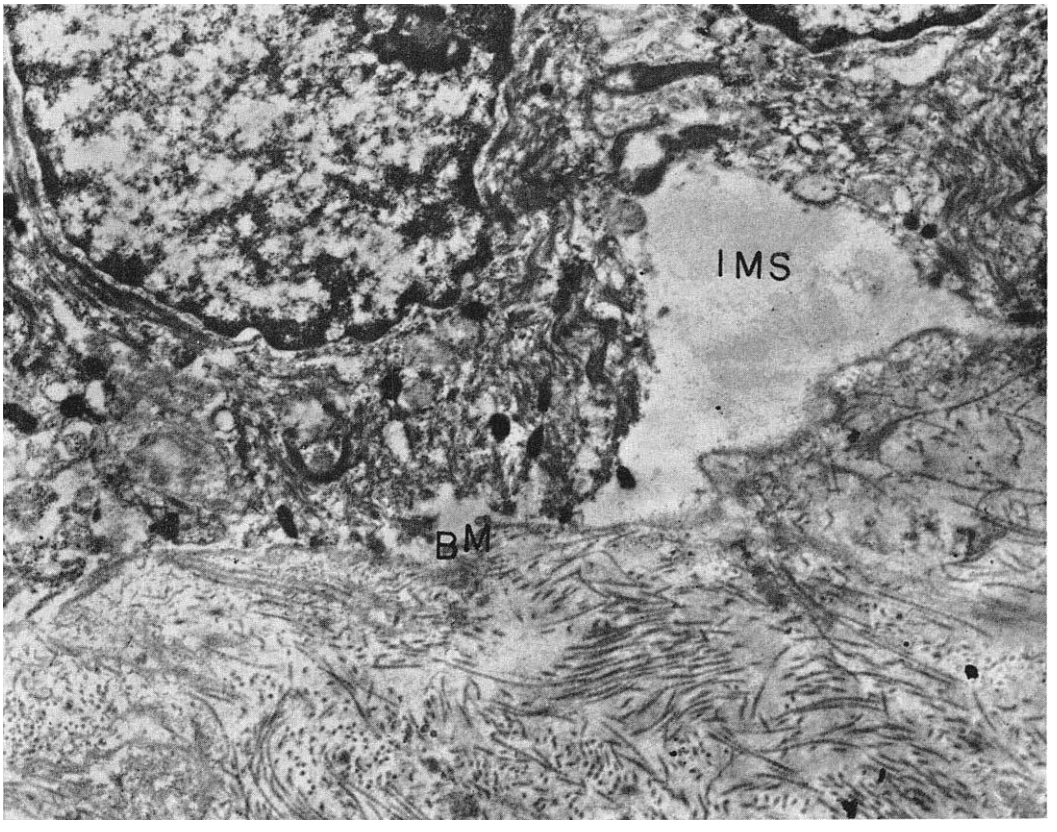

FIG. 9. *Epidermolysis bullosa hereditaria letalis*. Region of D–E junction. Note the early vesicle produced by widening of the intermembrane space (IMS), and the basal cell in direct contact with the basement membrane (BM). Approximately 14,640 ×.

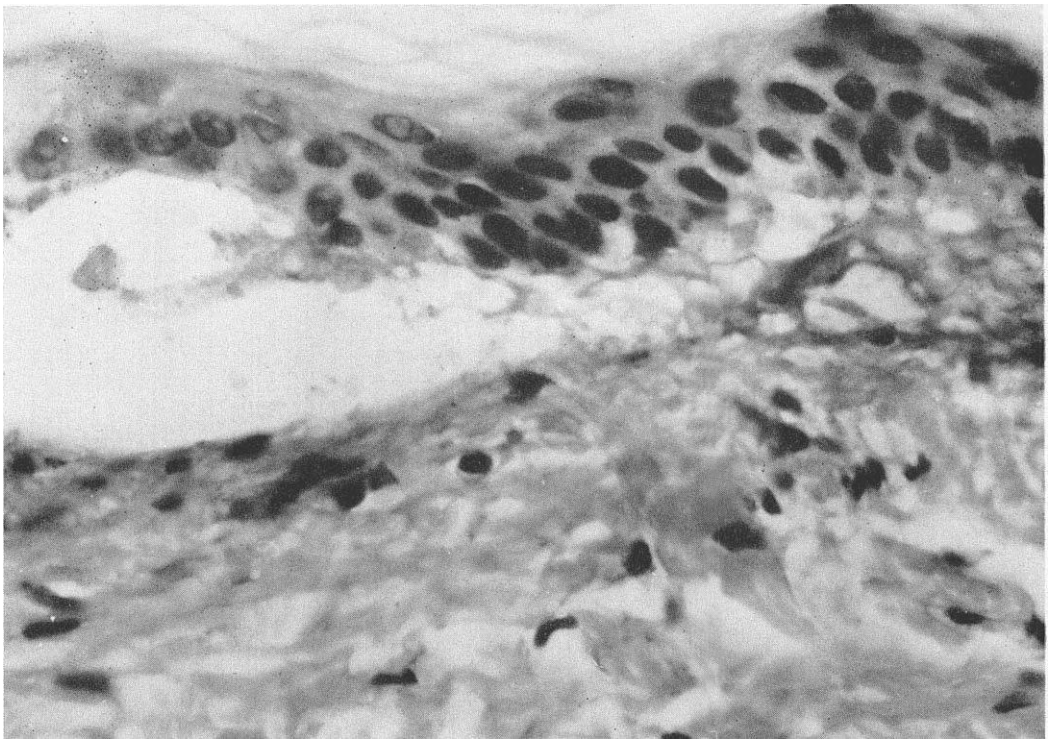

FIG. 10. *Epidermolysis bullosa dystrophica, recessive type.* Light micrograph. There is gross D–E separation and fragmentation of the upper dermis. PAS stain.

