AN ELECTRON MICROSCOPIC STUDY OF ACANTHOLYSIS AND DYSKERATOSIS IN PEMPHIGUS FOLIACEUS

WITH A SPECIAL NOTE ON PECULIAR INTRACYTOPLASMIC BODIES*

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Our interest in pemphigus foliaceus stems from previous electron microscopic studies of ancantholysis in pemphigus vulgaris (1) and of dyskeratosis and acantholysis in Hailey-Hailey's disease (2) and in Darier's disease (3). It was felt that a detailed study of the behavior of the desmosomes and the tonofilaments in pemphigus foliaceus might strengthen the concept that the desmosomes serve an important function in the process of keratinization (4).

This concept is based on the following observation: Desmosomes are present in all strata of the epidermis, including the horny layer, although their ultrastructure varies slightly in different layers of the epidermis (Fig 1, 1b and 1c). It was suggested that this persistence of the desmosomes up to the horny layer was not incidental, but served a special purpose. It was also postulated that the desmosomes not only serve as points of cohesion between epidermal cells but also function as points of anchorage for the tonofilaments which are widely accepted to be the precursors of keratin (5). Thus the desmosomes may provide orientation for the tonofilaments in the epidermal cells during their passage from the basal to the horny layer. And it is felt that proper spatial orientation of the tonofilaments is necessary for normal keratinization to take place. This concept formulated on observations on normal skin was then strengthened by investigations on the so-called acantholytic conditions, in which a loss of desmosomes and thereby a loss of their orienting function for the tonofilaments lead to errors in keratinization. In pemphigus vulgaris, a severe necrotizing injury of unknown etiology leads to complete destruction of the tonofilaments with ensuing loss of desmosomes. Death of epidermal cells follows. Therefore, keratinization could not proceed (1). Dyskeratosis in pemphigus vulgaris is very rare and was observed only in cases that had been treated with corticosteroids. In Hailey-Hailey's disease and in Darier's disease, the destructive changes in the epidermal cells are much less severe and the tonofilament-desmosome complex seems to be selectively altered—presumably by a genetic defect (2, 3). Whether in these two genetic disorders the tonofilaments are separated from their corresponding desmosomes, or fail to connect with them during their initial synthesis or whether both mechanisms prevail is not established. Whatever the etiology of the tonofilament-desmosome alteration, the tonofilaments are deprived of proper orientation by their desmosomes and alterations in keratinization ensue.

It seemed not unlikely that the *morphologic* changes in the epidermal cells in pemphigus foliaceus should lie in between the severe, generally destructive changes in pemphigus vulgaris and the much milder selective lesions in Hailey-Hailey's disease and Darier's disease: this indeed is the case, as will be shown in this publication.

In the course of the investigations designed to explore these possibilities, peculiar intracytoplasmic bodies were observed in epidermal cells. They will be described in detail and possible interpretations will be given.

MATERIAL AND METHODS

Seven patients with generalized pemphigus foliaceus were examined. Three of them were middle-aged females, one was an elderly woman, two were young men in their thirties, and one patient was a male teenager. No patients with the Senear-Usher type of pemphigus erythematosus were examined in this series since it was deemed advisable to investigate them separately. Biopsies were performed on all these patients during the active state of their eruption. Only small blisters measuring not more than 2 mm in diameter were taken by punch biopsy from several different locations, under minimal novocaine anesthesia. An attempt was made to select lesions which with reasonable

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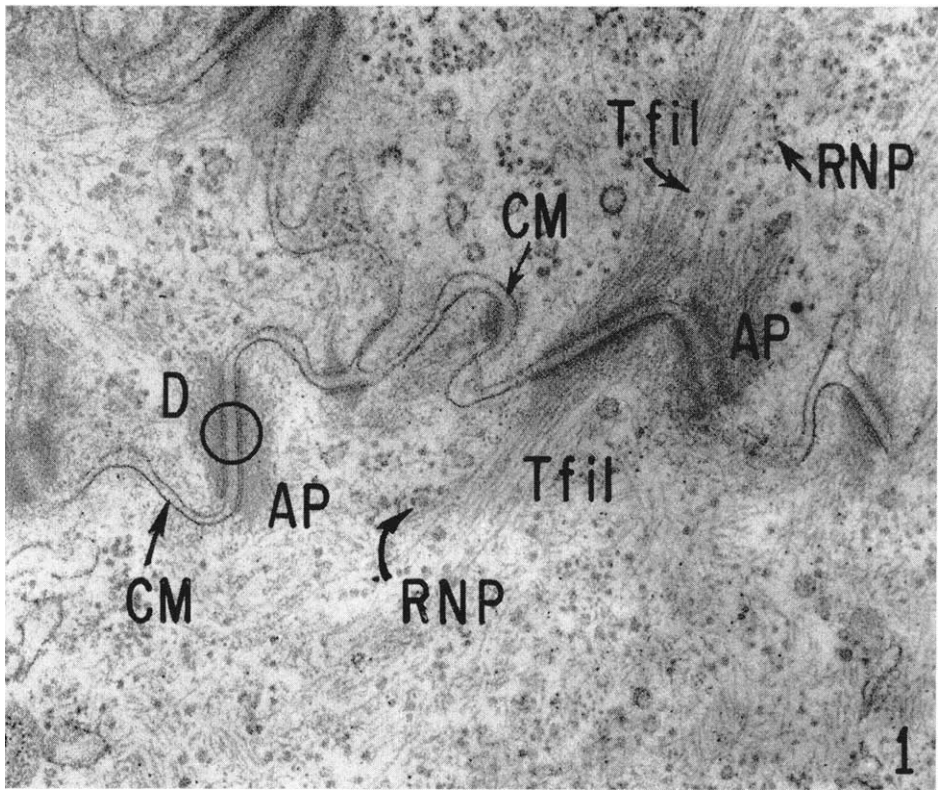

FIG. 1.A. Three epidermal cells in the lower Malpighian layer with several desmosomes (D) are shown. Tonofilaments (Tfil) "sprout" from these desmosomes at the attachment plate (AP). The desmosomes are seen in continuity with the cell membrane (CM). RNP particles are in close association with tonofilaments. Epon × 45000.

had been placed on therapy just one or two days prior to the removal of biopsy specimens.

