THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 65:191-200, 1975
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Vol. 65, No. 1
Printed in U.S.A.

STUDIES OF THE MECHANISM OF EPIDERMAL INJURY BY A STAPHYLOCOCCAL EPIDERMOLYTIC TOXIN

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Experimental animal models of the two forms of toxic epidermal necrolysis have been reviewed: a murine model of staphylococcal-induced epidermolysis and a hamster model of graft-versus-host disease. In the former, a protein exotoxin, epidermolysin, has been purified and characterized. The exotoxin has a molecular weight of approximately 30,000 and causes a split beneath the granular layer. It is effective at 3×10^{-12} moles. Epidermolysin does not require an intact complement system for its action since $B_{10}D_2$ mice deficient in C5 or mice injected with the decomplementing agent in cobra venom factor were susceptible to its epidermolytic effects. Neither are immunocompetent thymocytes required for the action of the toxin since hairless, athymic adult (nu/nu) mice are susceptible. A few reports of epidermolysis due to an exotoxin of group I *Staphylococcus aureus* have appeared. This toxin is antigenically different from the exotoxin of group II organisms.

A model of drug-induced toxic epidermal necrolysis has been described in hamsters, but the toxic principle released from sensitized lymphoid cells has not yet been characterized.

Toxic epidermal necrolysis is one of the most dramatic examples of epidermal injury. In the past, careful histopathologic examination has helped to clarify the causes. More recently, the development of an experimental animal model and the availability of a highly purified epidermolytic toxin, epidermolysin, have enabled us to explore the pathogenesis of the process and to attempt to determine how the toxin exerts its selective action on the epidermis.

In this paper we will review the epidermolytic form of toxic epidermal necrolysis, focusing on the protein exotoxin(s) elaborated by some phage group II *Staphylococcus aureus* which are responsible for the epidermal injury.

CLINICAL BACKGROUND

For nearly a century, physicians have observed, particularly in neonates or young children, the abrupt onset of blistering and exfoliative epidermal diseases which have been called by such names as "dermatitis exfoliativa neonatorum," "toxic epidermal necrolysis," or "erythrodermie bullouse avec epidermolyse." Recently these diseases have been linked with bullous impetigo and a staphylococcal scarlatiniform syndrome. A similar entity has been reported in adults with compromised immune function or overwhelming staphylococcal septicemia; thus this syndrome and its clinical variants extend over a wide age group.

In 1956, Lyell described a skin eruption which closely resembled scalding and postulated that "some circulating toxin specifically damages the epidermis and results in its necrosis" [1]. He called the resulting syndrome toxic epidermal necrolysis (TEN), defining necrolysis as "a neologism coined to combine the clinical appearance of epidermolysis with the histologic observation of epidermal necrosis" [2].

Cases of this syndrome had been previously reported under various names [3], but it is still most commonly referred to in English as Lyell's syndrome, toxic epidermal necrolysis, or the scalded-skin syndrome.

In 1878 Ritter von Rittershain reported 297 cases of neonates suffering from a skin eruption he named dermatitis exfoliativa neonatorum (DEN) [4]. In 1961 Frain-Bell and Koblenzer suggested a relationship between the DEN of von Rittershain and the TEN of Lyell [5]. Koblenzer later reviewed the literature and presented cases of his own to establish this relationship convincingly [6]. *S. aureus* was first isolated from a patient with DEN in 1898 [7]. Although von Rittershain did not associate DEN with infection, several authors have since established it as a staphylococcal disease [8-13].

On the other hand, TEN appeared to be a multifactorial disease. In 30 of the 128 cases reviewed by Lyell in Britain in 1967, a staphylococ-

Supported, in part, by PHS Training Grants 5T01 AM 05300 and AM 05512, and a grant from the Dermatology Foundation.

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cal infection seemed to be the predominant feature, in 36 a drug was implicated, in 28 a miscellany of diseases coexisted, and in 34 there was no clue to the cause [14]. In the 28 cases of miscellaneous disease, drugs were often given and may have been responsible for the complexity. Lyell felt that many of the children in the idiopathic group were suffering from an undiagnosed staphylococcal infection; all of the cases of staphylococcal origin were children under 10 years of age.

In the same year, Lowney et al reported 10 of their own cases and reviewed 139 published case reports [15]. They too observed that the staphylococcal cases occurred in children whereas the drug-related cases occurred in both children and adults. The mortality rates among the patients differed—7% among the 1- to 5-year-old group, 44% among the patients over 6.

