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DESMOSOMES IN PEMPHIGUS VEGETANS[1]

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Abstract. Desmosomes of a patient suffering from pemphigus vegetans were studied by electron microscopy and analysed by morphometry. The desmosomes were fewer in the patient's skin than in healthy individuals. The desmosomes of the patient were thin and short, showing pathological ultrastructures, viz. lack of median lines, single-sided attachment plaques and direct cell contact with some thickened cell membranes. These desmosomal changes were more manifest in the involved than in the uninvolved skin. The changes suggest deterioration or defective formation of desmosomes in pemphigus vegetans.

Pemphigus vegetans is a variant of pemphigus vulgaris. However, acantholytic cells characteristic of pemphigus vulgaris have not been found regularly in acanthotic rete ridges of this variant (11). The problem of whether or not the desmosomes in such areas would show a normal ultrastructure has remained unsolved hitherto.

MATERIAL AND METHODS

Patient. A 46-year-old female. Over the last 6 years the patient has suffered from brownish papillomatous eruptions in her axillae and genitofemoral regions. The mucous membrane of her oral cavity was reddish edematous. No blisters could be found. Peripheral blood showed eosinophilia. Skin biopsy of the femoral region showed acanthosis, with intra-epidermal abscesses of eosinophilic granulocytes and infiltrates of eosinophiles in the dermal papillae. Immunofluorescence microscopy showed intercellular precipitants of IgG and C_3 in the epidermis. Biopsy material for electron microscopy was obtained from the same area of the femoral region as for light microscopy and from normal skin on the extensor surface of the right upper arm.

Preparation for electron microscopy. Skin biopsy material was removed from the papillomatous eruption in her groin and from normal skin on the right upper arm. The specimens were fixed in a 6% glutaraldehyde solution of 0.2 mol cacodylate buffer, pH 7.2, with 7.5% sucrose. The specimens were then osmicated, dehydrated and embedded in Epon 812 *ad modum* Luft (12). Ultrathin sections were cut from acanthotic rete ridges in which neither eosinophilic granulocytes nor acantholytic keratinocytes had been found and from normal epidermis of uninvolved skin, by means of a Reichert ultramicrotome (Ultracut). The sections were stained with uranyl acetate and lead citrate and studied with a JEOL electron microscope 100 CX at 80 kV.

Morphometry of desmosomes. The desmosomes and semidesmosomes were measured for thickness and length. The thickness is the distance between both outer leaflets of the two cell membranes in the desmosome and, for semidesmosomes, the distance between the junction plate and the outer cell membrane. The distance was measured at the middle portions of the desmosomes. The length of both desmosomes and semidesmosomes was the length of the attachment plaques (Fig. 6). Desmosomes and semidesmosomes were enlarged ×60 000 for study. Using ×15 000 enlarged electron micrographs, the length of the desmosome-covered cell surface per unit length of cell surface in sections was also estimated.

The results were compared with the values obtained from biopsies of the breasts of 5 healthy middle-aged women studied by Hino et al. (10) (Figs. 7, 8, 9).

OBSERVATIONS

Involved skin

In the basal and lower Malpighian cell layers, keratinocytes showed numerous ribosomes, granular endoplasmic reticulum and mitochondria. A few tonofilament bundles were distributed irregularly in the cytoplasm (Fig. 1). Desmosomes showed abnormal ultrastructures (Figs. 2, 3), *viz.* (1) distinct attachment plaques with varying amounts of tonofilaments; (2) lack of median lines; (3) some desmosomes showing single-sided attachment plaques, while the opposite side was cell membrane with or without slight thickening of its inner leaflet; (4) simple contact of cell membranes. Semidesmosomes showed distinct substructures (Fig. 2), though some lacked the junction plates. In some places the basal lamina was interrupted by small pseudopods of basal cells. Anchoring fibrils appeared blurred.

