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MORBILLIFORM ERUPTIONS CAUSED BY PENICILLIN

A STUDY BY ELECTRON MICROSCOPY AND IMMUNOLOGIC TESTS*

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ABSTRACT

Although much has been learned regarding the immunology of penicillin eruptions, the advances are largely limited to reactions mediated by humoral antibodies. Therefore, we undertook a study of the more common, but less well-understood morbilliform eruptions which occur several days to weeks following penicillin therapy. Biopsies were taken from 4 patients with such eruptions and from 4 control subjects and studied by electron microscopy as well as light microscopy. The patients were skin tested with penicillin antigens and studied for hemagglutinating antibodies. The results showed no abnormalities in hemagglutinating antibodies, no detectable skin-sensitizing antibodies or delayed sensitivity to penicillin derivatives. Electron microscopy revealed striking differences between patients with penicillin eruptions and controls. The involved skins showed intercellular edema limited to the basal area and some intracellular edema. The basement lamina remained intact. Other morphologic changes presented interesting similarities to those seen in pemphigus vulgaris including clumped perinuclear tonofibrils and "tomb-stone-like" basal keratinocytes. Fluorescence studies revealed anti-epithelial antibodies in the blood serum of 2 of the 4 patients with morbilliform penicillin eruptions.

Great advances in the immunology of adverse reactions to drugs in general have been made since the discovery of and testing with degradation products of penicillin (1, 2). Most investigators agree that the anaphylactic reaction following sensitization to, and provocation by penicillin is associated with IgE skin-sensitizing antibodies to specific products of penicillin (3). Urticarial eruptions following penicillin are also associated with IgE antibodies that are frequently of penicilloyl-specificity. However, by far the greatest number of adverse cutaneous reactions following therapy with penicillin are exanthematic eruptions, particularly morbilliform. These are late in onset, that is, they first make their appearance 24 or more hours after administration of penicillin. They generally involve most of the body, or at least the arms and legs. Although there is evidence that in some cases rising titers of IgM penicilloyl-specific antibodies may be involved (4) or delayed hypersensitivity mechanisms (5), the mechanism of most of these eruptions remains unknown.

In an attempt to gain greater understanding of the pathogenesis of such eruptions, we undertook to study them by electron microscopy as well as the usual immunologic techniques.

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MATERIALS AND METHODS

Biopsies were taken from 8 patients of whom 4 had had recent penicillin therapy and developed cutaneous exanthems. All 4 had typical morbilliform eruptions which were late in onset and clinically were diagnosed as due to penicillin. They were taking no other drugs. Four additional subjects studied had had no recent penicillin. Two of these 4 had chronic dermatitis of indefinite etiology and 2 others were normal controls.

Six mm punch biopsies of involved skin were obtained using 1% Xylocaine as anesthetic. The excised skin was put in 5% glutaraldehyde in phosphate buffer for 1½ hours, washed for 5 minutes in buffer and then placed in 2% osmium tetroxide buffered to pH 7.4. The tissues were then dehydrated, using ethanol in graded dilutions, and embedded in Epon 812. Tissue sections measuring 1 micron, as well as of ultra thin size were cut on a Reichert Ultamicrotome. The ultra thin sections were stained with uranyl acetate followed by lead citrate and were then examined in an RCA-EMU 2E electron microscope. Sections were also cut and processed for light microscopy.

Skin tests for immediate wheal-and-flare reactivity were performed on all patients by stand-