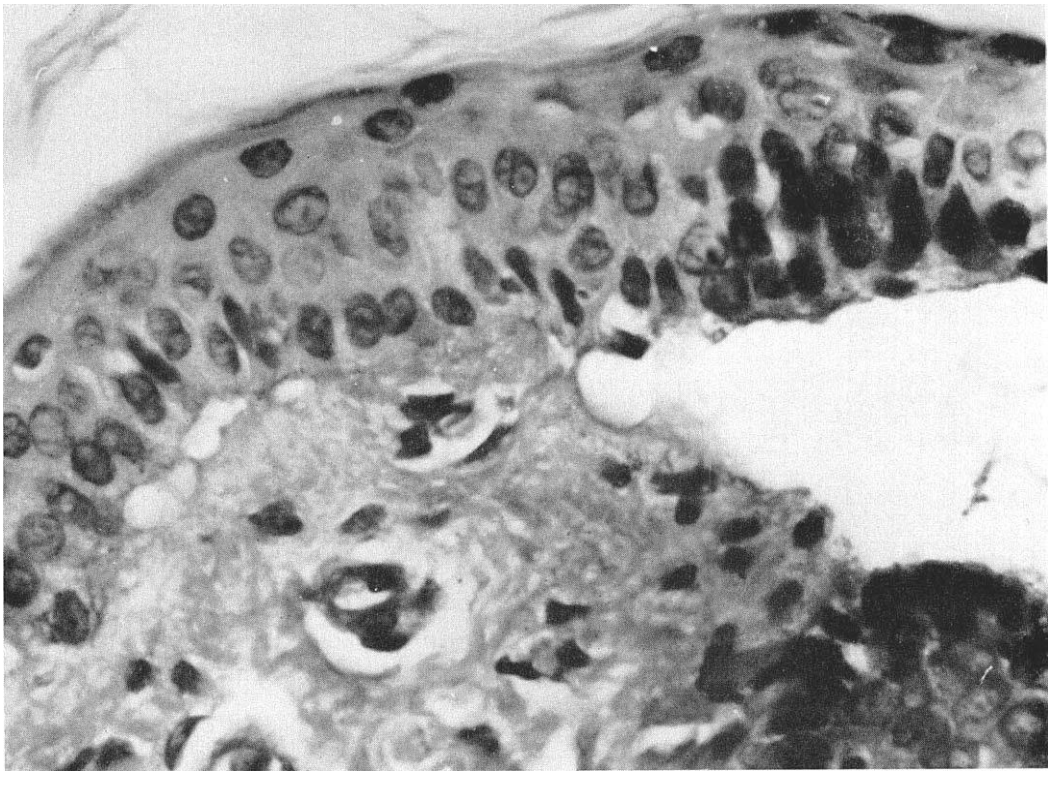

FIG. 11. *Epidermolysis bullosa dystrophica, recessive type.* Light micrograph of blister edge. Note the clean D–E separation and the early vesicles at the junction. PAS stain.

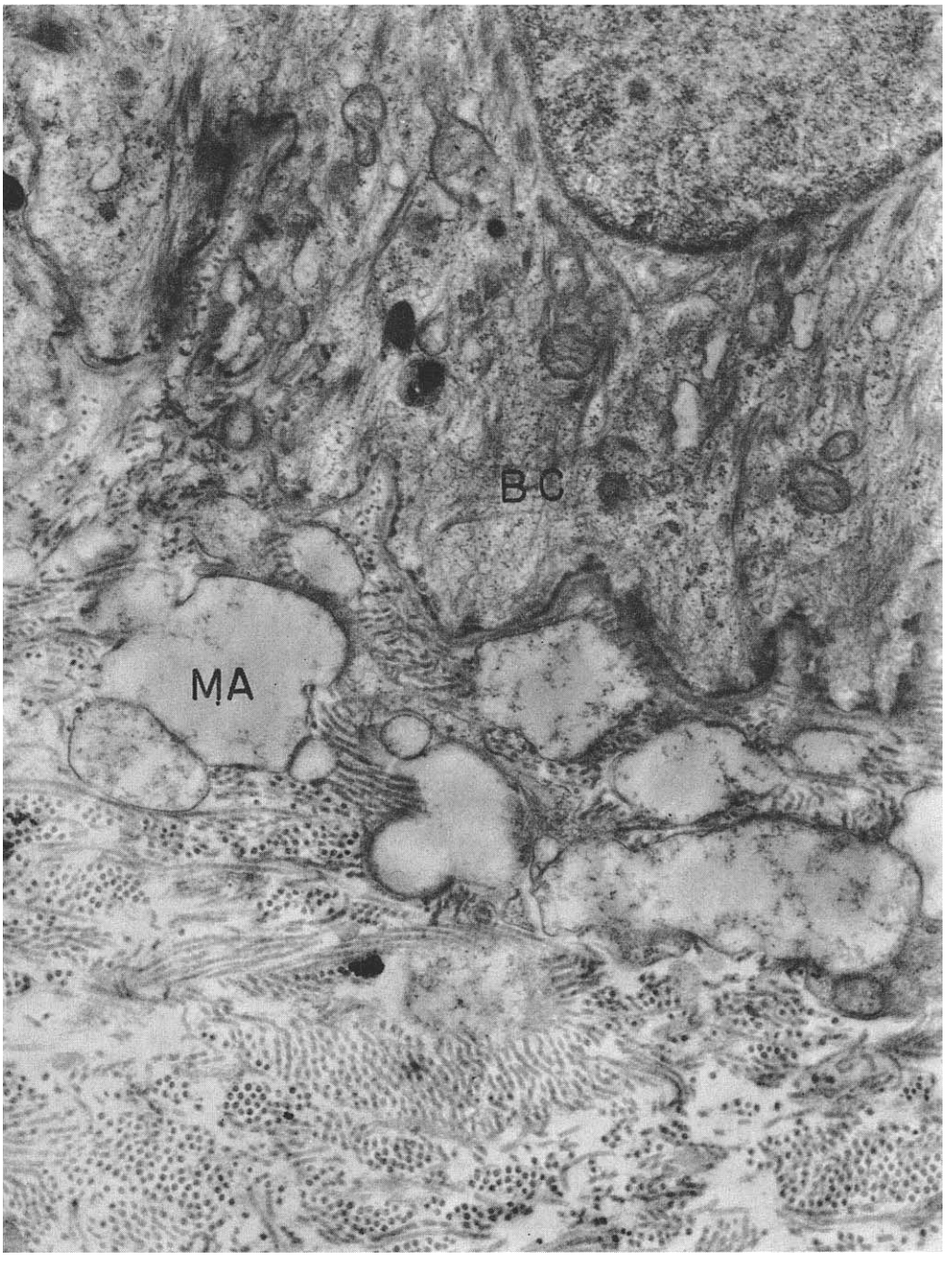

FIG. 12. *Epidermolysis bullosa dystrophica, recessive type.* "Non-traumatized" skin. Region of the D–E junction. Note the relatively healthy basal cell (BC) and the large macrophage cytoplasmic (MA) extensions below the D–E junction. Approximately 18,000 ×.