The biopsy specimens were fixed in buffered 1% osmium-tetroxide with sucrose for one hour and a half. Half the biopsy material was embedded in prepolymerized n-butyl-methacrylate with 1% Luperco. The other half of each specimen was processed in Epon as recommended by Luft (6) or Parsons (7). Sections were cut on a Porter-Blum microtome with diamond knives and after mounting on carbon-coated grids were stained with either uranyl acetate or lead citrate. Pictures were taken on a Siemens elmiscope.

RESULTS

When early lesions of pemphigus foliaceus were examined by electron microscopy, the dermis appeared unchanged. The basement membrane was intact (Fig. 2). The basal cells with the aid of the so-called junction granules adhered to the basement membrane in a normal fashion. The nucleus and the cytoplasm of the basal cells, however, often showed early changes that varied from specimen to specimen. Sometimes there was mild crenation in the nucleus, perinuclear retraction of the endoplasmic reticulum and swelling of the mitochondria (Fig. 2). Frequently the tonofilaments were separated from the attachment plate of their corresponding desmosomes (Figs. 3 and 4). The desmosomes, however, were usually present in the basal cell region, and were also distincly visible in the lower Malpighian area although the epidermal cell changes described above seemed more pronounced (Fig. 5). These mild changes in the basal cells and the lower Malpighian cells became more pronounced in the upper Malpighian stratum and in the sub-corneal layer.

In the upper Malpighian region of involved areas, one could frequently observe on many cell surfaces a diminished number of desmosomes and in some instances complete loss of desmosomes producing acantholytic cells which either floated freely or formed rows of detached

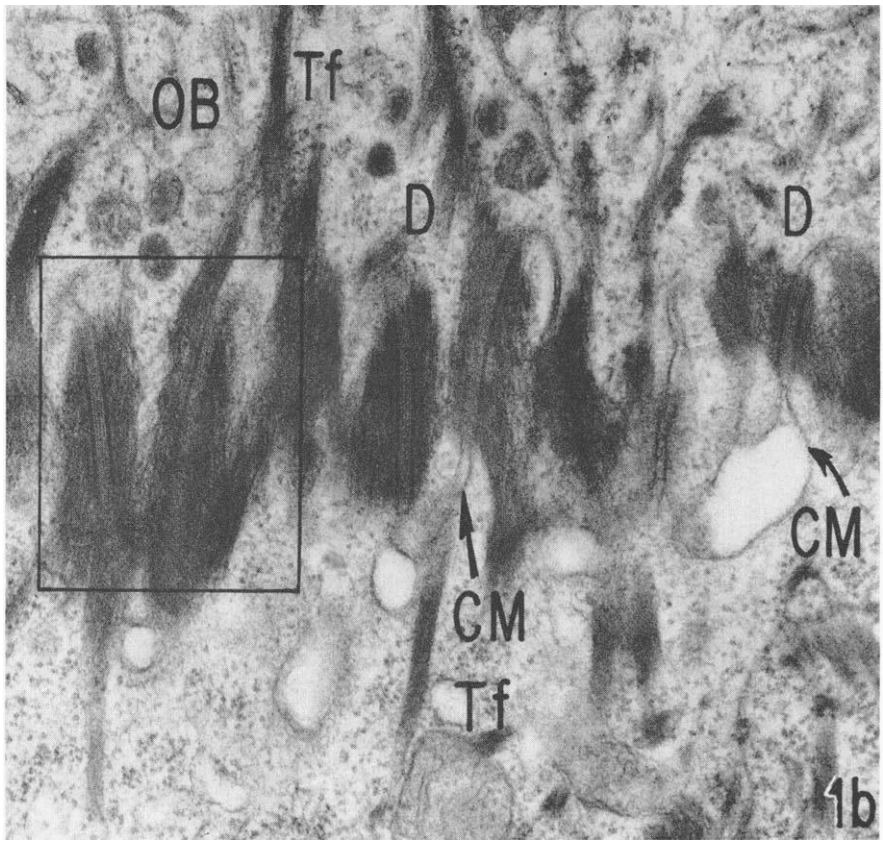

FIG. 1.B. Several desmosomes can be seen in the upper epidermal strata in this high power electron micrograph. Odland bodies in the upper portion are indicative of the lower granular layer. Tonofilaments are seen streaming from the attachment plate (AP) of the desmosomes. Two densely osmophilic lines and three fine interdesmosomal lines can clearly be distinguished in several of these desmosomes. The desmosome surrounded by a rectangular drawing is illustrated under still higher power in Figure 1C. Epon × 30000.

epidermal cells (Fig. 6). This loss of desmosomes appears to have been preceded by retraction of the tonofilaments from their attachment plate (Fig. 7). The tonofilaments which had separated from their corresponding desmosomes at the basal layer were discernible higher up in the Malpighian layer in packets (tonofibrils) without their usual linear arrangement, and without their proper orientation: Consequently they appeared as an irregular network of frequently interwoven packets, representing dyskeratosis (Fig. 8). Keratohyaline granules were present in many of these dyskeratotic areas. Tonofilaments were seen to be associated with them and to be coursing through them in an apparently normal fashion. Odland bodies were also present in the upper granular area and appeared to be similar on morphological grounds to the ones seen in normal skin (8, [Fig. 9]). The nuclei showed crenation with perinuclear halo formation due to retraction of the endoplasmic reticulum. The mitochondria were either swollen, contracted or partially dissolved. Complete epidermal cell degeneration, was however, only rarely observed.