In the upper Malpighian and granular cell layers, keratinocytes contained well formed attachment plaques with associated distinct tonofilament bun-

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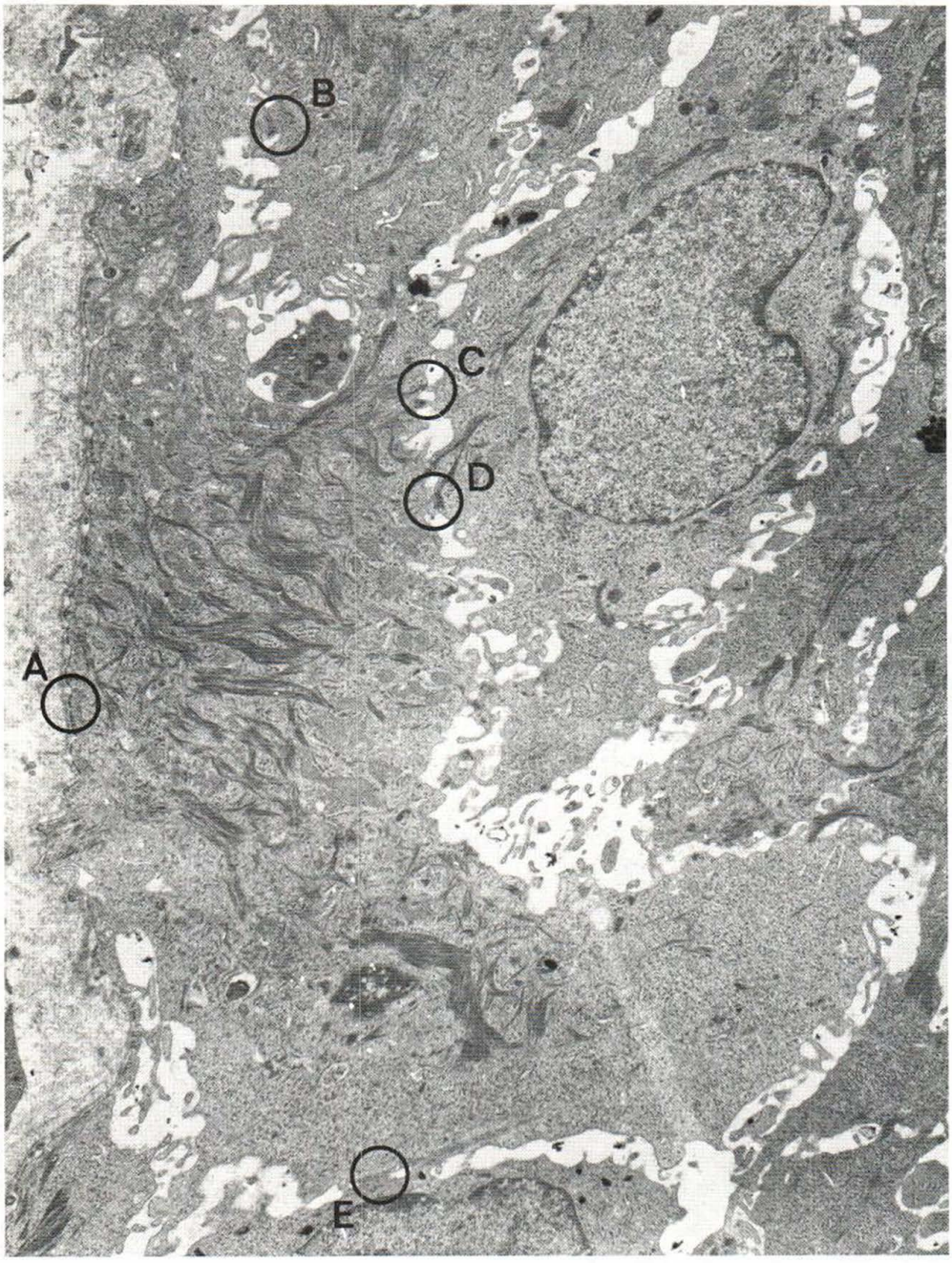


Fig. 1. Keratinocytes in basal and lower Malpighian cel layers of involved skin of the patient. Desmosomes and semidesmosomes within the circles, in alphabetical orde as in Fig. 2. ×6000.

dles (Fig. 4). Most of the desmosomes of these layers demonstrated no or, occasionally, dotted median lines (Figs. 4, 10). Tight junctions were also found. Keratinosomes and keratohyalin granules were seen in the granular cell layers.