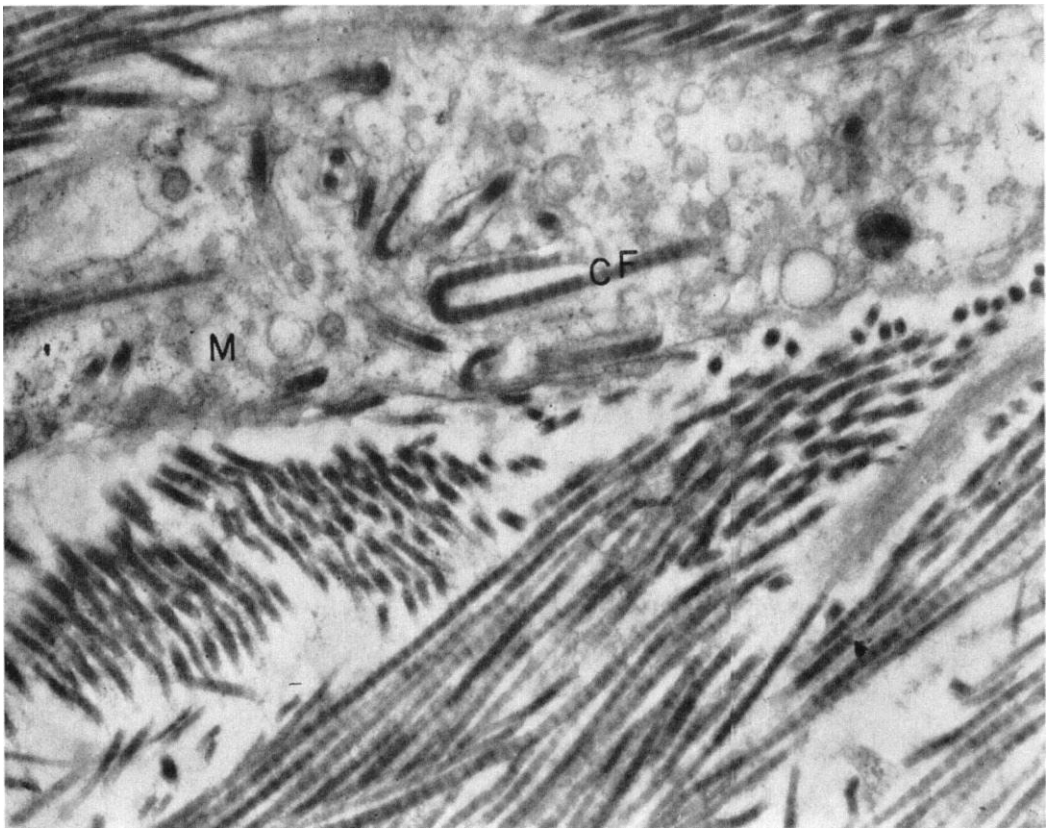

FIG. 13. *Epidermolysis bullosa dystrophica, recessive type.* "Non-traumatized" skin. Upper dermis. Note the large diameter, sometimes "looped" collagen fibrils (CF) within presumed macrophage (M). Most are membrane bound. Approximately 30,000 ×.

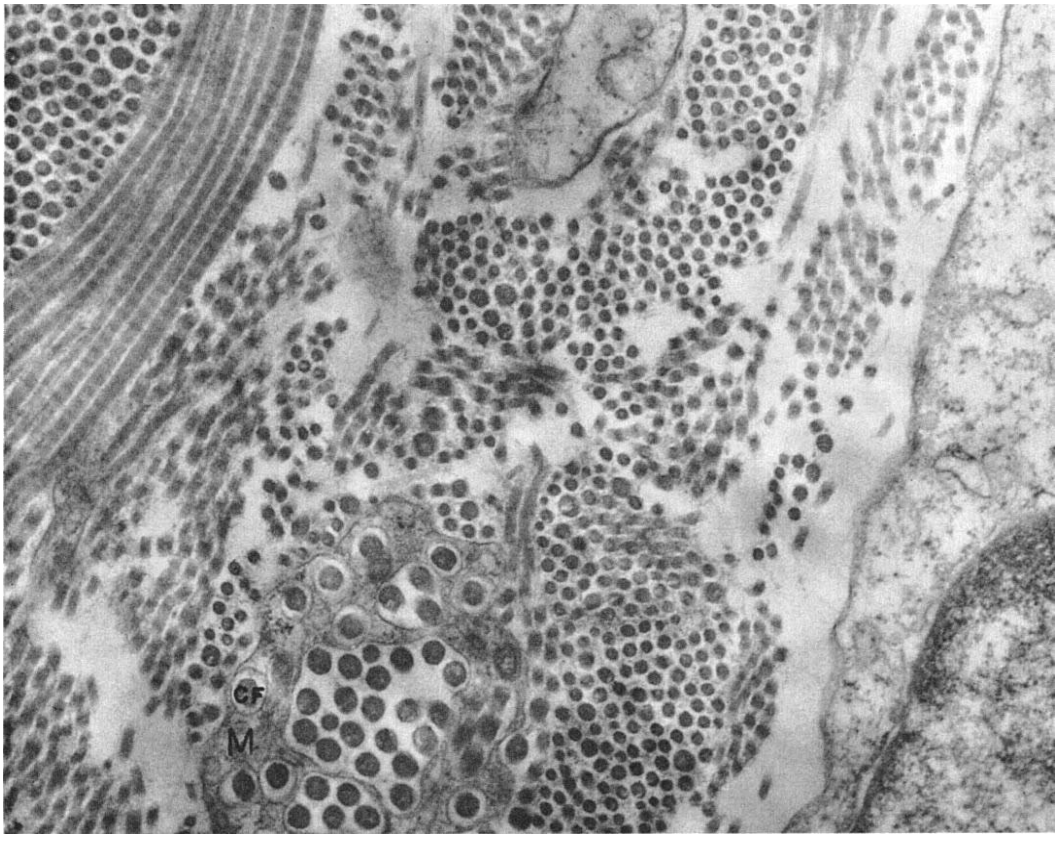

FIG. 14. *Epidermolysis bullosa dystrophica, recessive type.* "Non-traumatized" skin. Upper dermis. Note variation in collagen fibril (CF) diameter. Macrophage (M) contains a number of them within membranes. Approximately 30,000 ×.

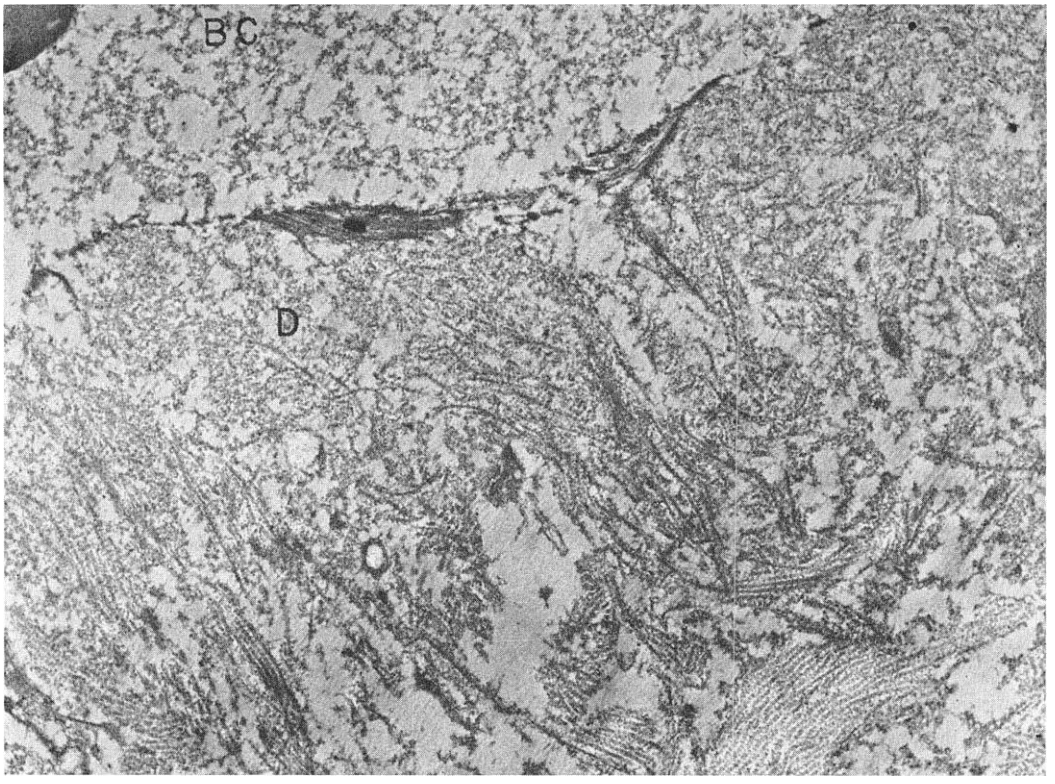

FIG. 15. *Epidermolysis bullosa dystrophica, recessive type.* Traumatized skin. Dermal side of blister. Note frankly necrotic collagen in blister cavity (BC) and marked degeneration of collagen in dermis (D) below. Approximately 13,420 ×.