Another notable finding in all the cases of pemphigus foliaceus which were examined was the presence of round bodies about 2000 Angstroms in diameter with a distinct external membrane and a definite fine sub-vesicular internal structure (Figs. 2, 3, 5 and 7). These bodies were seen in the cytoplasm but never within the nuclei. They could not be found with certainty outside the epidermal cells. They could be seen most frequently adjacent to acantholytic areas but were observed on occa-

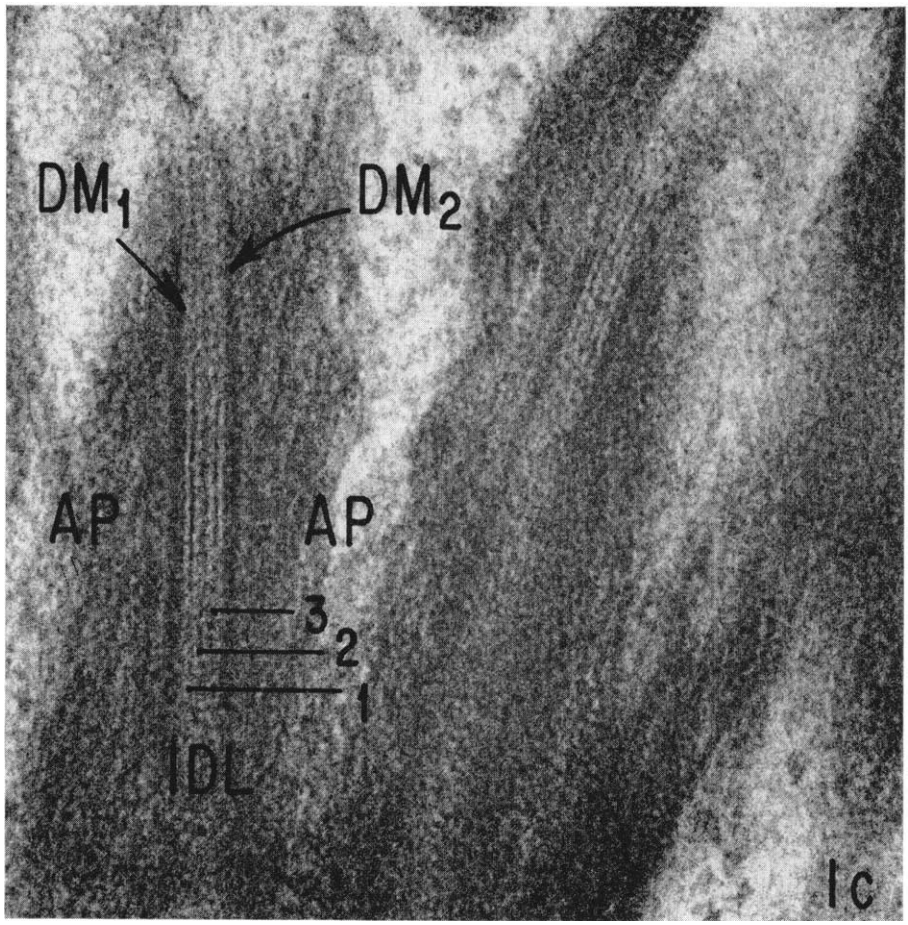

FIG. 1.C. The area in the rectangle in Figure 1B is shown here under highest magnification. Three fine interdesmosomal lines (IDL) can clearly be distinguished in the interdesmosomal substance. On both sides of these three fine lines is a heavily osmophilic dense double-layered membrane (DM) which forms the outer limitation of the attachment plate (AP). Epon × 100,000.

sion in epidermal cells close to the basement membrane. They were distinctly different in their structure from mitochondria (Figs. 2 and 5), and could also be distinguished from Odland bodies (8, [Fig. 9]) because they were larger and had a different internal structure. In this series of cases there was no unusual evidence of increased pinocytosis or transfer of any extracellular material to the interior of the cytoplasm in the form of lysosomes. On morphologic grounds, these peculiar bodies were comparable to vaccinia virus; the latter, however, tend to occur in much larger numbers (9).

DISCUSSION

Striking pathological changes in this series of cases of pemphigus foliaceus were confined to the epidermis. The earliest visible changes could be seen in basal cells. Marked changes appeared when the epidermal cells had reached the upper Malpighian or the sub-corneal strata. In the lower layers of the epidermis, cellular injury was mild and most desmosomes persisted. Despite the continued presence of the desmosomes, the arrangement of the tonofilaments was abnormal. They had begun to detach themselves from the attachment plates of the corresponding desmosomes. This finding is of importance because it bears out our previous observations that the first change in acantholysis appears to be detachment of the tonofilaments from the attachment plate of their corresponding desmosomes with subsequent disappearance of the desmosomes. The tonofilaments which had be-