Uninvolved skin

In the basal and lower Malpighian cell layers, keratinocytes showed cluping tonofilaments, mitochondria, ribosomes and endoplasmic reticulum. The cell surface exhibited numerous vil-

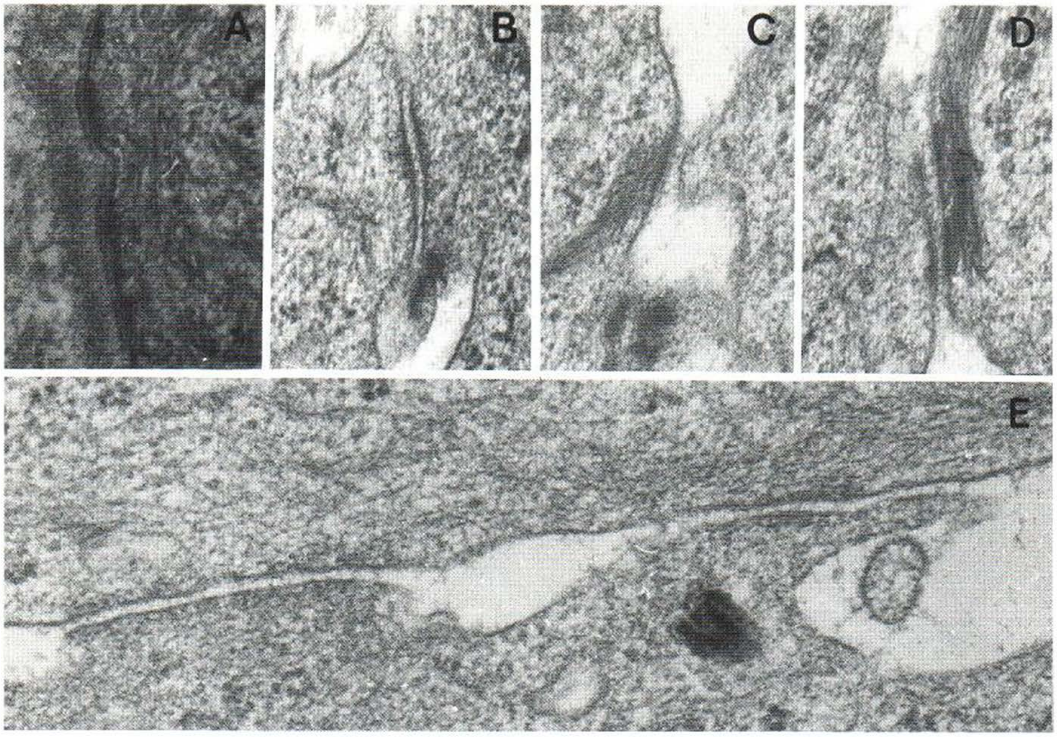


Fig. 2. (*A*) Complete semidesmosomes. (B–E) Incomplete desmosomes without median lines. (B) Cell membrane thickening with poor tonofilament attachment. (C) Single sided attachment plaque of a desmosome. The section was tilted at 50 degrees at right angles to the long axis of the desmosome. (D) Attachment plaque and tonofilaments are distinct on the right side while poor on the left side. Double layers of the cell membrane are clearly seen in both sides. (E) Desmosomes show faint attachment plaques. ×60 000.