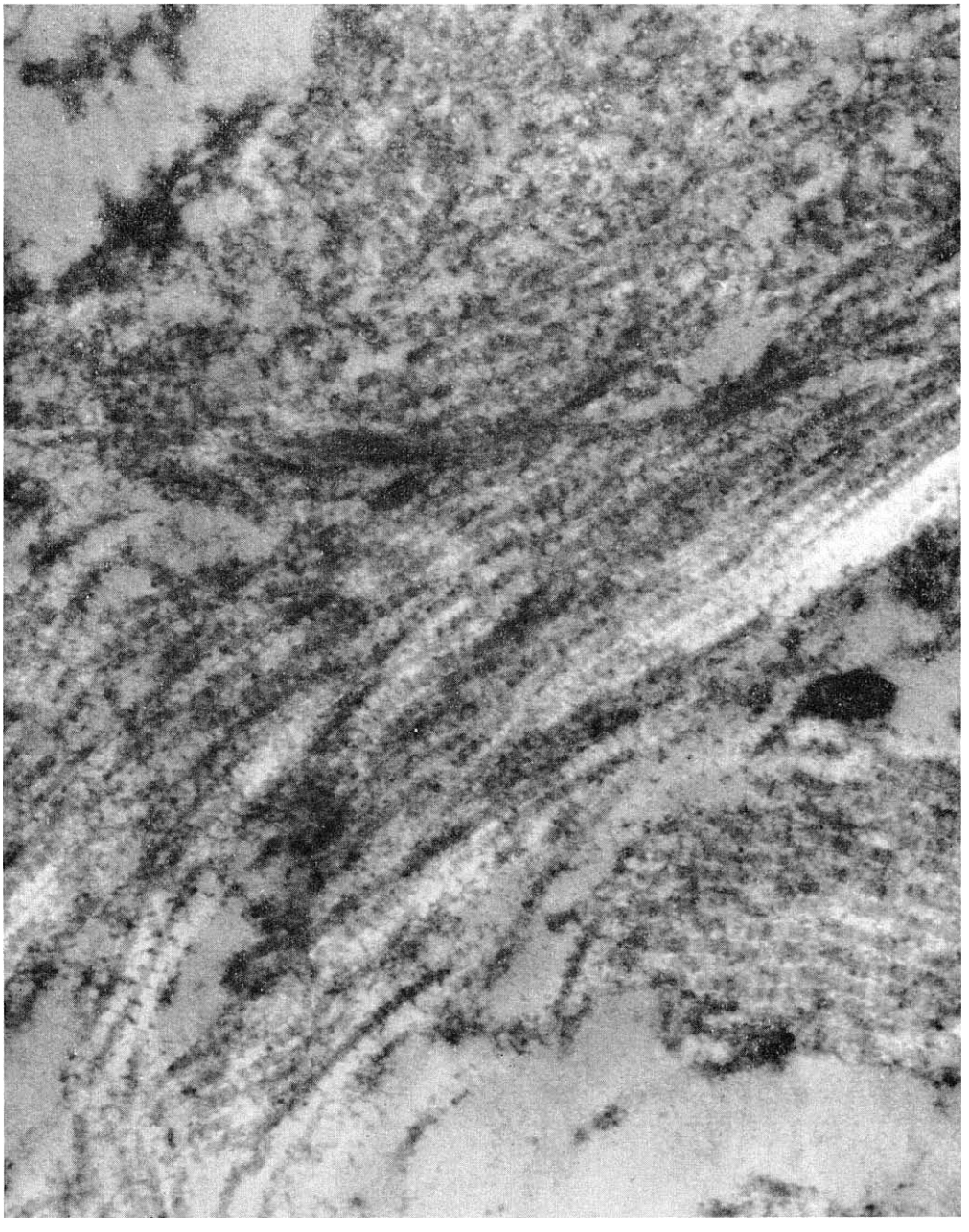

FIG. 16. *Epidermolysis bullosa dystrophica, recessive type.* Traumatized skin. Dermis. A higher magnification of degenerating collagen. Note the apparent perpendicular projections at major periods. Approximately 38,000 ×.

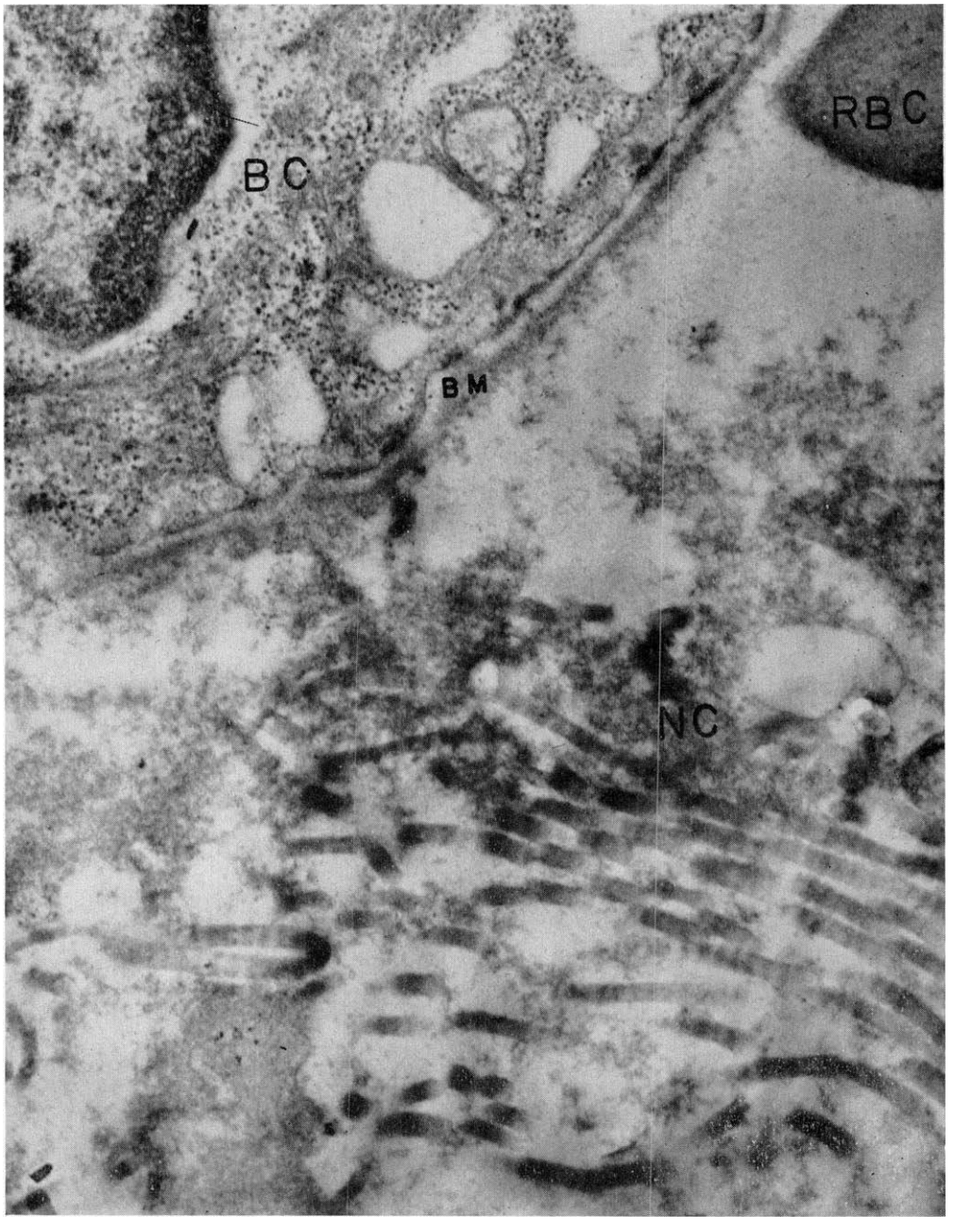

FIG. 17. *Epidermolysis bullosa dystrophica, recessive type.* Traumatized skin. Blister.edge. Note necrotic collagen (NC), intact basement membrane (BM) and red blood cell in blister cavity. Approximately 29,600 ×.