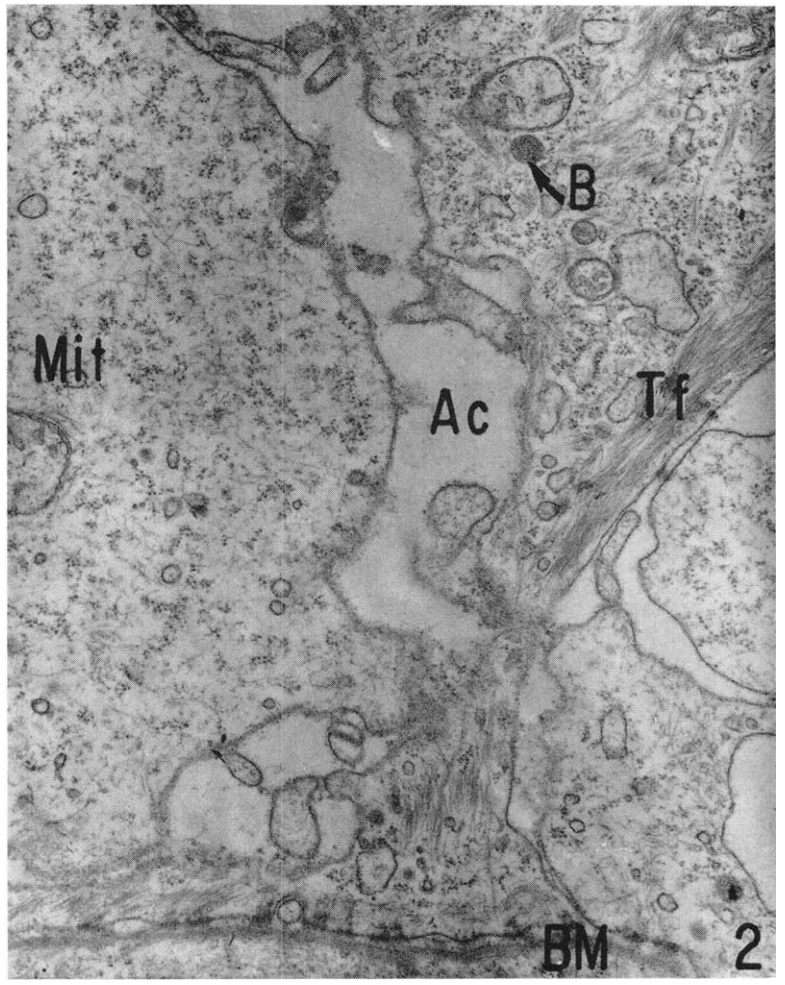

FIG. 2. Early acantholysis at the basal layer in pemphigus foliaceus. The basement membrane (BM) is intact and the basal cells are seen adhering to the basement membrane with the aid of the so-called junction granules. The desmosomes have largely disappeared in this particular area shown here and the tonofilaments have lost their orientation. The mitochondria (MIT) show signs of swelling particularly in the left cell shown on this electron micrograph. A peculiar intracytoplasmic body (B) can be seen in the upper right hand corner. Epon × 20000.

gun to retract in the lower Malpighian strata were arranged as irregularly interwoven packets of dyskeratotic material in the upper Malpighian region. In the dyskeratotic material, keratohyaline granules were present, and some of the disorderly arranged tonofilamentous packets were associated with them. Odland bodies seemed to be present in normal amounts. Accompanying these changes in the upper Malpighian area was a striking decrease or total loss of desmosomes. This led to acantholysis and the formation of lacunae in the upper strata of the epidermis. In the epidermal cells in this region there was usually retraction of the perinuclear cytoplasm resulting in halo formation. The mitochondria showed moderate changes by either swelling or contracting or even by partial dissolution. These morphologic alterations indicated mild cellular injury.

The epidermal abnormalities of pemphigus foliaceus seen by electronmicroscopy seem to be less severe than the destructive changes of pemphigus vulgaris (1) and more severe than the relatively mild selective lesions of Hailey-Hailey's disease (2) and Darier's disease (3). The dyskeratosis which follows the initial separation of the tonofilaments from their desmosomes is less striking than that seen in either Hailey-

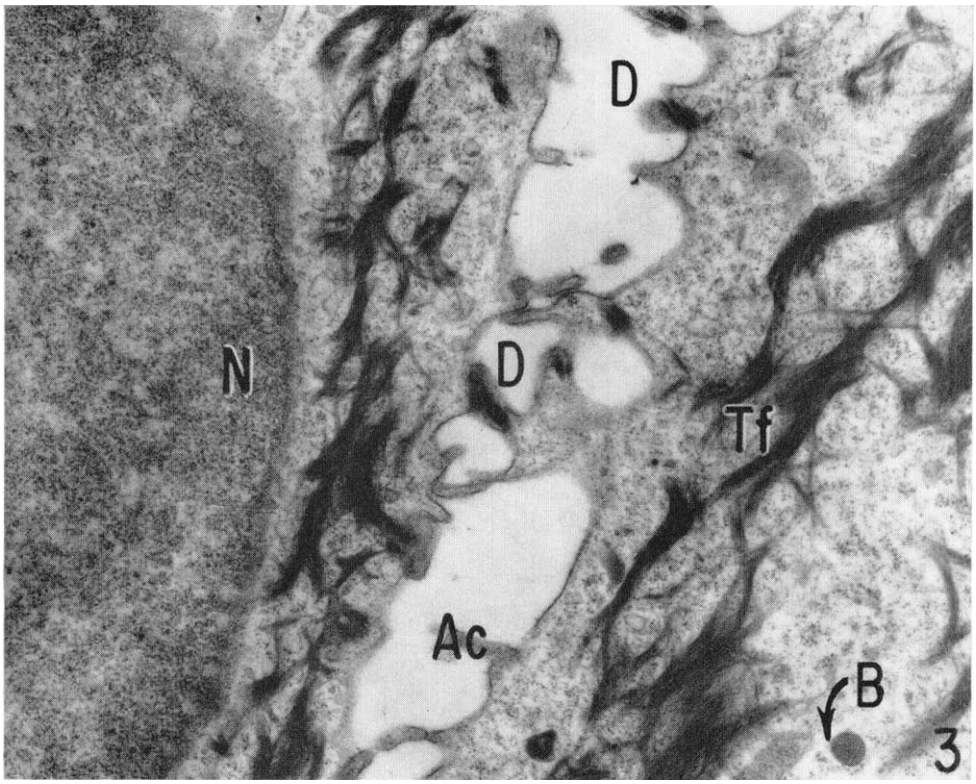

FIG. 3. Early acantholysis in the lower Malpighian layer. Two desmosomes (D) are seen persisting in this area. However, the tonofilaments (Tfil) have separated from their attachment plates and are seen as irregular aggregates of tonofilaments into tonofibrils. A peculiar intracytoplasmic body (B) is shown in the right lower corner of this photomicrograph. Epon × 20000.