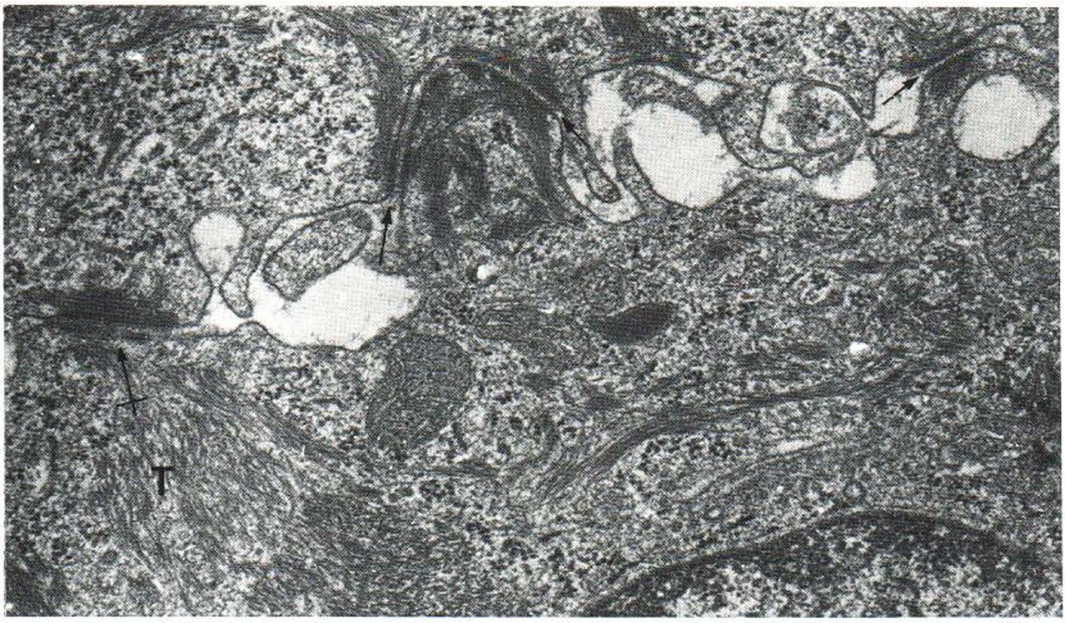


Fig. 3. Keratinocytes of the lower Malpighian cell layers of involved skin of the patient. *T* indicates rough bundles of tonofilaments. Arrows indicate desmosomes without median lines. A desmosome indicated by an arrow with a cross, shows an attachmant plaque on one side only, with poor tonofilaments. ×60 000.

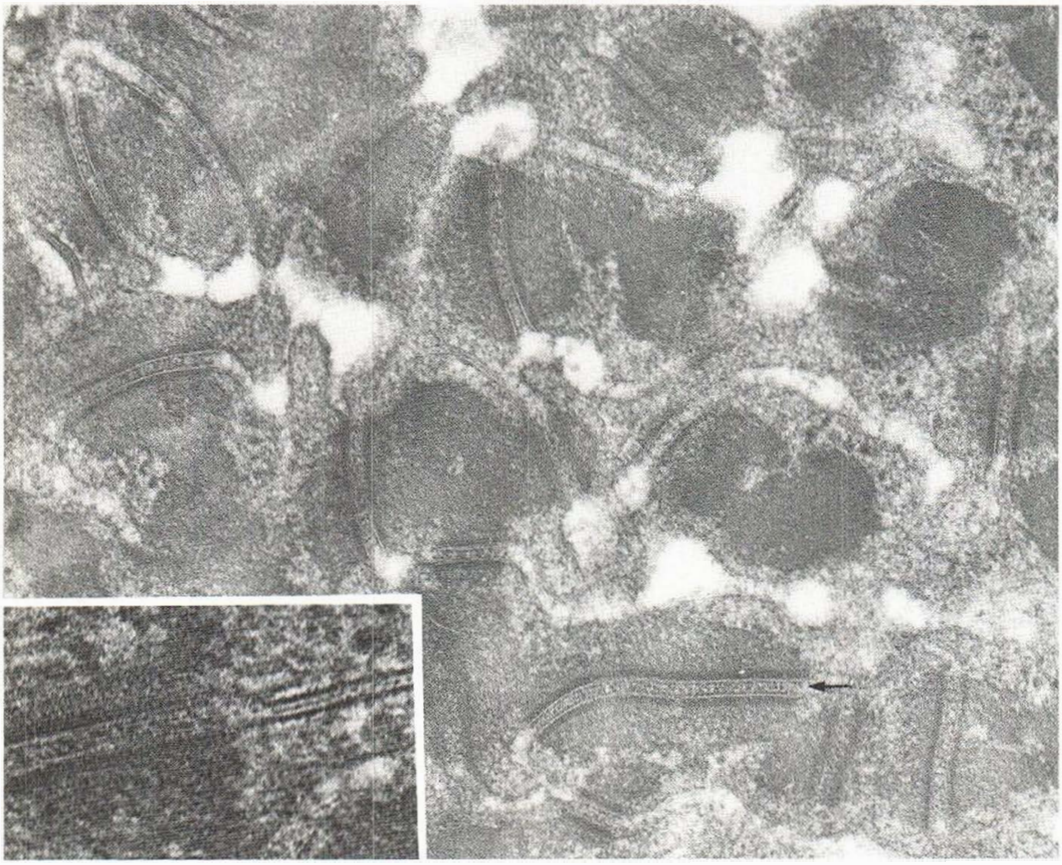


Fig. 4. Keratinocytes of the outer Malpighian cell layer. Most desmosomes have no median lines, though attachment plaques are distinct. An arrow indicates dotted median line. ×60 000. *Inset.* Gap junction shows distinct median line. ×120 000.

lus-like protrusions which occasionally showed short desmosomes on their tops (Fig. 5). The desmosomes showed distinct attachment plaques but median lines were lacking (Fig. 10). Semidesmosomes were numerous, showing normal ultrastructures. No abnormal desmosomes with single-sided attachment plaques were found.