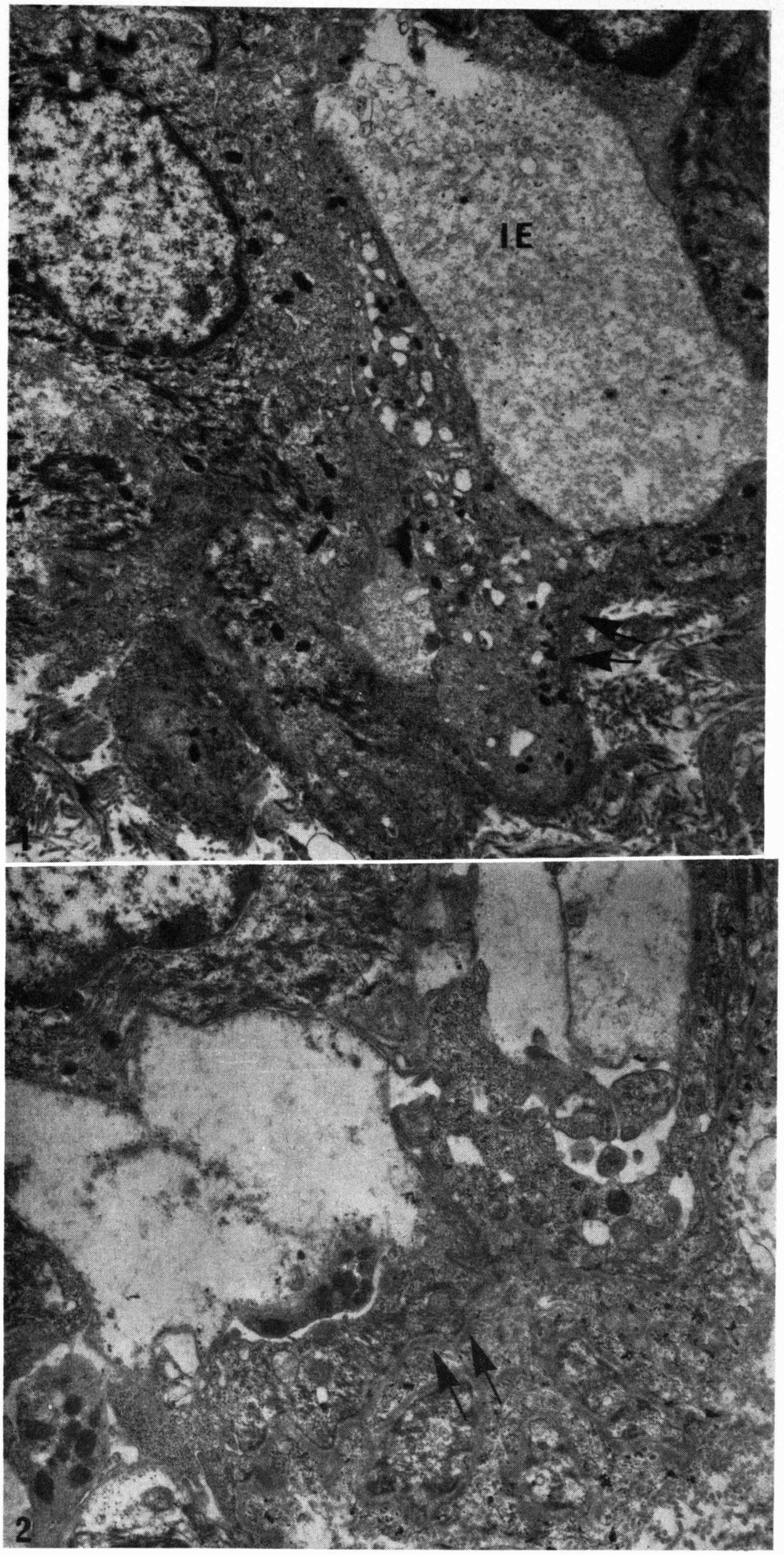


FIG. 1. An electron micrograph of an early lesion in a patient with a penicillin reaction. Intercellular edema (IE) is noted between basal cells as well as an intact basement lamina (arrows). × 7,425

FIG. 2. A later stage in the evolving exanthem showing disruption of the basal cells. Again the basement lamina is intact (arrows). × 11,880