Hailey's disease or Darier's disease, but nevertheless resembles it in certain ways, *i.e.*, the tonofilaments after separation from the desmosomes are no longer visible in an orderly oriented fashion in linear, discrete bundles, but appear as aggregates of packets in a disorderly arranged network.

In all four "acantholytic" conditions, acantholysis is due to loss of desmosomes which is apparently preceded by the above described alteration in the tonofilaments. The association of acantholysis and dyskeratosis can now be readily explained, because both phenomena are due to injury to the same subcellular structure, *e.g.*, the tonofilament-desmosome complex. A possible explanation for the detachment of the tonofilaments from their desmosomes and the subsequent loss of desmosomes will be given below.

The etiology and pathogenesis in all four so-called acantholytic diseases (pemphigus vulgaris, Hailey-Hailey's disease, Darier's disease, and pemphigus foliaceus) are most likely different. The pathologic alterations in the cytoplasm of the epidermal cells vary greatly in extent and severity. The changes in the tonofilament-desmosome complex, however, are morphologically closely related, and appear as the earliest epidermal cell alteration. This is actually quite predictable, since the tonofilament-desmosome complex is a highly organized structure and is thus readily susceptible to a great variety of injurious stimuli. And it appears that different injuries produce morphologically similar end results in this specialized structure (4), which lead to acantholysis and dyskeratosis. It is only in pemphigus vulgaris that acantholysis is seldom accompanied by dyskeratosis. The severe necrotizing injury in this disease apparently abolishes the viability

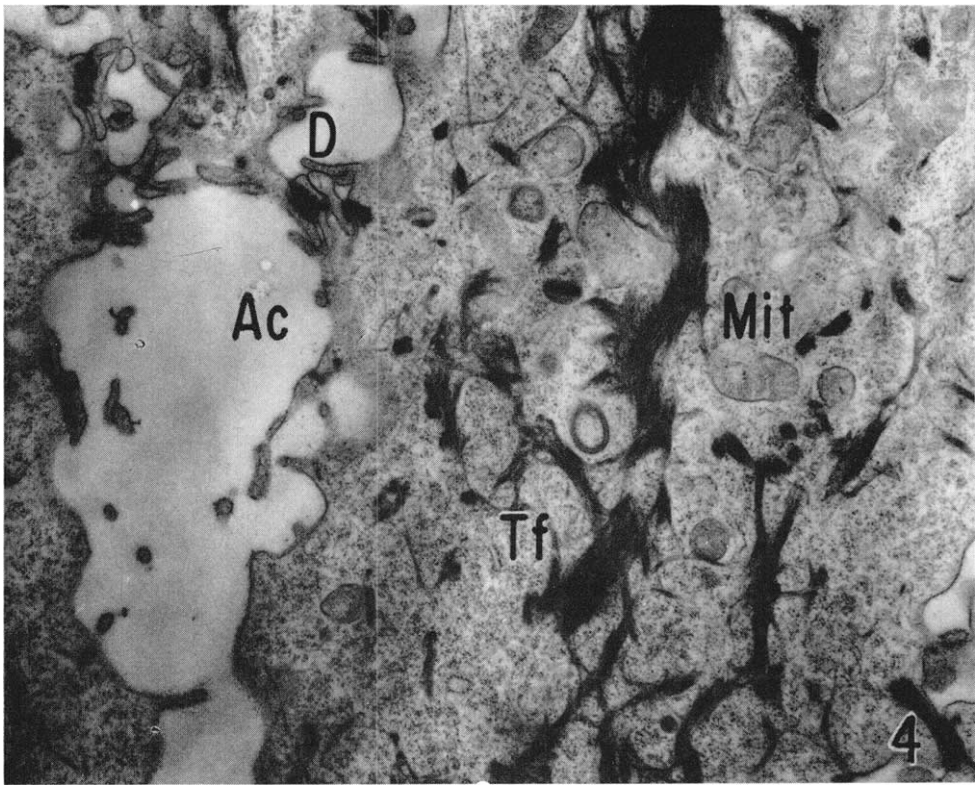

FIG. 4. Acantholysis in the lower Malpighian layer in pemphigus foliaceus. Two desmosomes (D) are still persisting. The tonofilaments, however, have entirely retracted from the cell surface and are shown in an irregular pattern throughout the cytoplasm of the epidermal cells. The mitochondria (MIT) show signs of mild injury. Epon × 16000.

of the epidermal cells which is necessary for keratinization to proceed. By contrast, in pemphigus foliaceus, the injury is not severe enough to destroy the viability of the epidermal cells and thus abolish keratinization altogether. Epidermal cell injury, however, seems to be severe enough to disrupt the tonofilament-desmosome complex to interfere with the normal orientation of the tonofilaments and to hinder their transformation into keratin. As a consequence of these alterations, acantholysis and dyskeratosis ensues.

The orienting function of the desmosomes for tonofilaments has also recently been shown by electronmicroscope studies on embryological epidermal cells (10, 11). First desmosomes appear at points of contact between epidermal cells and then tonofilaments "sprout" from the attachment plates of these newly formed desmosomes.

Interpretation of the peculiar intracytoplasmic bodies observed in this series of cases of pemphigus foliaceus

The peculiar bodies described under *Results* were seen in all seven patients examined in this series. One of the possible interpretations of these peculiar bodies is that they represent virus-structures. If these bodies were an infecting virus, they could easily cause sufficient injury to the epidermal cells to disrupt the highly specialized desmosome-tonofilament complex, but still permit the cell to survive. With the breakdown of the desmosome-tonofilament complex, acantholysis as well as dyskeratosis would ensue. It is obvious that electronmicroscopy can not demonstrate whether these peculiar bodies are in fact viruses; or if viruses, whether they are the cause of the disease. Proof of this sort can only be obtained by other types