In the outer Malpighian and granular cell layers, the desmosomes appeared larger in the most superficial layers, some showing indistinct median lines.

Morphometrical studies

The desmosomes were thinner in the involved areas than those of the uninvolved areas (Fig. 7). The desmosomes of the patient were also thinner than those of the healthy individuals (Fig. 7) (10). The thicknesses varied widely in the basal and lower Malpighian cell layers of the involved areas. The semidesmosomes were the thinnest in the involved skin, but the differences were not so great as in the desmosomes. Both the desmosomes and the semidesmosomes were shorter in the patient than in the healthy individual (Fig. 8) (10). The length of the cell area of desmosomal coverage was smaller in the patient than in the normal individuals. The desmosomal contact was the shortest in the lower Malpighian cell layers of involved skin (Fig. 9).

DISCUSSION

Acantholysis is a phenomenon in which keratinocytes become free after detaching desmosomes. In pemphigus vulgaris (4, 5, 7, 15) and vegetans (7, 8), acantholytic keratinocytes show small indistinct attachment plaques of the detached desmosmes. Braun-Falco and Vogell (3, 4) described decreased

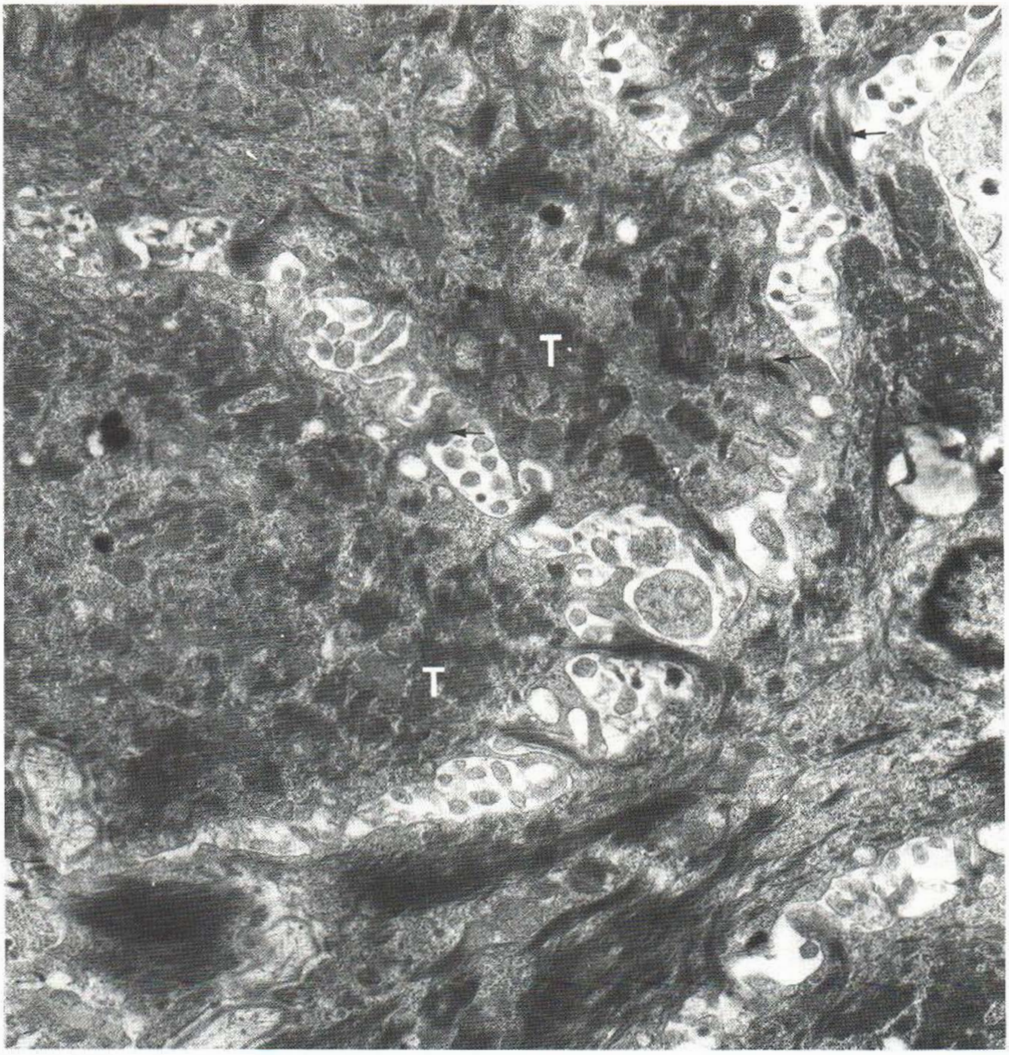