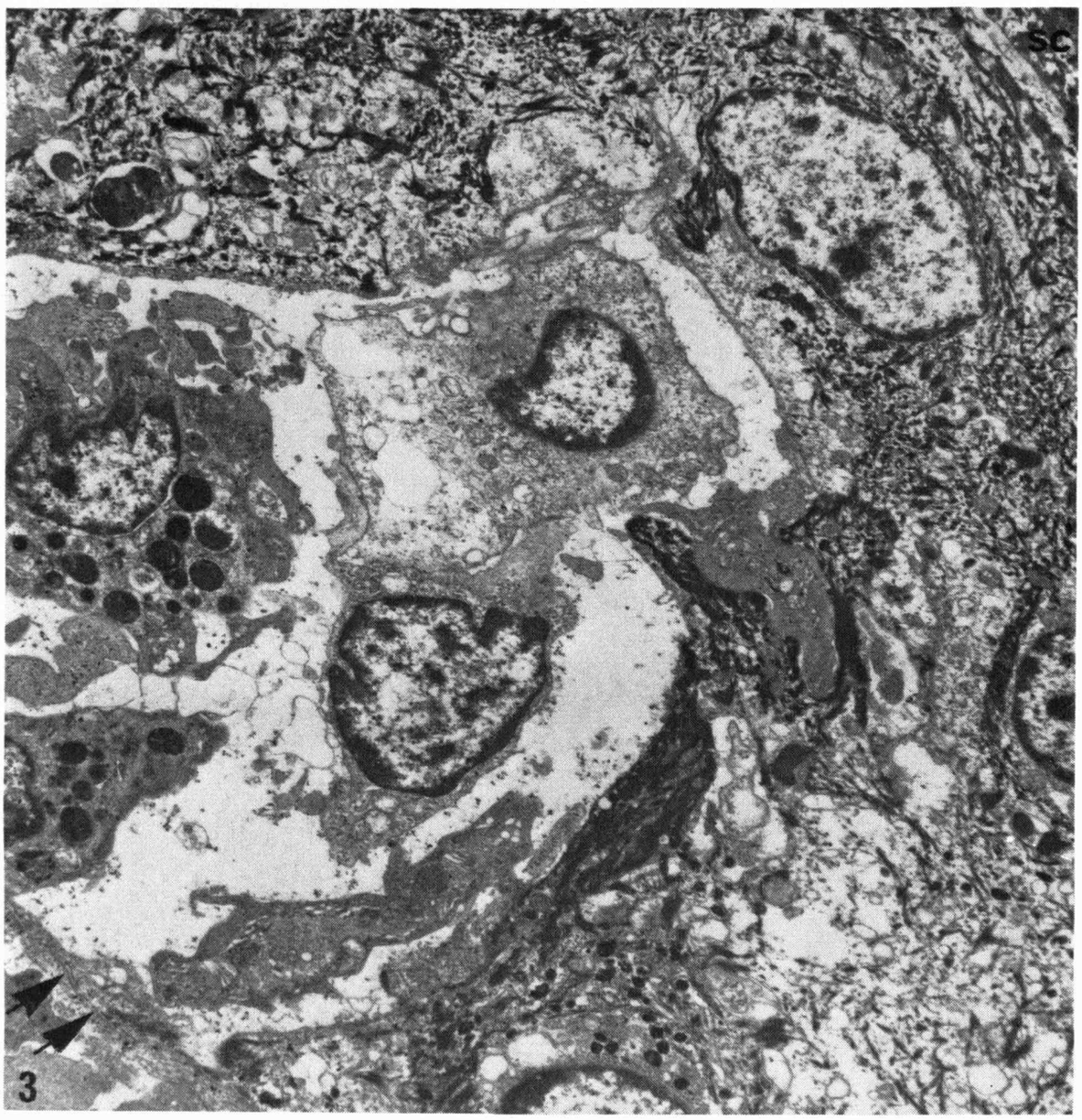


FIG. 3. A patient with a florid eruption shows much cellular disruption. Basement lamina (arrows) is intact. Many macrophages and inflammatory cells are observed. The stratum corneum (SC) is also noted. × 4,579

ard techniques (2, 4) with penicilloyl-polylysine and the "minor determinant" mixture of penicillin (4). Blood was obtained for penicilloyl-specific hemagglutinating antibody and was analyzed by standard techniques (6).