Staphylococci cultured from cases of TEN had already been identified as organisms of phage group II, usually type 55 or 71 [16,17], and had been associated with Ritter's disease [9–13] and bullous impetigo [11,18–20] as well as with a scarlatiniform eruption [21–24]. In 1970, Melish and Glasgow proposed that dermatitis exfoliativa neonatorum, staphylococcal TEN in children, staphylococcal scarlet fever, and bullous impetigo were clinical manifestations of infection with phage group II staphylococci and represented a spectrum of diseases with a single etiology [25].

HISTOPATHOLOGIC OBSERVATIONS

Before the pathogenesis of TEN could be understood, the pathologic changes which accompany the disease had to be described. In his first case, Lyell reported that the pathology was confined to the epidermis where necrosis of the epidermal cells resulted in blister formation between the epidermis and dermis; in one case, however, the necrosis was more superficial and formed an intraepidermal split [1]. Koblenzer observed an intraepidermal split in the children he studied [6]; Lowney et al also observed an intraepidermal split in small children with staphylococcal infection but a subepidermal split in the older children [15]. While agreeing that the intraepidermal split was typical of staphylococcal TEN, Lyell postulated that the nonstaphylococcal cases had the deeper split [26]. Thus, by 1969 staphylococcal TEN was generally regarded as being histologically and, in some respects, clinically different from nonstaphylococcal TEN.

EXPERIMENTAL MODELS OF EPIDERMAL NECROLYSIS

Murine Model of Staphylococcal TEN

Evidence that a circulating toxin is implicated in staphylococcal TEN is now firmly established. In 1967, both Lowney et al [15] and Samuels [27] suggested that staphylococcal TEN results from some diffusible toxic product of the staphylococci. Lowney et al [15], who observed that only rarely were staphylococci cultured from the site of the bullae or peeling, postulated a circulating toxin arising from the site of infection, often the pharynx, ears, conjunctiva, or skin elsewhere. Arbuthnott and co-workers suggested, erroneously as it turned out, that the delta hemolytic toxin was responsible since, in England, most of the strains of staphylococci isolated from cases of TEN produced the delta toxin [28].

Our knowledge of this disease advanced considerably when in 1970 Melish and Glasgow [29] injected group II staphylococci that had been isolated from cases of staphylococcal TEN, bullous impetigo, or staphylococcal scarlatiniform eruption into newborn mice and epidermolysis ensued. The experimental lesions developed from the formation of a Nikolsky sign to widespread peeling. Histologic sections were characterized by the same kind of cleavage plane within the epidermis at the level of the stratum granulosum that is found in human patients. Since the organisms could not be cultured from the site of the peeling or even seen histologically near this area, these workers hypothesized that the products of the staphylococci were being disseminated from the site of infection and were causing lesions elsewhere. They developed an experimental model in which they established that group II staphylococci are the etiologic agents of staphylococcal TEN.

Hamster Model of Grant-versus-Host TEN

In 1968, Billingham and Streilein developed an animal model for studying the drug-induced variety of TEN [30], and Streilein discussed that work at the Symposium in 1969 [31]. They observed that graft-versus-host (GVH) reactions in the hamster resulted in an explosive, necrotic epidermolysis with large sheets of intact epidermis peeling from the affected animals. Cutaneous reactions are characteristic of other animal and human cases of GVH, but the TEN-like picture seems to be peculiar to the hamster. TEN, however, has been associated with a GVH reaction in humans [32,33] and has occasionally complicated GVH reactions in rats [34] and monkeys [35]. When sensitized lymphoid cells from donors of either parental strain are injected intracutaneously into adult F_1 hybrid hamsters, the GVH reaction, including TEN, occurs. Streilein showed that the skin is not the antigenic stimulus for this reaction and hypothesized that when the leukocytes of the hybrid are attacked by the injected lymphoid cells of the parents, a pharmacologically active macromolecular agent is released which damages the epidermis [31]. He supported this hypothesis by finding that serum obtained during the acute GVH reaction was cytotoxic toward suspensions of epidermal cells grown in vitro. In this connection, serum obtained from one of the patients with GVH complicated by TEN produced cytotoxic effects in vitro against epithelial cells [36]. Therefore, in the drug-induced form of TEN there is some evidence to support Lyell's hypothesis that a circulating toxin damages the epidermis.

THE STAPHYLOCOCCAL EXOTOXIN, EPIDERMOLYSIN

Isolation and Purification

From the supernatant of cultures from certain phage group II strains of *S. aureus*, Arbuthnott, Kent, Lyell, and Gemmell [37] and Kapral and Miller [38] isolated and partially purified a factor which caused epidermolysis in newborn mice. Later, Melish, Glasgow, and Turner [39], Kondo, Sakurai, and Sarai [40], and Dimond and Wuepper [41] purified this extracellular product which has been called "exfoliatin" [38] or "epidermolytic toxin" [37]. Since the primary histologic finding in this model, as in human patients, is a lysis within the epidermis which is characterized by a separation of the cells rather than by profuse scaling or exfoliation, we believe the toxin is epidermolytic and have proposed that it be called "epidermolysin" [41].