Fig. 5. Basal and lower Malpighian cell layers of uninvolved skin. Tonofilament bundles (*T*) appear more distinct than those of involved skin. A few desmosomes (*arrows*) and numerous villi are seen in the intercellular spaces. No median lines of the desmosomes are seen. ×15 000.

numbers of desmosomes and their simple ultrastructures, i.e. thickened cell membrane in the lesions as well as in the uninvolved skin of pemphigus vulgaris patients. Morphometry of the present study demonstrated a definite deterioration of desmosmomal connection in both involved and uninvolved skin of a pemphigus vegetans patient.

Wilgram (15) maintained that detachment of tonofilaments from the attachment plaques was the

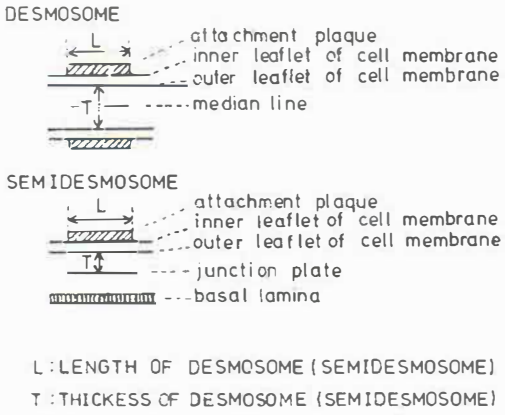
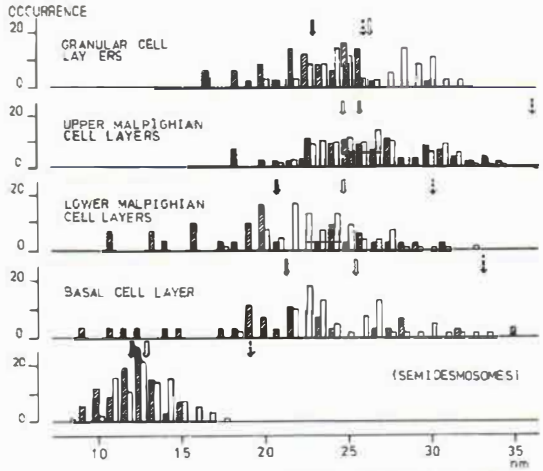


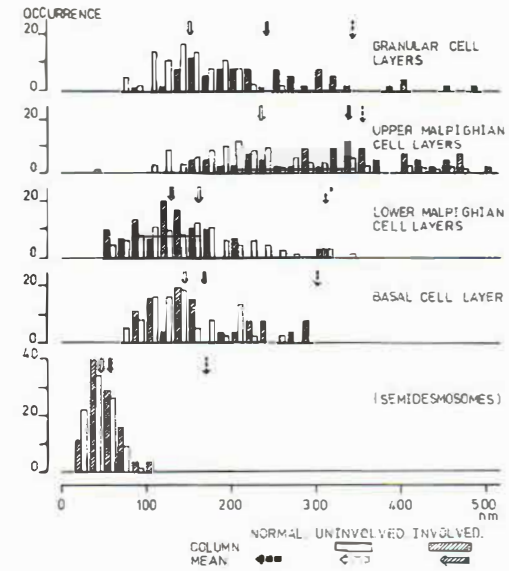
Fig. 6. Length and thickness of desmosomes and semidesmosomes.



	Normal Skin		Uninvolved Skin		Involved Skin
Granular Cells	26.0± 10.0 (145)	≠	26.3± 3.0 (35)	>	22.8± 2.8 (49)
Upper Malp. Cells	36.0± 7.3 (127)	>	24.7± 2.8 (110)	≠	25.7± 3.5 (45)
Lower Malp. Cells	30.0± 8.0 (57)	>	24.7± 2.8 (65)	>	20.7± 5.3 (31)
Basal Cells	33.0± 9.3 (97)	>	25.3± 3.5 (37)	>	20.8± 6.3 (26)
(Semidesmosomes)	19.0± 4.7 (232)	>	13.0± 1.9 (90)	>	12.0± 1.5 (66)

Mean ± Standard Deviation (nm.) (Numbers). P<0.05
≷ Significant Difference. ≠ Insignificant Difference.