RESULTS

(1) *Electron microscopy*. Comparison of the epidermis from patients with penicillin reactions with that of the controls showed striking differences. Sections from control patients showed normal epidermis with closely packed basal cells and keratinocytes in the stratum spinosum, and an intact basement lamina. Sections from penicillin eruptions showed disruption of the epidermal basal cells in varying degrees. In the earliest lesions examined in all cases there was intercellular edema (Fig. 1) and some intracellular edema in the basal cells. The tonofilament-desmosomal complex and basement lamina remained intact. The dermis had some macrophages present near the basement lamina. There was no edema present nor any change in the dermal components. Examination of skin material which had been taken from patients with more extensive changes, at a later stage in the evolving exanthem, showed disruption of the basal cells, and invasion by macrophages into areas of intercellular edema. Examination of the basement lamina whether from the edematous area or adjacent sections, revealed it to be intact (Fig. 2). Examination of sections from a patient with the most florid eruption showed much cellular disruption extending from the basement lamina to the mid-epidermis (Fig. 3) and many macrophages and inflammatory cells. This finding was never observed in the upper strata of

the epidermis. The extensive morphologic disruption of keratinocytes in the lower strata appeared to produce a bulla. Again, the basement lamina was intact in all sections examined.

Many of the basal cells underwent degeneration exhibiting pyknotic nuclei and perinuclear clumping of the tonofibrils (Fig. 4). However, it is interesting to note that within the clumped tonofibrils the structural integrity of the desmosome remained intact (Fig. 5). In addition, as the intercellular spaces widened, there was a serous exudate located within them.

(2) *Light microscopy.* Of the 4 patients with penicillin eruptions, 2 showed no epidermal abnormalities; the other 2 showed mild intercellular edema in a few areas. The dermis had enlarged blood vessels and inflammatory cells. The 2 normals showed no changes; the 2 patients with chronic dermatitis showed changes which were predominantly dermal, and mild degeneration of the epidermal basal layer, also some acanthosis and parakeratosis.

(3) *Fluorescence microscopy.* Studies by standard indirect immunfluorescent technique (7) using guinea pig esophagus as substrate gave moderate and strongly positive tests for anti-epithelial antibodies in serum from 2 of the 4 patients (titers to 1/20) who had morbilliform eruptions from penicillin.*

(4) *Skin and hemagglutination tests.* All patients had negative immediate and delayed skin tests for wheal-and-flare reactivity to all penicillin antigens. Hemagglutination assays revealed relatively low titers of IgM antibodies in all cases.