Some workers have produced toxin by growing the bacteria in semisolid nutrient agar under 20% CO_2 [37] or in yeast extract–trypticase soy broth under 10% CO_2 [38,40]; others have inoculated Medium 199 in dialysis sacs and implanted them within the rabbit peritoneum [39,41] for a few days. Obvious differences in the quantity of toxin produced by various strains have been reported [38].

Simple schemes for purifying toxin include adsorption chromatography to hydroxylapatite [37], isoelectric focusing in polyampholytes [39,42], and chromatography upon diethylaminoethyl cellulose [40] or Sephadex [38], or carboxymethyl Sephadex after block electrophoresis in Pevikon [41]. Although epidermolysin is effectively separated by these procedures from most proteins in culture supernatants, the α-hemolytic toxin is extremely similar in size and charge behavior. The α-hemolytic toxin and epidermolysin are separable after electrophoresis in polyacrylamide gels [40, 41] or isoelectric focusing [39,42].

We performed zone electrophoresis at the isoelectric point of the α-toxin, pH 9.0, as our first step in purification [41] and obtained good separations of these two proteins (Fig. 1). After electrophoresis in Pevikon, fractions containing epidermolysin were pooled, dialyzed, and chromatographed on carboxymethyl Sephadex C-50 in phosphate buffer, pH 6 (Fig. 2). Proteins were eluted from the cation exchanger by a sodium chloride gradient. Epidermolysin eluted as the major protein peak and was measured by bioassay in newborn mice. Its purity was ascertained in polyacrylamide gel electrophoresis (Fig. 3).

Characterization of Epidermolysin

When stored at 4°C or −20°C, epidermolysin remains stable for several months. It is remarkably heat stable and resists heating to 60°C for 1 hr or to 100°C for 20 min [38,40], but boiling for longer periods leads to decreased activity [38,40].

The toxin is said to be stable in buffers from pH 5.0 to 9.0 but precipitates below pH 4.0 [39,41]. The substance is not irreversibly denatured at this pH, however, since its activity is restored when it is resolubilized in acetate buffer containing sodium dodecyl sulfate [41].

The electrophoretic behavior of epidermolysin is like that of a beta globulin [39,41]: it sediments at approximately 3S in gradients of sucrose [39], and its isoelectric point was found by two laboratories to be 6.7 to 7.0 [39,42] but 4.0 to 4.5 by two others [38,40].

The molecular weight of epidermolysin has been investigated by several methods. By gel permeation chromatography on Sephadex G-50, results of 23,500 [38], and 24,000 [40] have been obtained. Electrophoresis in acrylamide gels containing sodium dodecyl sulfate have given results of 28,600 in one laboratory (Fig. 4) [41] and 33,000 in another [41]. By analytical ultracentrifugation, we have obtained a value of 32,500, assuming epidermolysin to be a globular protein with a partial specific volume of 0.74 [41]. Evidence that the toxin is a protein has been provided by the digestion of epidermolysin with proteolytic enzymes; when measured by bioassay, its activity was reduced or destroyed by incubation with pronase [38], trypsin [38,39], or pepsin [30].

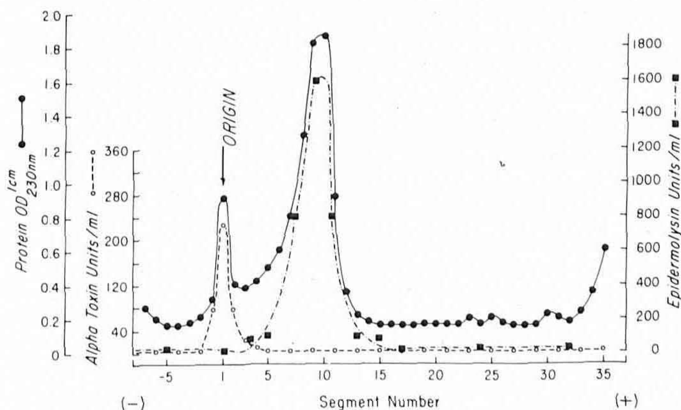

FIG. 1. Zone electrophoresis of *Staphylococcus aureus* (strain EV) culture filtrate in Pevikon containing 0.05 M Tris buffer, pH 9.