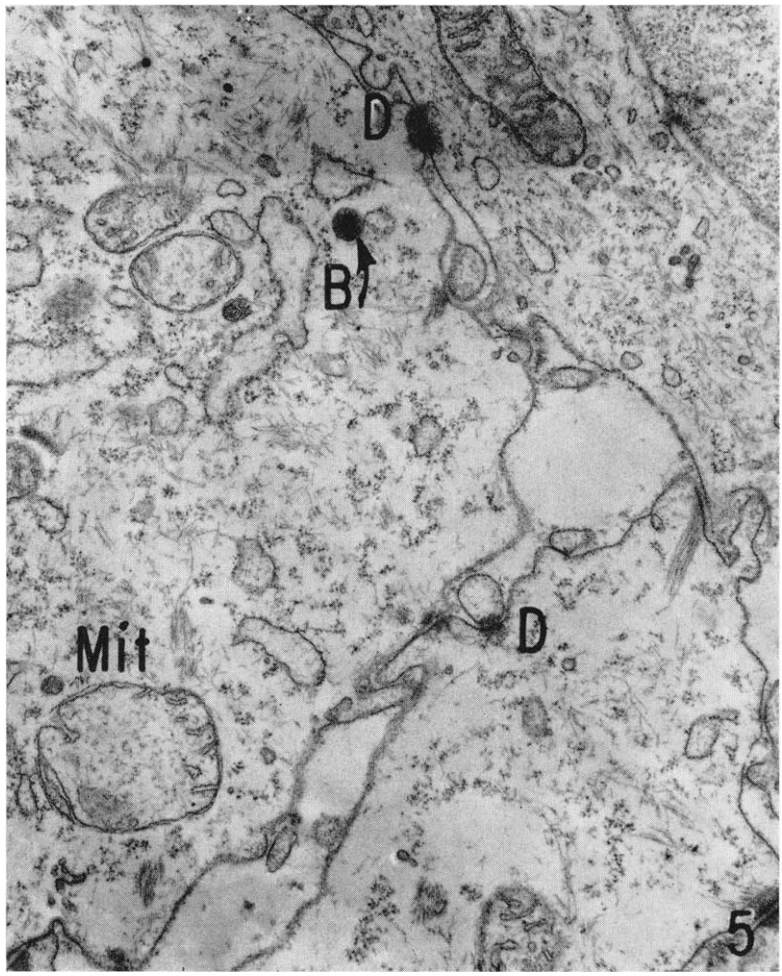

FIG. 5. This electron micrograph shows the earliest change in pemphigus foliaceus in an area that was more heavily involved than those shown in Figures 2–4. Desmosomes (D) are still seen persisting but tonofilaments do not connect with their attachment plates anymore. The tonofilaments are seen instead as irregularly oriented strands throughout the cytoplasm. This particular picture shows that desmosomes do persist while tonofilaments have retracted from them. Thus this picture tends to prove that tonofilamentous separation preceded disappearance of the desmosomes. In the right middle portion of this picture some tonofilaments are still seen inserting into an apparently intact desmosome. Mitochondria (MIT) show signs of definite swelling indicative of cellular injury. A peculiar intracytoplasmic body (B) is seen in the upper portion of this electron micrograph. Epon × 20000.

of technics. The arguments concerning the virus etiology of pemphigus vulgaris have been reviewed by Nasemann and Marchionini in 1957 (12, 13). In 1962, Crosti and co-workers reported that they had cultured a virus obtained from the skin of "pemphigus" (14). These authors did not differentiate between pemphigus vulgaris and pemphigus foliaceus. Furthermore, they reported finding a similar virus in patients with dermatitis herpetiformis and with erythema multiforme, thus weakening their argument. Yet the possibility that pemphigus foliaceus might be caused by a virus was made as early as 1931 by Urbach and Reiss (15). The finding of the present study should at least renew interest in the concept of the virus etiology, since these peculiar bodies were observed in all biopsy specimens taken from

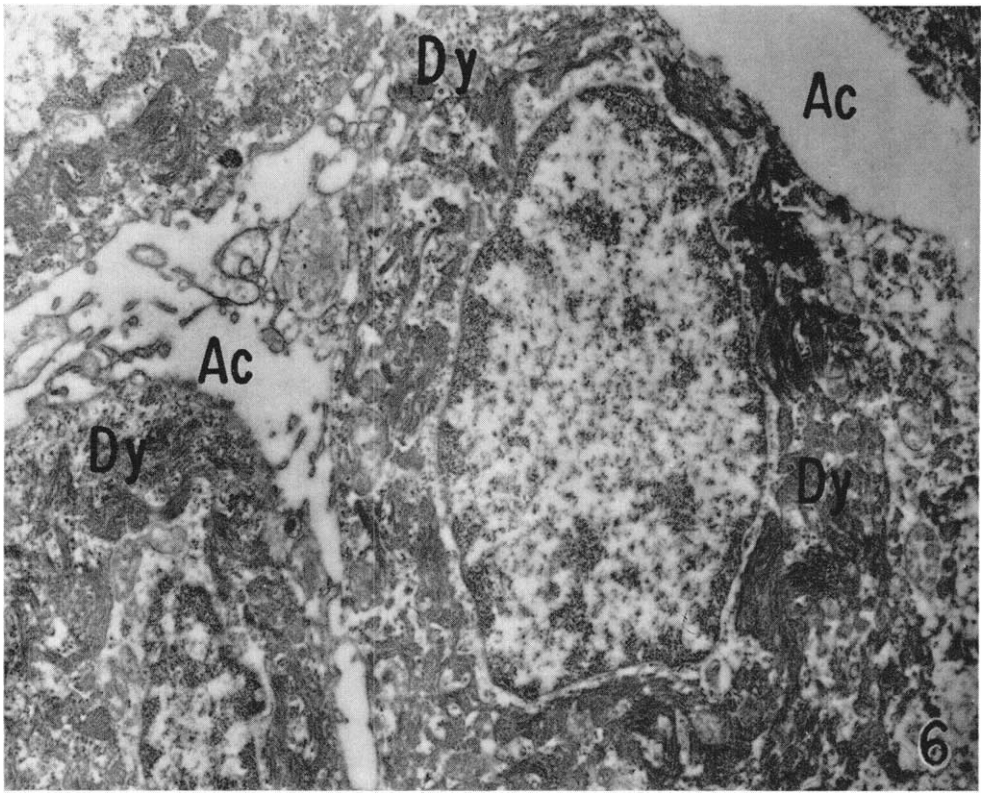

FIG. 6. An advanced state of acantholysis near the granular layer in pemphigus foliaceus. The desmosomes have all disappeared on the three cells shown here. The tonofilaments have all retracted into an irregular network of tonofibrils around the nucleus, forming dyskeratotic material (DY). Methacrylate × 20000.