DISCUSSION

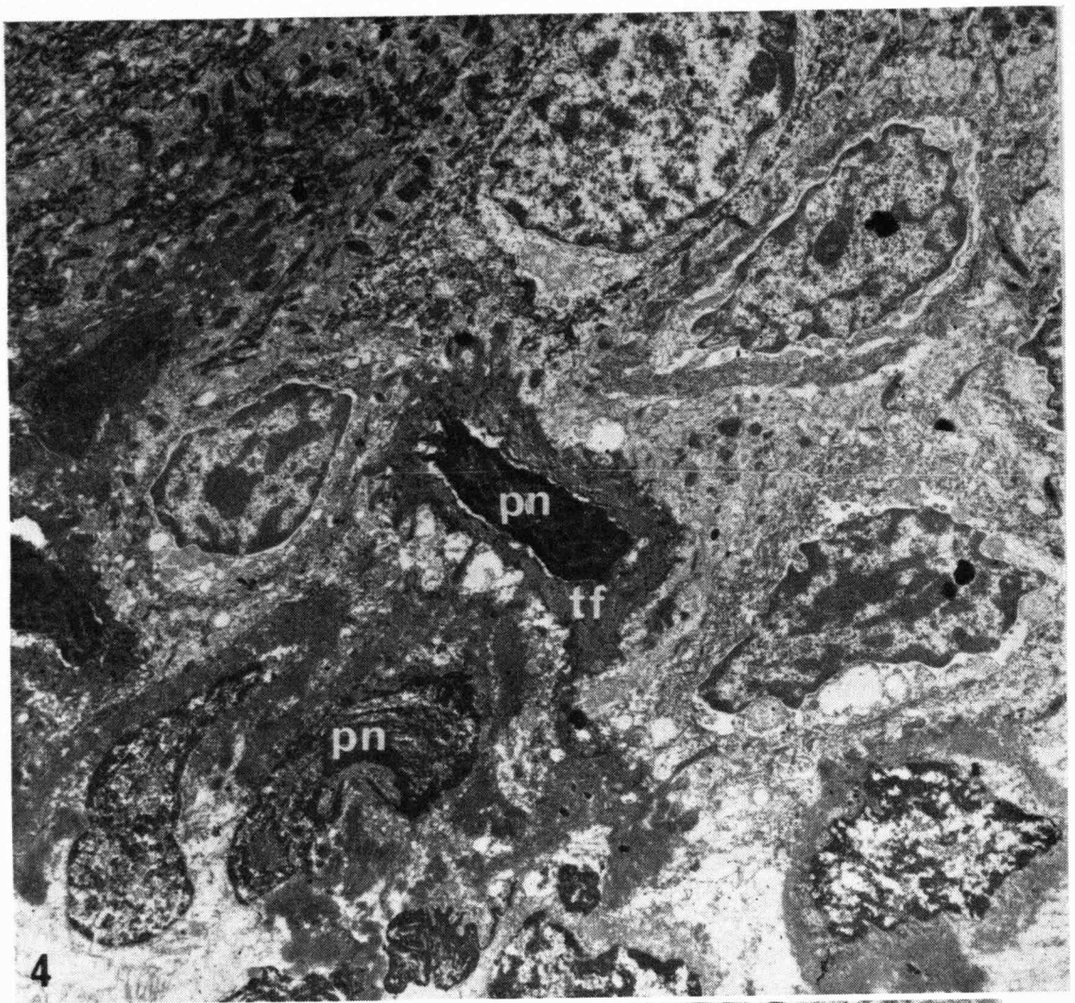
The results of this study by electron microscopy of lesions in patients with morbilliform reactions to penicillin show that the primary changes appear to be in the basal-cell-layer of the epidermis. At this point it is still impossible to know whether the initiating event is intercellular or intracellular. In either case the insult is limited to the area just above the basement lamina. This results in a loss of normal cellular architecture with exocytosis of inflammatory cells. The lesion appears to extend to the mid-epidermis. The basement lamina remained intact in all cases, and no extension to levels above the middle level of the epidermis was noted.

The morphologic findings present interesting similarities to those seen in pemphigus vulgaris and ultraviolet-exposed epidermis. Wilgram, *et al.* (8), pointed out in an earlier study that the tonofilament-desmosome complex may be an area of early insult in pemphigus vulgaris. The early involvement of desmosomes in pemphigus vulgaris was also noted by Braun-Falco and Vogell (9, 10). Hashimoto and Lever (11, 12) have presented evidence that the dissolution of the intercellular cementing substance between keratinocytes is an initiating event in pemphigus with acantholysis the result. In the present study of morbilliform eruptions following penicillin, we have noticed widened intercellular spaces in the lower epidermal strata and a serous exudate filling these spaces. In addition, the basement lamina remains intact and even though the basal cells undergo degeneration, the perinuclear clumping of tonofibrils resembles the description given of a "tombstone row" for the configuration of basal cells in pemphigus vulgaris described by others (13, 14) (Fig. 4). Hashimoto and Lever (12) have recently stated that the occluding zonules have a high degree of persistence even in the advanced degeneration of pemphigus. Our study of drug eruptions also demonstrates that the desmosomal attachments of the perinuclear tonofibrils (Fig. 5) are intact.

Although much has been learned in the penicillin system regarding antigen and antibody and their roles in producing adverse effects due to drugs, relatively little has been discovered regarding morbilliform eruptions which frequently follow penicillin therapy. Based on our results, we speculate that repeated introduction of penicillin or other drugs can result in a formation of haptene-skin-complexes with formation of auto-skin antibodies. This is not a new idea since it has been known for years that immunization with simple chemicals results not only in antibody directed towards the chemical, but also towards carrier proteins (15).