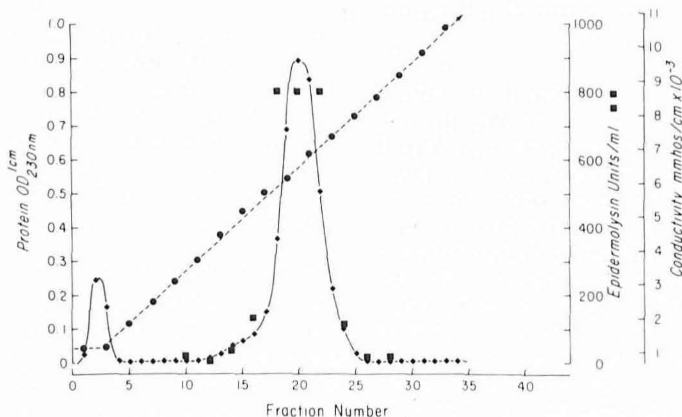

FIG. 2. Chromatography of epidermolysin, recovered from zone electrophoresin in Pevikon, upon carboxymethyl Sephadex C-50 in phosphate buffer 0.01 M, pH 6. Adsorbed proteins were eluted with a gradient of 0.2 M NaCl in the phosphate buffer.

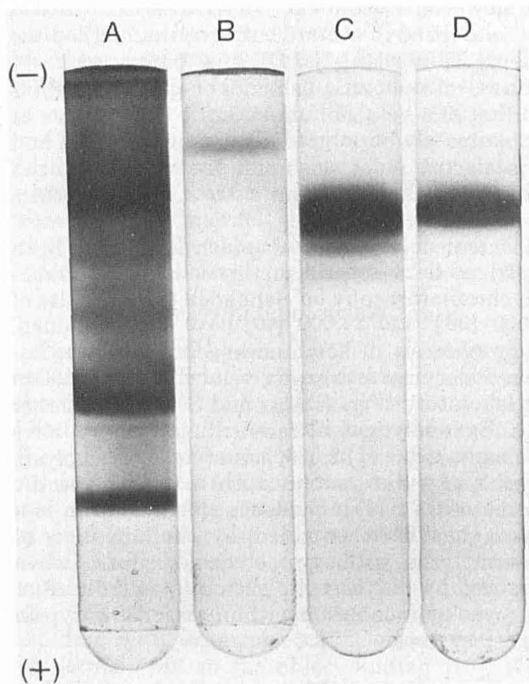

FIG. 3. Polyacrylamide gel electrophoresis of crude staphylococcal culture filtrate (*A*), fractions recovered from zone electrophoresis containing α-toxin (*B*) and epidermolysin (*C*), and, finally, highly purified epidermolysin recovered from CM Sephadex chromatography (*D*).

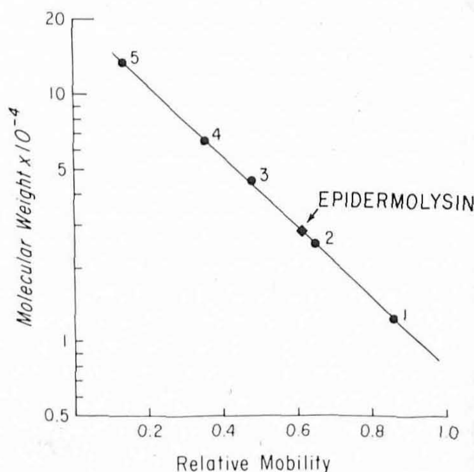

FIG. 4. Acrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) of staphylococcal epidermolysin relative to marker proteins of known molecular weights. The marker proteins were horse heart cytochrome C (*1*), cytochrome C dimer (*2*), ovalbumin (*3*), bovine albumin monomer (*4*) and dimer (*5*). The molecular weight of epidermolysin 28,600.

Epidermolysin is immunogenic, and precipitating antibodies to the protein have been raised in rabbits (Fig. 5) [40,41,43]. These antibodies, passively administered to neonatal mice, neutralize the effect of toxin in vivo [43]. The toxin appears to be a single polypeptide chain, not associated with a subunit structure. It migrates as a single moiety in acrylamide gels after it has been reduced by dithiothreitol with SDS [41,42].

Measurement of Epidermolysin

The bioassay, first described by Melish and Glasgow [29], remains the only functional test for the activity of epidermolysin. When toxin was recovered in supernatants, it was again measured by bioassay [38,44]. Because of different methodologies, the unit of epidermolysin measured by this method has not been standardized and therefore resists comparison by the various investigators. Kapral and Miller [38] injected 0.02 ml of toxin in 1-day-old mice and defined an exfoliating dose (ED_{50}) as that which gives a positive Nikolsky sign in 50% of the group after 3 hr. Having injected 0.1 ml in mice 1 to 5 days of age which had been randomized, Melish and her co-workers defined an exfoliating unit (EU) as the reciprocal of the dilution which causes exfoliation in at least one of each group of three mice after 2 hr [39]. Finally, Kondo et al, who injected 0.05 ml in mice of unstated age, defined their unit (EU) as the reciprocal of the minimum dose which gives a positive Nikolsky sign in 100% of the group after 3 hr [40].