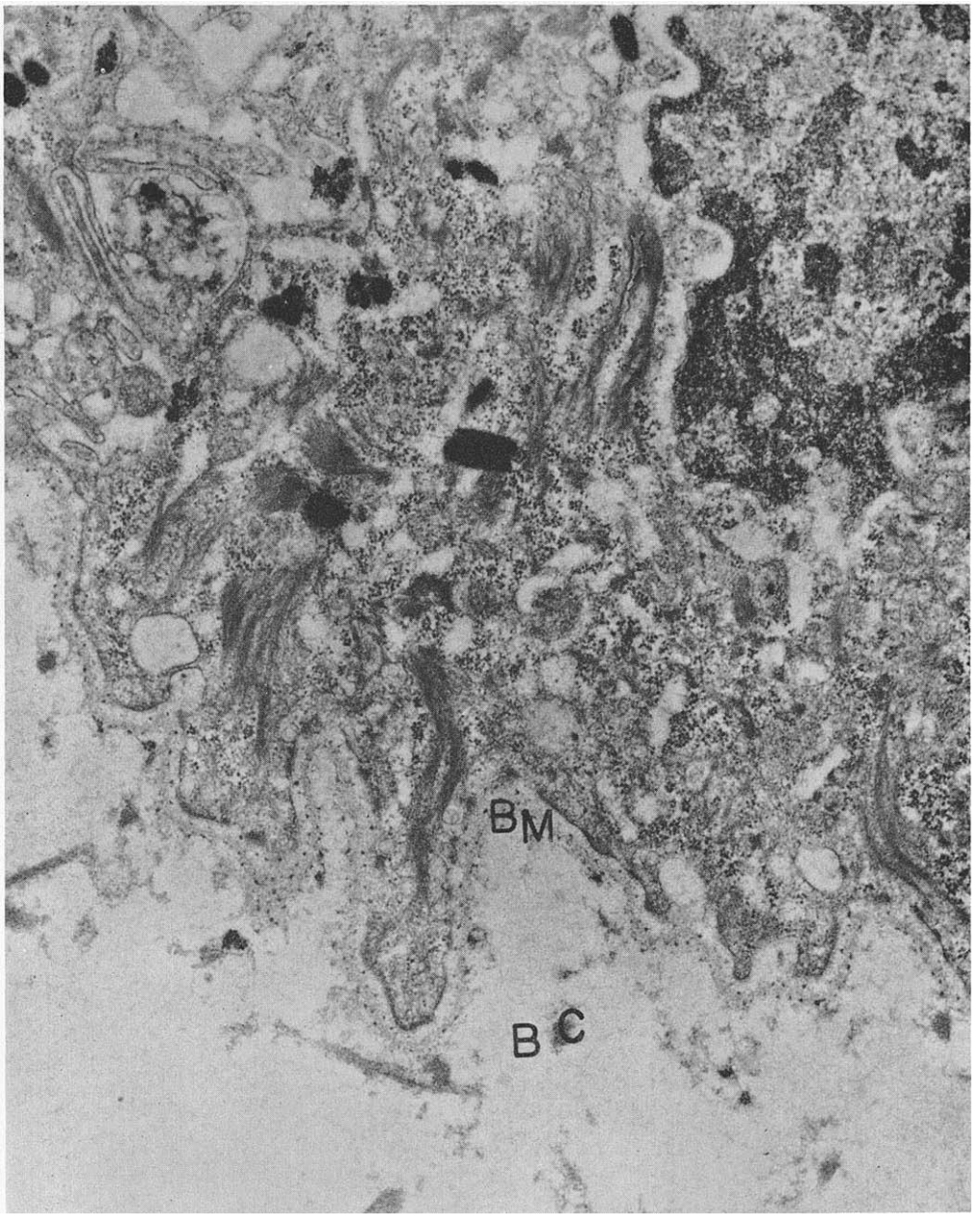

FIG. 18. *Epidermolysis bullosa dystrophica, recessive type.* Traumatized skin. Epidermal side of blister. Note intact basement membrane (BM) studded with RNP like-particles and blister cavity (BC) below. Approximately 22,800 ×.