In the case of penicillin, several carrier proteins have been already identified including human gamma globulin (16, 17) although by no means are all of these known. Accordingly, it is quite possible that some skin proteins serve as carriers for penicillin allergy and after re-

* Recently, Go has reported anti-epithelial antibodies in serum from patients taking Kynex: Go, M.: Uber ein mit der indirekten Immunfluorescenzmethode nachweisbares epidermo-cutanes Fluorescenzphänomen unter Sulfamethoxy-Pyridin (Lederkyn®, Kynex®), Der Hautarzt, *20:* 222, 1969.



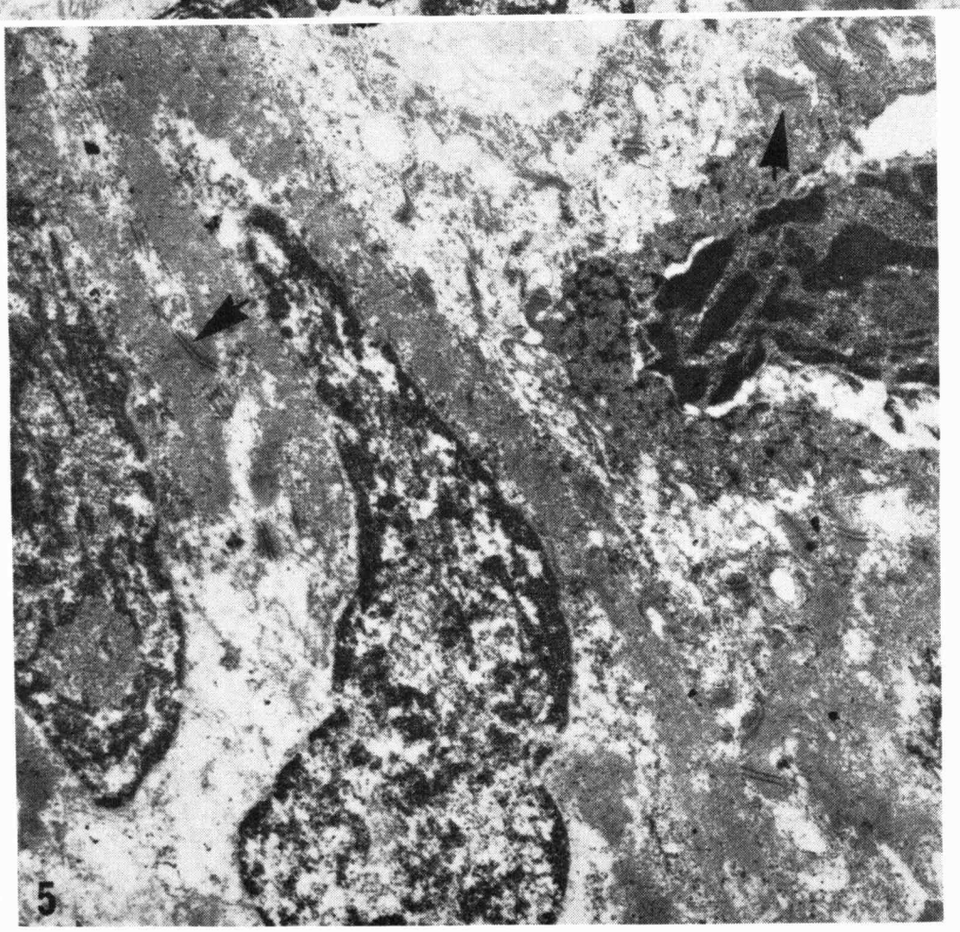


FIG. 4. Degeneration of the basal cells exhibiting pyknotic nuclei (pn) and perinuclear clumping of the tonofibrils (tf). × 7,425

FIG. 5. An electron micrograph showing the structural integrity of the desmosomes (arrows) within the perinuclear clumping of the tonofibrils. × 11,880

peated insults with penicillin, sufficient antibody directed against skin might be formed to produce the initial stages of a pemphigus-like picture. Further investigations to confirm this hypothesis, of course, are required and should involve additional studies of anti-epithelial antibodies as well as studies of patients with other types of drug eruptions.

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