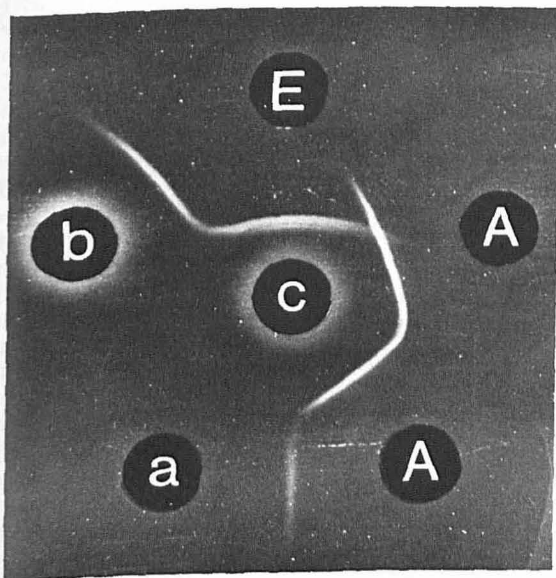

FIG. 5. Antigenic distinction of staphylococcal α-toxin and epidermolysin by double diffusion in agar. Antigens were placed in wells indicated by *E* (highly purified epidermolysin) or *A* (α-toxin, Burrowes Wellcome); antisera were placed in wells *a* (antibody to α-toxin), *b* (antibody to epidermolysin), and *c* (a 1:1 mixture of *a* and *b*).

We have subsequently investigated the dose-response in mice of specified age after the injection of known quantities of epidermolysin in a volume of 0.02 ml [44]. Part of the data is given in Table I to show how important the age of the recipient is on the time for a localized Nikolsky sign to become evident. We have observed a linear response between 15 and 90 min in 1-day-old mice when the injected dose was between 2.6 and 0.1 μg of epidermolysin. Presumably, a major cause of these differences in mice of various ages is the time required for diffusion of the toxin from its subcutaneous depot to the epidermis.

It is generally agreed that less than 1 μg of epidermolysin causes a positive Nikolsky sign in newborn mice. In our laboratory, approximately 100 ng (about 3×10^{-12} moles) cause a positive Nikolsky sign 90 min after subcutaneous injection in a 1-day-old mouse; these results indicate the potency of this agent. This is the same general range of biologic effectiveness of histamine, bradykinin, or the anaphylatoxin fragments derived from serum complement components.

Neither clinical nor investigative studies have fully exploited the measurement of epidermolysin antigen even though such measurement of epidermolysin or of antibody to epidermolysin by sensitive immunochemical techniques could probably be useful for diagnostic purposes or for defining individuals at risk.

To date, among newborn rats, rabbits, chickens, mice, and human patients, only the last two respond to the toxin [38]. Something about the maturation of mouse and human epidermis seemed to account for the fact that only newborn mice and children were susceptible [37,39]. More

TABLE I. *Effect of mouse age on the epidermolysin bioassay*

Epidermolysin injected (μg in 0.02 ml)	Time (min) for Nikolsky sign[a]		
	1 day[b]	3 days	5 days
2.6	15	20	25
0.65	50	60	70
0.16	75	110	145

[a] Mean of duplicate determinations.
[b] Age of recipient mice, in days.

recently, hairless adult mice [45,46], epilated adult mice [47], and, most importantly, adult human beings have been shown to be susceptible [47]. It is therefore not surprising that in the past two years several cases of TEN caused by group II staphylococci have been reported in adults [48–54].

Multiple Molecular Forms of Epidermolysin

Epidermolysin is found in multiple molecular forms [39–41]. In our preparations, fractions obtained from Pevikon electrophoresis contained only one or two minor substances which were examined by polyacrylamide gel electrophoresis (PAGE) and were at first thought to be contaminants. When these were subjected to chromatography upon CM-Sephadex, two peaks of protein were seen. Epidermolytic activity was measured by bioassay in both peaks, and each protein had the same apparent molecular weight (28,600) as determined by acrylamide gel electrophoresis in SDS. To determine whether we were dealing with electrophoretic variants of the toxin [41], we performed electrophoresis in PAGE on a fraction which contained about equal amounts of each moiety. The gels were cut, the proteins were eluted in buffer, and two distinct zones of epidermolysin were identified by bioassay. The two substances were immunochemically identical. Whether these different species of epidermolysin resulted from gene duplication or postsynthetic alteration of the gene product has not yet been determined.