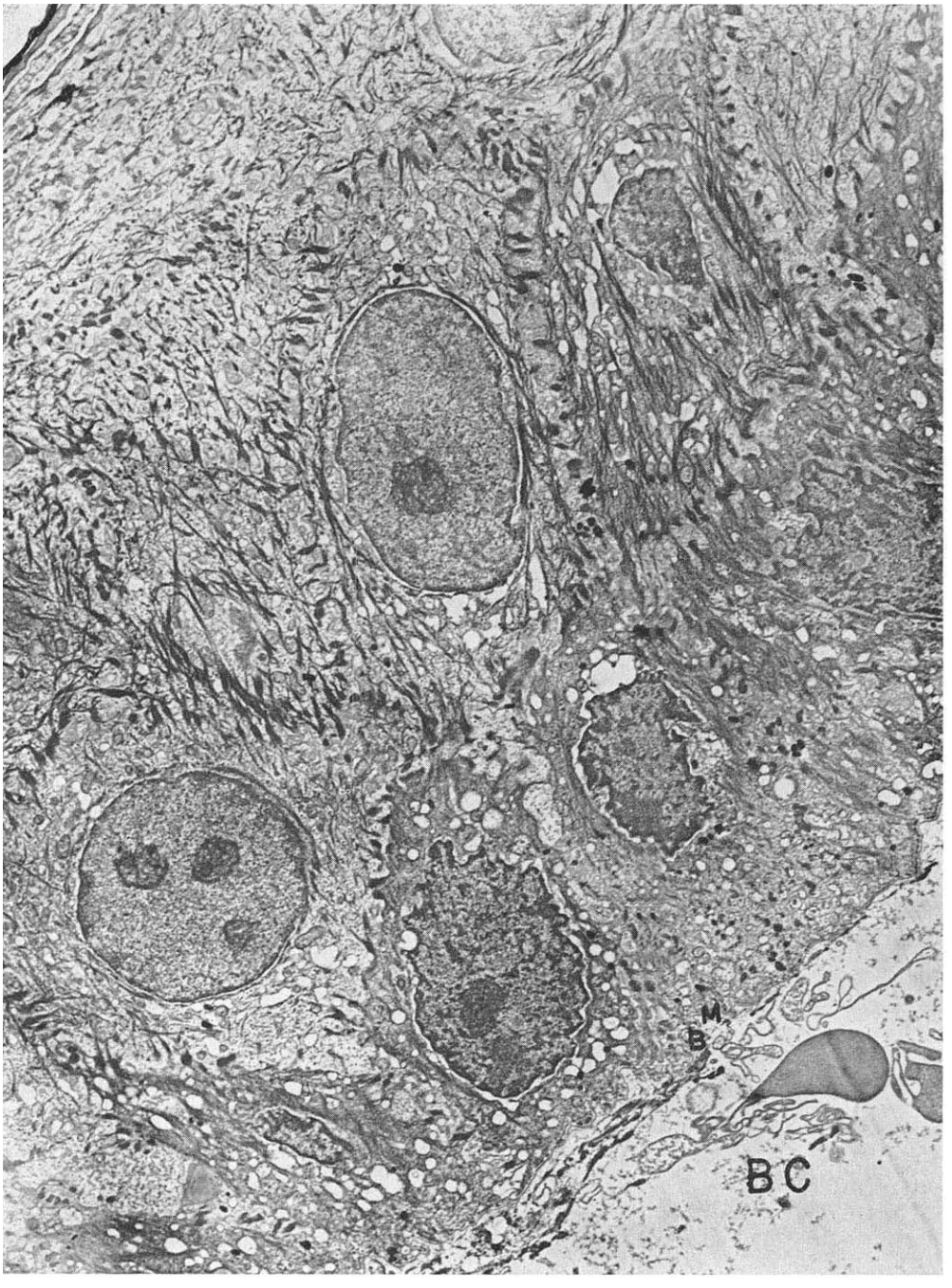

FIG. 19. *Epidermolysis bullosa dystrophica, recessive type.* Traumatized skin. Blister. Low magnification view of whole, intact epidermis. Note relatively normal appearance, especially of superficial layers. Intact basement membrane can be distinguished (BM) just above blister cavity (BC).

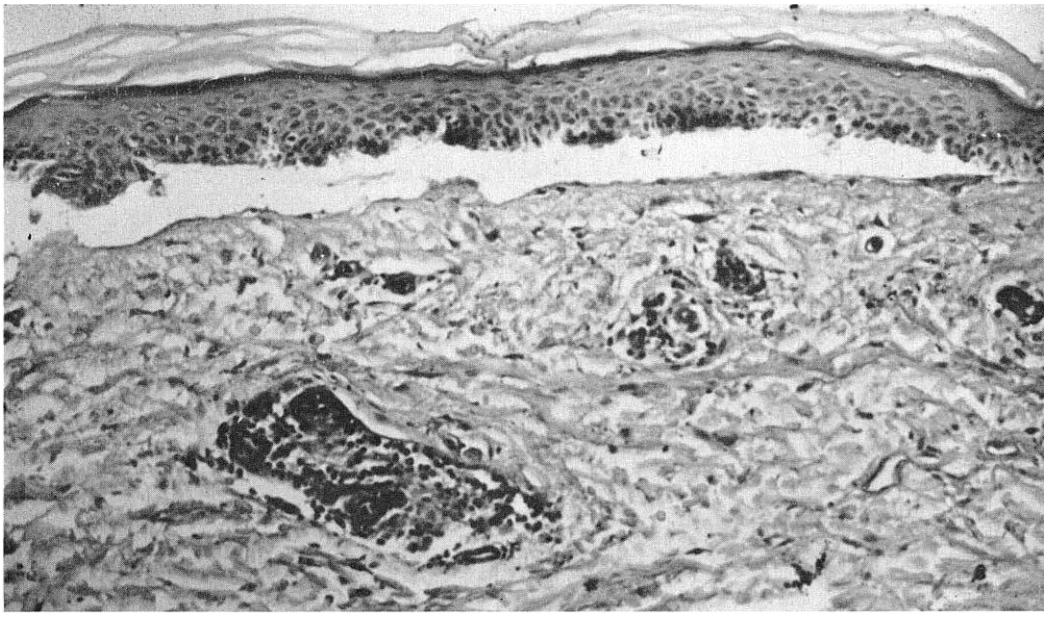

FIG. 20. *Porphyria cutanea tarda.* Light micrograph. PAS stain. Note PAS positive material on both epidermal and dermal side of blister, and the marked vascular reaction.

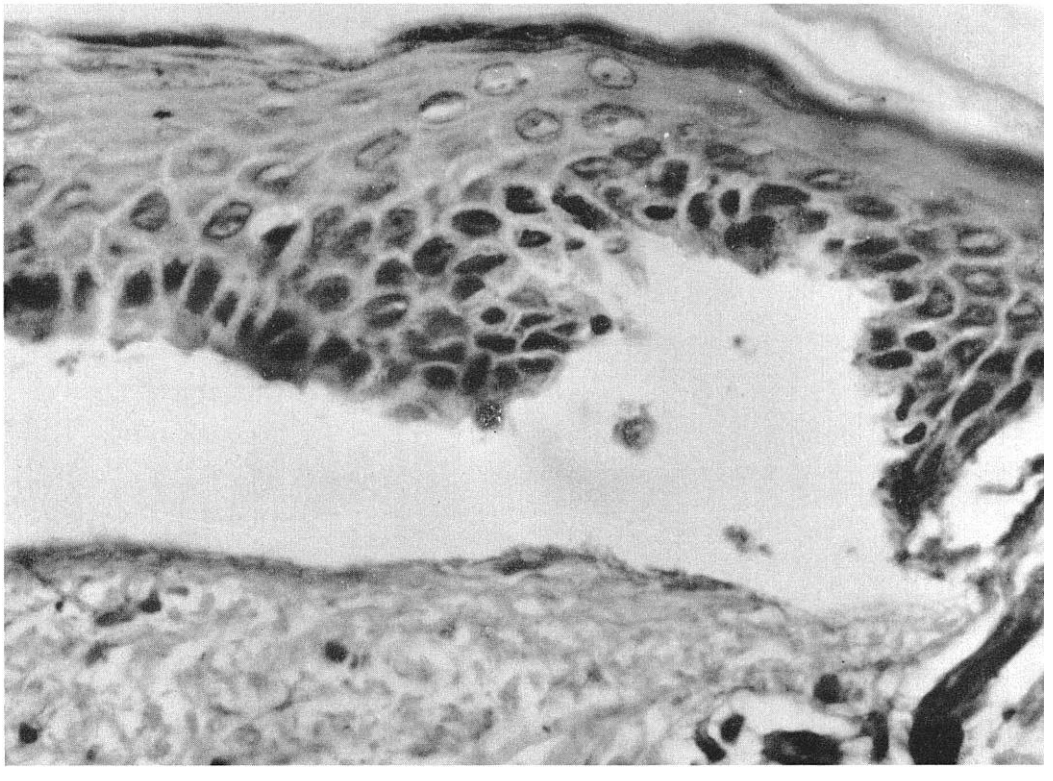

FIG. 21. *Porphyria cutanea tarda.* Light micrograph. Note PAS positive material on both sides of the blister and the mild spongiosis.