seven patients with pemphigus foliaceus. On morphologic grounds these bodies compare well with vaccinia virus (9), but they have not been observed in clusters of large numbers to justify a morphologic designation of virus with certainty.

There are other possible interpretations of these bodies: a) They might represent altered mitochondria, although normal mitochondria are usually much larger than the bodies seen here; b) These bodies could be a product of cellular injury peculiar to pemphigus foliaceus, but of a non-viral nature; c) The possibility has been considered that these peculiar bodies represent lysosomes, but their distinct external membrane and their consistent size do not match the lysosomal structures seen under a variety of other circumstances; d) One should consider that similar bodies might also be observed in other skin conditions that have not been examined so far. However, such bodies have as yet not been found in a large and varied amount of epidermal pathology that has been subjected to electronmicroscopy. A definite interpretation of the true nature of these peculiar bodies awaits further work with tissue culture technics, as well as increased experience in the electron microscopy of diseases of the epidermis. Finally, it should be noted that similar bodies were not seen by us in a series of five cases of pemphigus vulgaris (1) although virus bodies were looked for. (This point is under reinvestigation.) The present finding of these peculiar bodies, however, might suffice to revive an interest in the possibility of a viral etiology of pemphigus foliaceus.

SUMMARY

Seven cases with generalized pemphigus foliaceus were examined by electron micros-

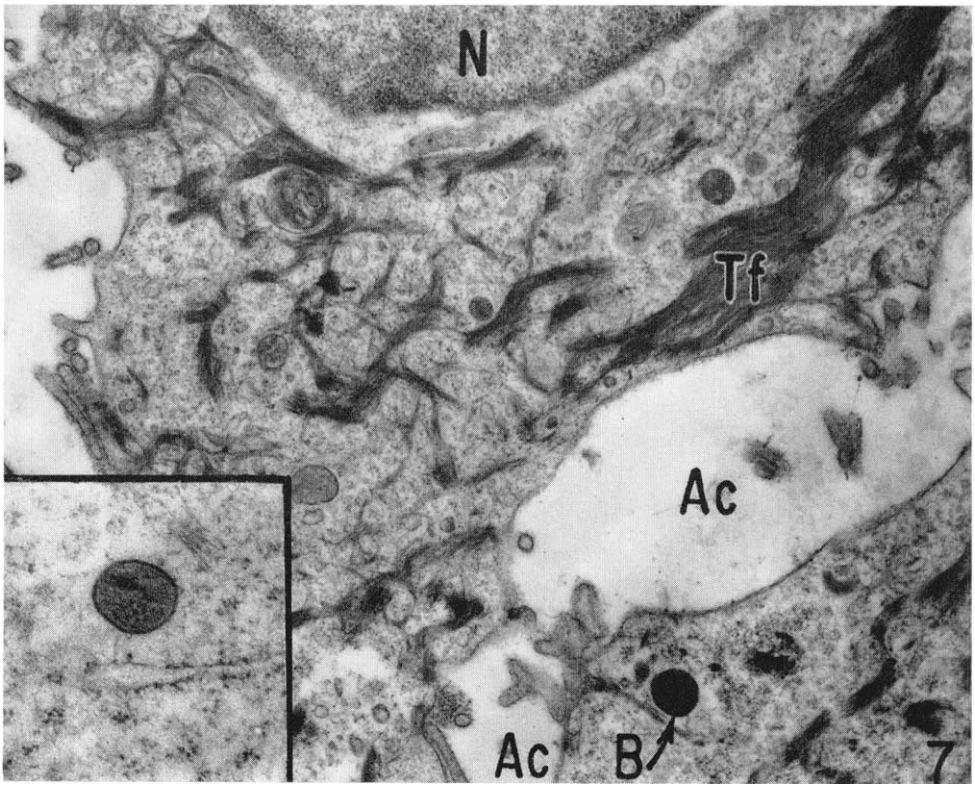

FIG. 7. Advanced acantholysis near the granular layer in pemphigus foliaceus. No desmosomes can be seen on the two cells shown here. The tonofilaments have lost their orientation and are illustrated as an irregular network of fibrils. A peculiar intracytoplasmic body (B) is shown in the right portion of this electron micrograph. It is illustrated under higher magnification in the insert in the left lower corner of this picture. Epon × 17000. Insert Epon × 50000.