Melish et al [39] suggested that epidermolysin exists in polymeric forms with different molecular weights. Kondo et al [40] described four substances, obtained from strain Zm of *S. aureus*, phage group II, type 55/71, which had exfoliating activity but different specific activities. Each of these separable moieties had the same molecular weight and was neutralized by antibody raised against one of them [40].

Toxins from Group I Staphylococci

Recently, the production of an exfoliating principle by staphylococcal strains not belonging to phage group II has been reported [40,55,56]. McClosky described a patient with a scarlatiniform eruption and necrotizing fasciitis from which *S. aureus*, phage type 85, was isolated. The organism produced epidermolysis in newborn mice [55]. Of the 52 isolates which were phage typed in a large group of children with toxic

epidermal necrolysis by Rasmussen, only one was group I, type 29; the remaining 51 were identified in phage group II [56].

Kondo et al recovered group I *S. aureus* from 1 patient with TEN and from 3 others with bullous impetigo [40]; all four organisms were sensitive to phage type 79. These authors showed that these strains produced a substance which had epidermolytic activity in newborn mice and also showed that the epidermolytic principle differed from epidermolysin since it was not neutralized by an antiserum to epidermolysin and did not precipitate with antibody to epidermolysin by double diffusion in agar.

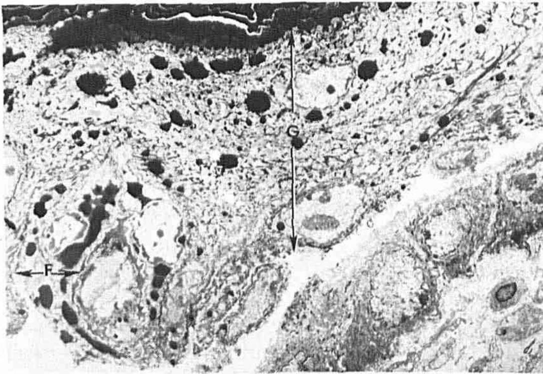

FIG. 6. The epidermis of a 3-day-old mouse 60 min after the injection of epidermolysin. Cleavage occurs below the granular cell layer of the epidermis (*G*) and the follicular infundibulum (*F*) (× 1,700).

Genetic Control of Epidermolysin Synthesis

Whether the epidermolytic toxin is under the genetic control of the bacteria, the bacteriophage, or plasmids that reside in the bacteria, poses another problem. Some bacterial toxins, e.g., diphtheria toxin, staphylococcal enterotoxin, streptococcal erythrogenic toxin, and probably staphylococcal alpha toxin, are under the control of a bacteriophage that has lysogenized the bacteria [57]. Plasmids are extrachromosomal genetic elements composed of double-stranded DNA, which may replicate independently of bacterial chromosomes; probably they are adventitious inhabitants of the cells and occur in all staphylococcal cells. Best established are the plasmids which contain genetic information for synthesizing penicillinase, but other plasmids are known to confer resistance to tetracycline and other antibiotics. The ability of staphylococci to synthesize delta toxin is also thought to be controlled by a plasmid [58]. Some recent work by Rogolsky et al [59] and Warren et al [60] has suggested that the genetic control of epidermolysin synthesis is not exerted by a bacteriophage but rather by the extrachromosomal genes of a plasmid.

FIG. 7. Keratinocytes in the plane of cleavage 45 min after epidermolysin injection. Peripheral cytoplasm (*) is edematous, desmosomes (*single arrows*) appear intact, and the intercellular spaces (*double arrows*) are not dilated (× 24,430).

Ultrastructural Studies of Epidermolysis

Electron microscopic studies of staphylococcal TEN in the mouse were first reported by Lillibridge, Melish and Glasgow [61] who suggested that the initial event was the disappearance of what they called "extracellular bubbles" between the cells of the stratum granulosum followed by the widening of the intercellular spaces and the splitting of the desmosomes. They also suggested that the "bubbles" are analogous to Odland bodies (lamellar granules, membrane coating granules, keratinosomes) and contain proteolytic enzymes which are released through the action of the toxin and then act on adjacent desmosomes to produce interdesmosomal cleavage.

In our ultrastructural studies, the lamellar granules in both the control and toxin-treated animals remained intact and were normally and abundantly distributed between the cells of the granular layer [62]. Wherever we observed epidermal cleavage, the cleft followed a plane immediately beneath the lowest granular cells except in sections containing developing hair follicles where the cleavage plane passed beneath the granular cell layer of the follicular infundibulum (Fig. 6).