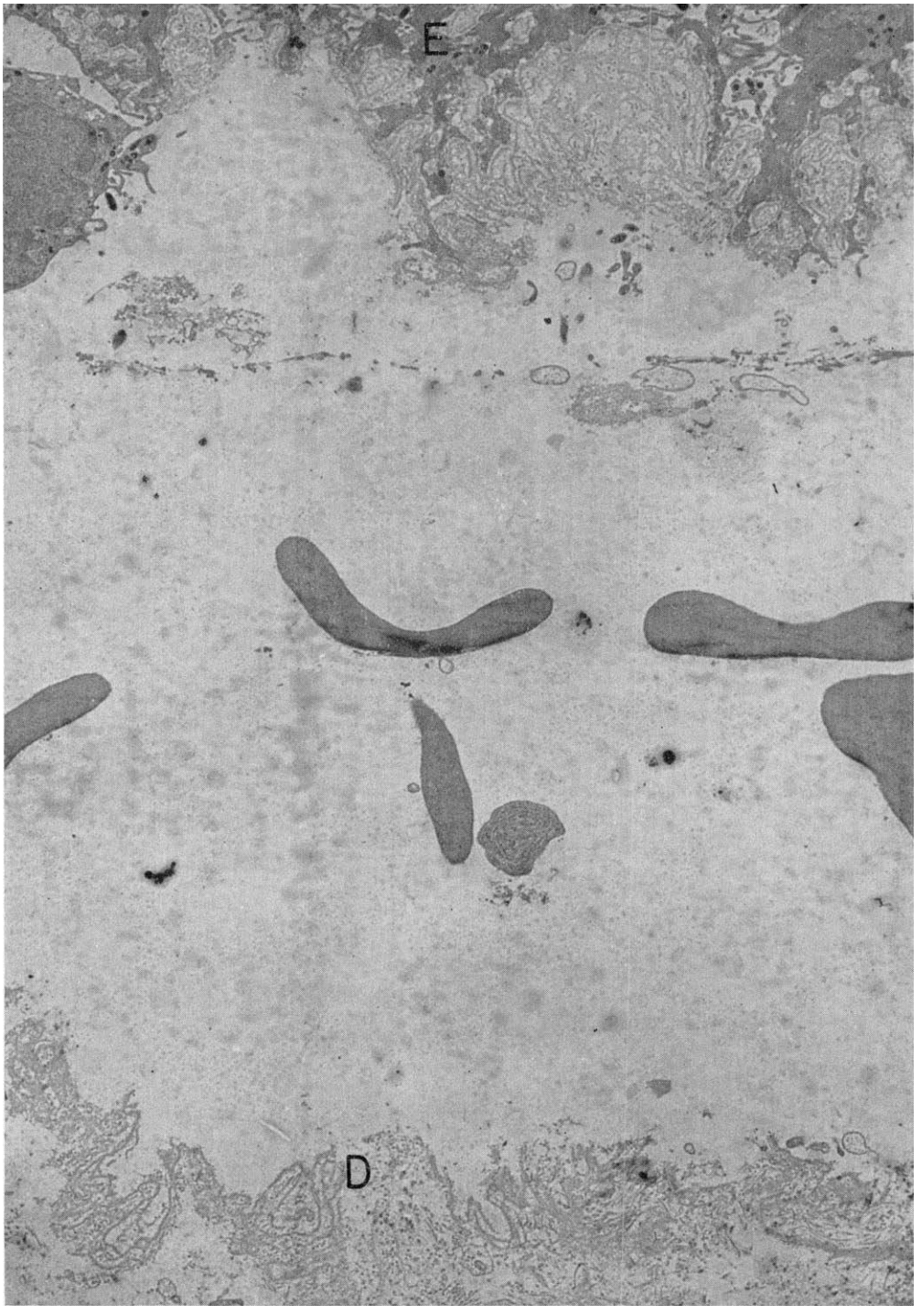

FIG. 22. *Porphyria cutanea tarda.* Low magnification electron micrograph. Note connective tissue adhering to the epidermal side (E) of the blister, the edematous appearing dermal side (D) of the blister, and the red blood cells in the blister cavity. Approximately 5850 ×.

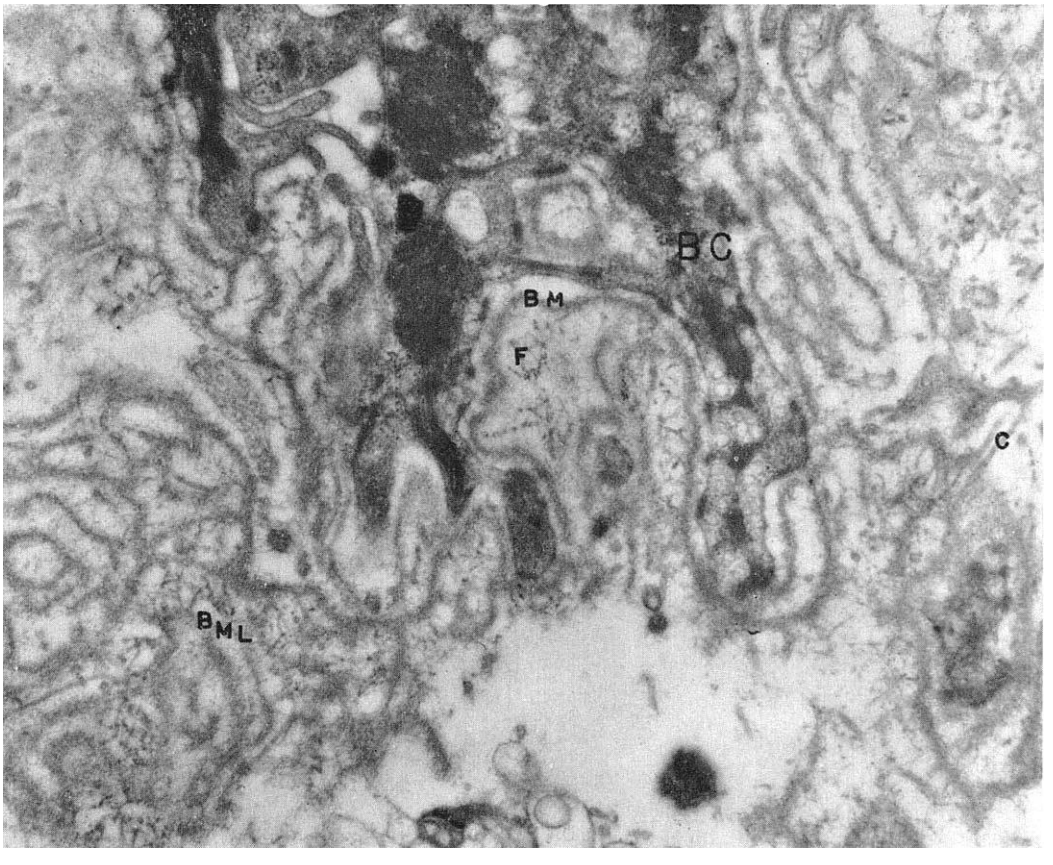

FIG. 23. *Porphyria cutanea tarda.* Blister. Epidermal side. Note the relatively intact basal cell (BC) and the basement membrane (BM) banded filaments (F) and basement membrane—like (BML) material. Few collagen fibrils (C) are present. Approximately 24,400 ×.