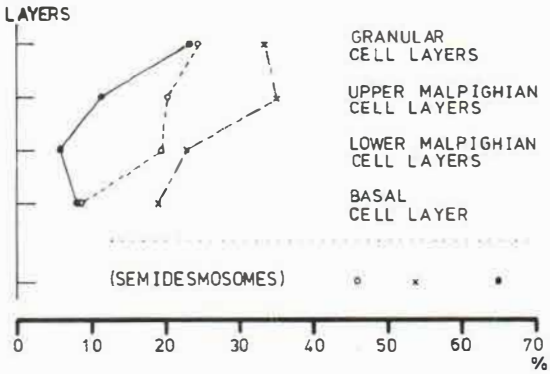
Fig. 7. Thickness of desmosomes.



	Normal Skin		Uninvolved Skin		Involved Skin
Granular Cells	344.0±129.0 (145)	>	153.7± 42.5 (35)	<	242.2± 89.5 (49)
Upper Malp. Cells	354.0±105.0 (127)	>	238.9± 87.8 (110)	<	338.3±107.3 (45)
Lower Malp. Cells	311.0±104.0 (57)	>	160.5± 65.2 (65)	>	130.3± 50.3 (31)
Basal Cells	301.0±114.0 (97)	>	143.8± 44.6 (37)	≠	168.3± 60.3 (26)
(Semidesmosomes)	171.0± 63.0 (232)	>	47.7± 15.0 (90)	<	53.2± 18.5 (66)

Mean ± Standard Deviation (nm.) (Numbers). P<0.05
≷ Significant Difference. ≠ Insignificant Difference.

Fig. 8. Length of desmosomes.



	INVOLVED.		UNINVOLVED.		NORMAL SKIN
GRANULAR CELL LAYERS	23.4	≠	24.6	≠	33.5
UPPER MAL. CELL LAYERS	11.4	<	20.4	<	35.5
LOWER MAL. CELL LAYERS	5.7	<	19.5	≠	22.8
BASAL CELL LAYER	8.2	≠	8.7	<	19.1
(SEMI-DESMOSOMES)	65.1	≠	46.0	≠	53.8

$$X = \frac{\text{Total Length of Desmosomal Length}}{\text{Total Length of Cell membrane}} \times 100\ (\%)$$

≷ Significant Dif. ≠ Insignificant Dif. (P<0.05)

Fig. 9. Desomosmes and cell membrane.

Cell layers	Desmosomes without median lines (%)	Desmosomes with dotted median lines (%)	Desmosomes with linear median lines (%)
Uninvolved skin:			
Granular cell layers	57.1	42.9	-
Upper Malpighian cell layers	55.4	24.6	20.0
Lower Malpighian cell layers	73.8	21.6	4.6
Basal cell layer	91.9	5.4	2.7
Involved skin:			
Granular cell layers	83.7	16.3	-
The other cell layers	100.0	-	-

Fig. 10. Median lines of desmosomes.

first step in the changes. Desmosomes then gradually disappeared. Hashimoto et al. (9) reported that dissolution of the extracellular cement of polysaccharide nature was the main event in the pathogenesis. Attachment plaques and tonofilaments are normal in the early stage of the pathological process. However, the present results of morphometry and abnormal ultrastructures of the desmosomes indicated that formation of desmosomes

was influenced by unknown factors in both involved and uninvolved skin of the patient. Lack of median lines of desmosomes is a striking sign of the defective desmosomes.

Serum globulin of pemphigus vulgaris patients can bind to epithelial cell membranes (6) the pemphigus antigen being in the keratinocyte cell membrane (13). Previous investigators have produced single-sided desmosomes, identical with those found in the present study, by organ culture of rhesus monkey skin in medium which was supplemented with serum of a patient with pemphigus vulgaris (1, 2). Serum IgG of a pemphigus patient has also been added to an organ culture of human skin (14) and acantholytic changes were produced in the epidermis. These results suggested that serum IgG of a pemphigus patient might be one of the factors inhibiting desmosome formation.

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