After epidermolysin had been injected into a subcutaneous site on the back, specimens were removed at 15-min intervals. The dose was selected to give a positive Nikolsky sign 45 min after injection. No attempt was made, however, to abrade the skin or to create a plane of cleavage so that the ultrastructural changes could be evaluated in situ. The specimens obtained from normal control mice and from those injected with epidermolysin were identical at 15 and 30 min; in those obtained at 45 min, early changes had appeared along the plane between the granular and malpighian layers. In the cells adjacent to this zone, a marked swelling of the peripheral cytoplasm between desmosomal attachments was observed, but the width of the intercellular spaces remained normal (Fig. 7). By 60 and 75 min, the intercellular spaces had widened along the zone of cleavage. Initially intact, the desmosomal attachments subsequently separated through the interdesmosomal contact zone (Fig. 8); those which formed the cleft border had numerous microvilli and half desmosomes along their free surfaces (Fig. 9). Still later, some cell membranes were disrupted, and the alterations in cell organelles suggested cytolysis.

Elias et al have reported similar observations in specimens obtained from human patients [47]. The fact that the cells separated before the leakage of horseradish peroxidase or thorium dioxide into the separating cells suggests that separation occurred before cytolysis [63]. He also showed that surface coats of keratinocytes stainable by ru-

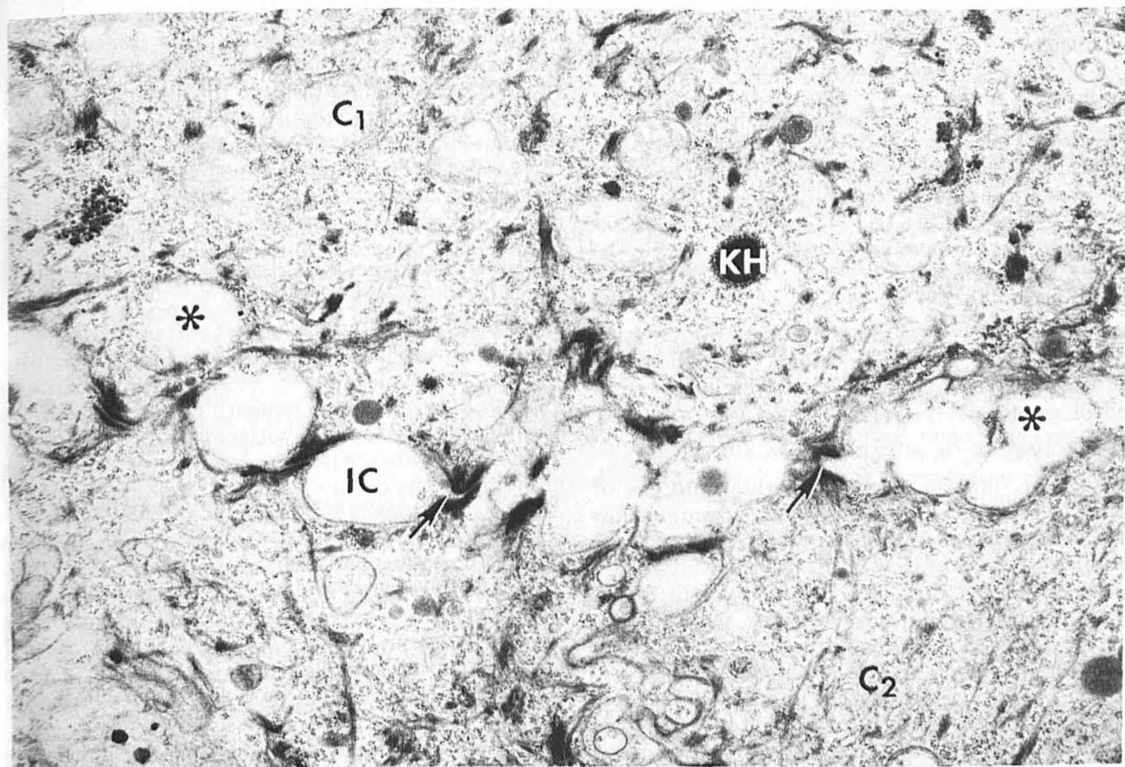

FIG. 8. The lowermost granular cell (C_1) and a cell from the malpighian layer (C_2) 60 min after the injection of epidermolytic toxin. Swelling of the peripheral cytoplasm (*) is prominent, intercellular spaces (*IC*) are wide, and desmosomes have started to separate (*arrows*). A keratohyaline granule (*KH*) marks the granular cell (× 18,950).

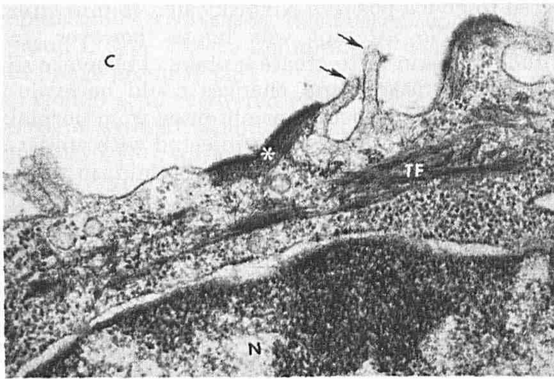

FIG. 9. Part of an epidermal cell bordering the epidermal cleft (C) 75 min after the injection of epidermolysin. The nucleus (*N*) and tonofilaments (*TF*) are intact. Microvilli (*arrows*) and half desmosomes (✱) are present along the free cell surface (× 34,000).