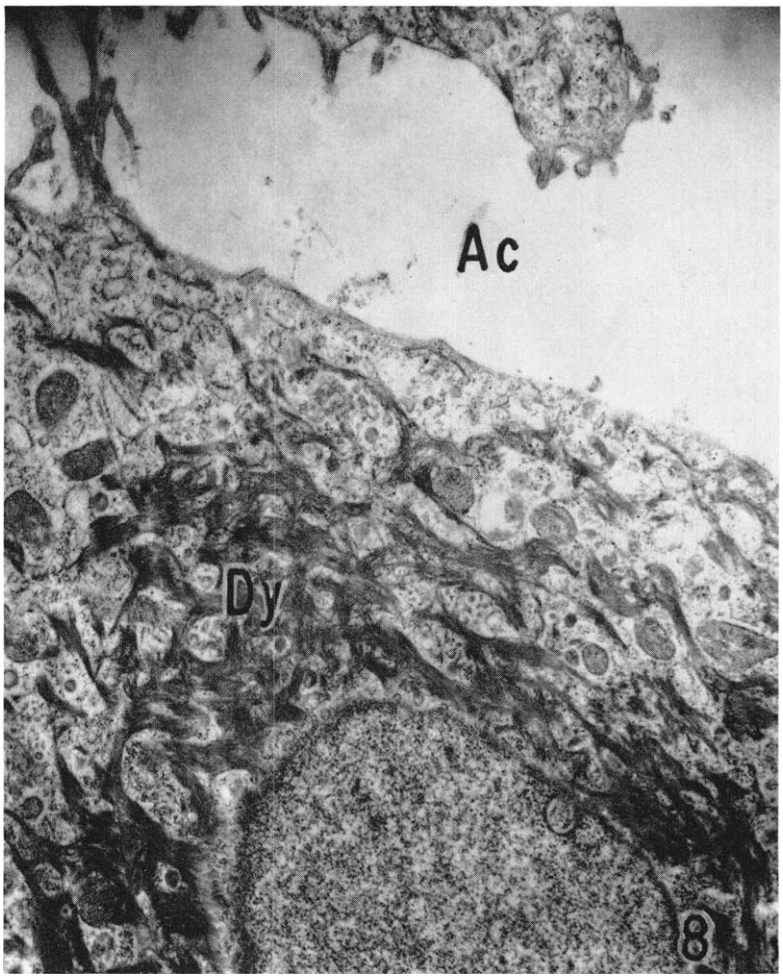

FIG. 8. Advanced acantholysis with complete loss of desmosomes in the sub-granular area in pemphigus foliaceus. The tonofilaments form an irregular network of interwoven fibrils after their retration from the desmosomes. This network of fibrils represents dyskeratosis (DY). Epon × 18000.

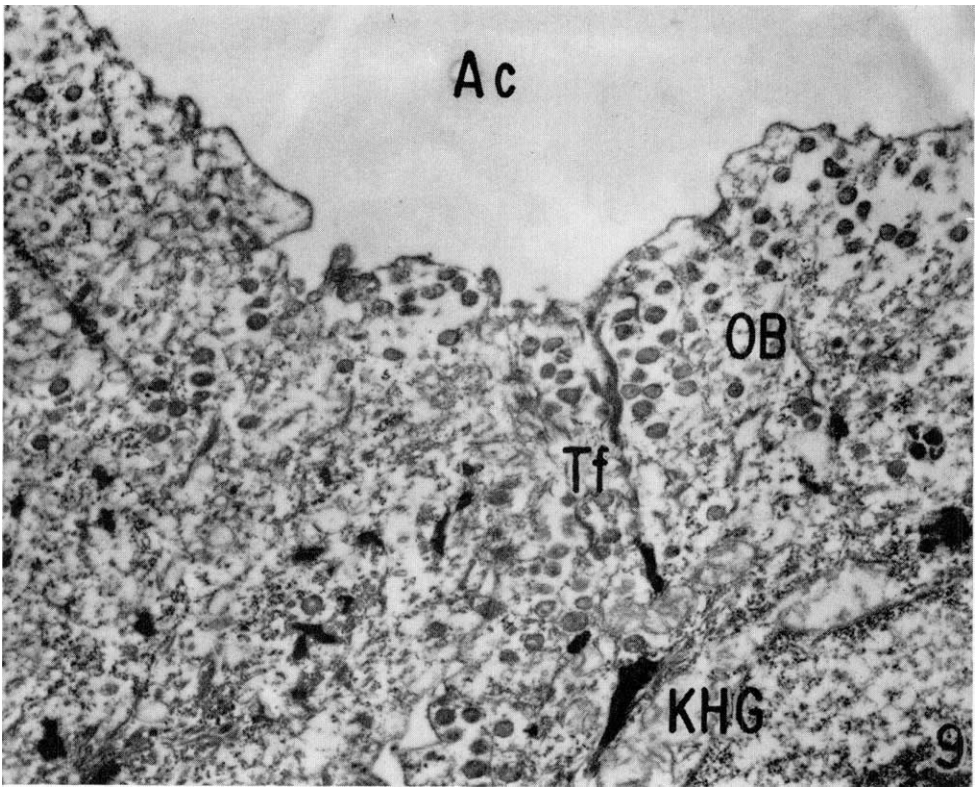

FIG. 9. Acantholysis (AC) in a sub-corneal lacuna in pemphigus foliaceus. The area of the lacunae is represented by the space AC. The granular cells are characterized by numerous Odland bodies (OB) and keratohyaline granules (KHG). Tonofilaments (Tf) are seen to be associated with keratohyaline granules. Methacrylate × 15000.

copy. No case with pemphigus erythematosus was included in this series. The dermis and the basement membrane appeared normal. Striking pathological changes were confined to the epidermis. The earliest change appeared to be a separation of the tonofilaments from the attachment plate of their corresponding desmosomes in the basal region. Acantholysis and dyskeratosis in the Malpighian and granular layer were due to injury of the same subcellular structure, *e.g.*, the tonofilament-desmosome complex. Because epidermal cell injury was mild cellular viability and attempts at keratinization were retained to some degree. Epidermal cell injury, however, was sufficiently severe to cause a detachment of the tonofilaments from their desmosomes. This was followed by: 1) a loss of desmosomes leading to acantholysis, and 2) loss of proper orientation of the tonofilaments leading to dyskeratosis.

These findings of the tonofilament-desmosome relationship in pemphigus foliaceus seem to support the concept that the desmosomes have an orienting function for the tonofilaments and thus serve a purpose in the process of keratinization.

Peculiar bodies were observed in the cytoplasm of epidermal cells in all seven cases of pemphigus foliaceus. Different interpretations are given for these peculiar bodies. The opportunity is taken to revive interest in the possibility of a virus etiology for pemphigus foliaceus.

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