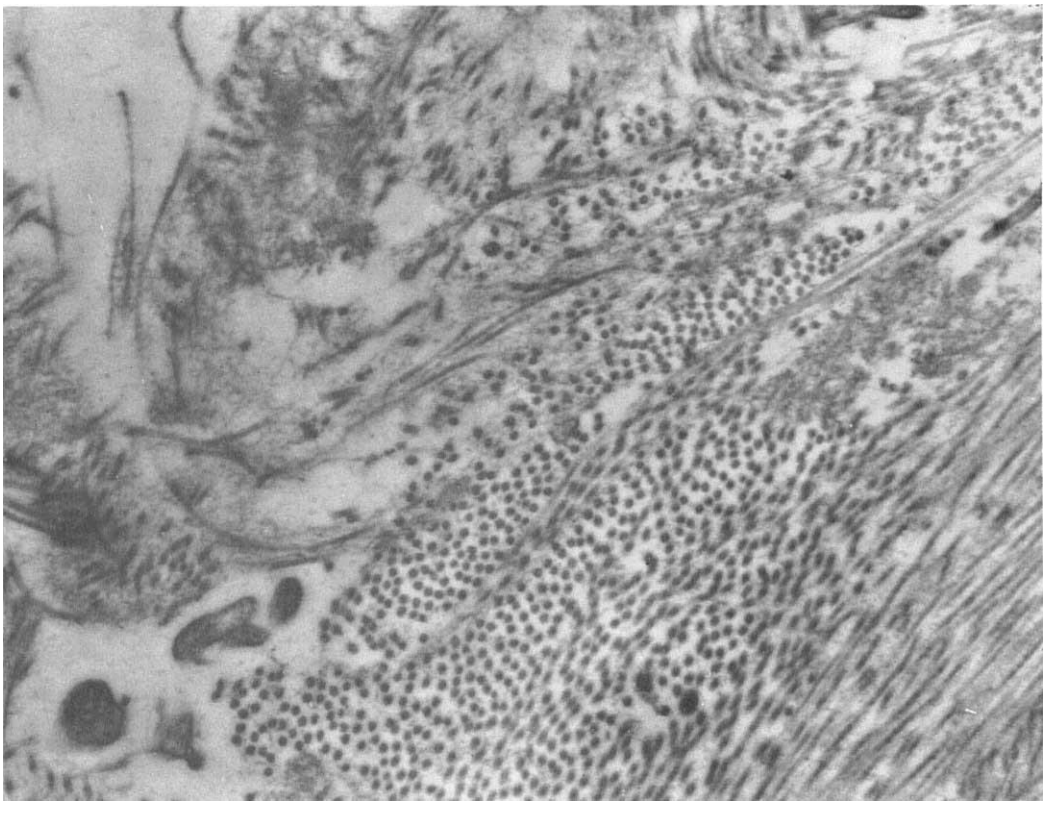

FIG. 24. *Porphyria cutanea tarda*. Upper dermis. Area of transition between degenerative connective tissue and relatively normal connective tissue. Note the many fine filaments in the degenerative areas and the wide spacing between collagen fibrils. Approximately 14,640 ×.

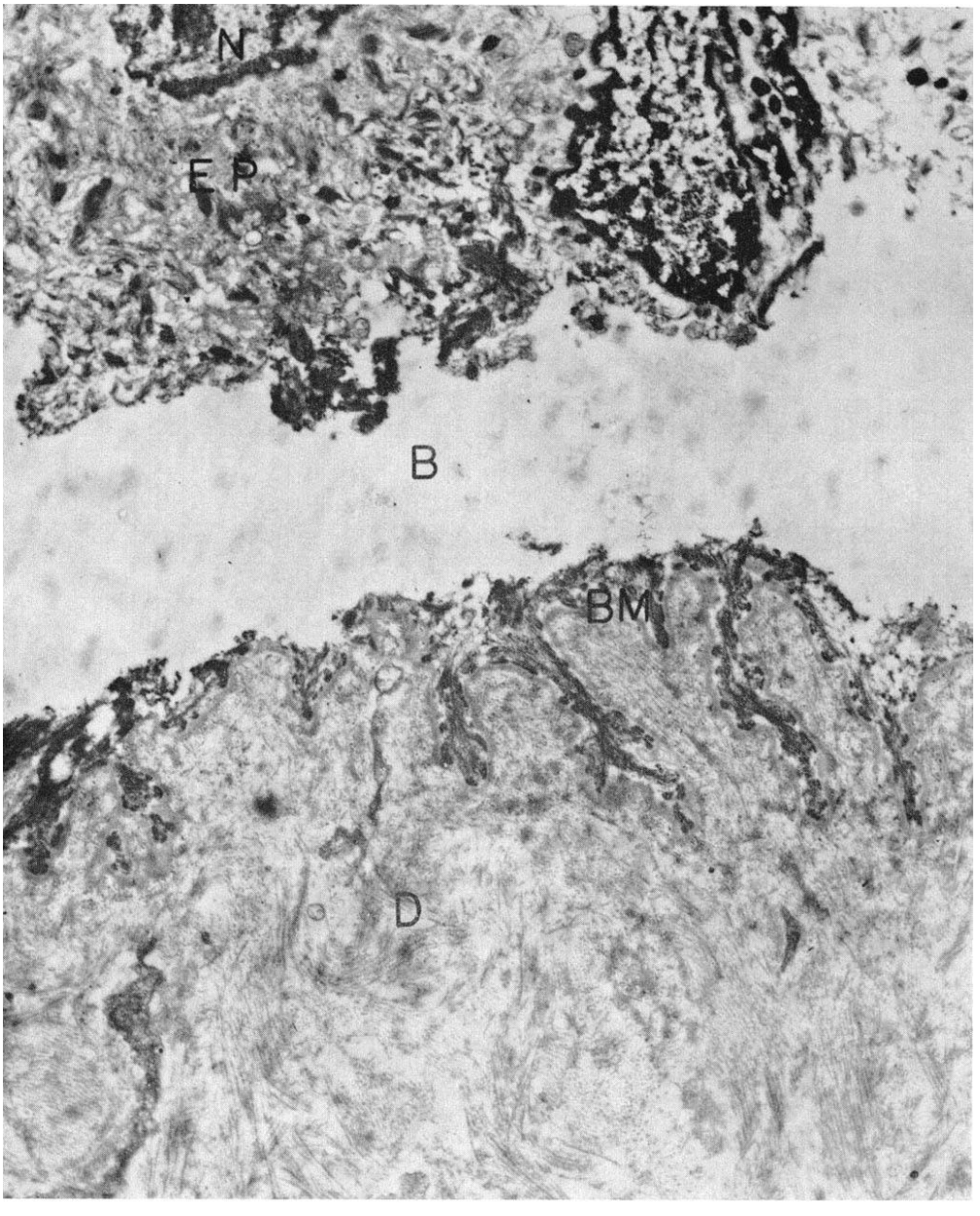

FIG. 25. *Epidermolysis bullosa simplex*. The region of the D–E junction. An 8 minute old, fully developed blister. Note that cleavage has occurred within the basal cells of the epidermis (EP). On the dermal side of the blister intact cytoplasmic projections of the basal cells remain in their usual relationship to the dermis (D). The basement membrane is intact (BM). Approximately 7,600 ×.

after porphyrin excretion and/or UVL exposure is decreased. If it is possible to induce blister formation by UVL alone or by UVL plus infrared irradiation, as recently reported by Runge and Watson (10) it would be of interest to know whether these blisters are pathologically similar to trauma induced blisters. It is reasonable to assume that all successful methods of blister formation induction in porphyria act directly or indirectly on the same substrate, adding "insult to injury". *In vitro* experiments aimed at testing this hypothesis are being conducted.

SUMMARY

Blisters in epidermolysis bullosa hereditaria letalis, epididermolysis bullosa dystrophica (recessive type), and porphyria cutanea tarda all show grossly dermal-epidermal separation by light microscope examination. Electron microscope examination reveals distinct pathologic features in each disease. These pathologic features also allow the three conditions to be easily differentiated from a previously studied disease in the group, epidermolysis bullosa simplex. Pathogenetic hypotheses based on these data indicate certain areas of study likely to be fruitful.

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