TABLE II. *Effect of epidermolysin in selected strains of mice*

Strain tested (no.)	Treatment[a]	C3[b]	C5[b]	Epidermolysis[c]
B10/D2 new line (6)	Saline	Norma	Normal	6/6
B10/D2 new line (6)	CoF	Low	Low	6/6
B10/D2 old line (4)	Saline	Normal	None	4/4
B10/D2 old line (4)	CoF	Low	None	4/4
Nude (athymic) (12)	None	ND[d]	ND	2/2

[a] Saline or cobra venom factor (Cordis) 1 U/gm injected intraperitoneally 24 hr prior to epidermolysin.

[b] Mouse C3 and C5 determined by double diffusion in agar.

[c] Number positive/number tested.

[d] ND, not done.

thenium red were not removed by epidermolysin, an indication that cleavage cannot be attributed to the removal of stainable surface acid mucopolysaccharides.

MECHANISM OF ACTION OF EPIDERMOLYSIN

To learn more about the normal process of keratinocyte adhesiveness and to determine how epidermolysin so dramatically disrupts this normal function of epidermis, we initiated a study of its mechanism of action. We considered four major hypotheses: (a) epidermolysin exerts an enzymatic activity specific for an epidermal substrate which is required for normal cell adherence; (b) epidermolysin has a cytotoxic effect by which it disrupts cellular function directly or activates a humoral mechanism which leads to cytolysis by an indirect action (c) epidermolysin labilizes lysosomal enzymes and thus leads to autolysis; (d) epidermolysin exerts a biophysical effect which disrupts normal cytoadherence.

Although there is no evidence that epidermolysin is an enzyme, a prototype of those enzymes which release epidermal cells from one another is trypsin [64], which cleaves polypeptide chains at carboxyterminal lysine or arginine. For example, trypsin cleaves the major glycoprotein of erythrocyte membranes (glycophorin) and thus releases much of the carbohydrate moiety into the fluid phase [65]. We have been unable to inhibit epidermolysin with trypsin inhibitors from soy beans or lima beans or by the tissue trypsin inhibitor Trasylol [41]. Additional evidence is available from the fact that few enzymes can withstand boiling to the same degree as epidermolysin. Finally, we have preliminary evidence that, unlike most enzymes, epidermolysin binds firmly to the cell surface of keratinocytes. When epidermolysin or bovine albumin was conjugated with the fluorochrome dye Fluorescamine and incubated with cyrostat sections or mouse skin, Fluorescamine-epidermolysin was seen to bind at the surface of keratinocytes [41].

There is also no evidence that epidermolysin exerts a direct cytotoxic effect. We considered whether it could exert an indirect cytotoxic effect by activating serum complement. If the terminal components C5–9 were activated in the vicinity of keratinocytes, membrane damage with decreased cellular adhesiveness might ensue. This possibility was investigated in newborn mice deficient in C5 (B10/D2 "old") or intact in C5 (B10/D2 "new"). In addition, groups of these mice were treated with the decomplementing factor in cobra venom (CoF) which depletes C3 and C5 via the alternative (properdin) pathway of complement activation. Complement components dependent upon C5, or C3 in the case of CoF-treated animals, were not required for epidermolysin action (Tab. II).

A third possible mechanism of epidermolysin action is its effect on epidermal lysosomes, which might lead to the release of constituents that are injurious to the cell membrane.

A purely biophysical effect of epidermolysin could alter electrostatic charges between cells and lead to their separation by frictional forces. Such a discrete cleavage plane beneath the granular layer is not seen, for example, with chaotropic ions of the Hofmeister series which exert their effect at the junction of the dermis and epidermis [66]. Nevertheless, this hypothesis deserves to be explored.

Despite mounting evidence for or against these four hypotheses, the mechanism of action of epidermolysin remains elusive. We are, however, confident that future investigations will not only produce data on the biochemical basis for epidermolysin action but lead to improved methods of studying other bullous diseases and to a better understanding of the normal epidermis.

We are indebted to Mrs. Cinda Lobitz, Mrs. Judith Pedersen, Ms. Joyce Beeman, and Ms. Yvette Frutiger for skillful technical assistance. Dr. Marvin Rittenberg kindly supplied the antiserum to mouse C5.

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