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THE STAPHYLOCOCCAL SCALDED SKIN SYNDROME: STUDIES ON CLINICAL FACTORS, PATHOGENESIS, MICROBIOLOGY, AND BIOCHEMICAL ASPECTS

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I. Introduction

Toxic epidermal necrolysis is one of the most acutely devastating skin diseases. In recent years considerable progress has been made in differentiating two distinct forms of the disease utilizing careful histopathologic study and newly developed animal models. In this paper we will focus upon the staphylococcal form of toxic epidermal necrolysis, also termed the staphylococcal scalded skin syndrome. A review at this time seems justified because: (1) considerable confusion still exists about the clinical and histologic features which clearly divide toxic epidermal necrolysis into two unrelated disease groups; (2) the incidence of the staphylococcal scalded skin syndrome appears to be increasing and physicians of numerous specialties can expect to encounter various forms of the disease sooner or later; and (3) the development of an animal model in 1970 produced much new information about the disease and its pathogenesis, and an overview of this information here may stimulate interest and further work in this field.

II. Terminology and Definitions

It is now generally recognized that toxic epidermal necrolysis (Lyell's Disease,TEN) comprises two distinct clinico-pathologic entities. Both forms are characterized by skin tenderness followed by widespread exfoliation and denudation (74). One form of varied etiology (e.g., 9,10,16-18,67,71,83,84) occurs chiefly in adults (Fig. 1), and carries a high mortality rate from attendant fluid, electrolyte, and colloid loss because the entire epidermis is damaged (83) (Figs. 2,3). Thus, the pathogenesis and attendant poor prognosis are similar to those of a massive second-degree burn. Very little is known about the pathogenesis of the entity, though the microscopic pathology, as well as some immunopathologic studies, suggest an asssult on

epidermal basal cells (19,79,83). The association of toxic epidermal necrolysis with graft-vs-host disease in man (83) and certain experimental animals (rat, hamster, monkey) (15) suggests an immunologic mechanism for at least some forms of this entity (15,100,111).

The second form, here termed the staphylococcal scalded skin syndrome (SSSS), primarily afflicts children under the age of five (Table 2) Fig. 4-6) (20,47,50,64,67,76,97). Though exfoliation may be as widespread as in non-staphylococcal toxic epidermal necrolysis, the mortality rate is low. This form is now firmly linked to underlying infection by staphylococci nearly always of phage group 2 (4,13,25,45,48,82), and their elaboration of distinctive substance(s) which cleave the epidermis beneath the stratum granulosum (Fig. 7) (35,56,62,111). In order to avoid future confusion between these two unrelated entities we will retain the terms toxic epidermal necrolysis (TEN) for the usual adult form and staphylococcal scalded skin syndrome (SSSS) solely for the staphylococcal disease.

III. Historical Aspects

The staphylococcal scalded skin syndrome was first recognized as a distinct clinical entity by Ritter von Rittershain in 1878. Ritter observed 297 cases during a 10-year period while directing a foundling hospital in Prague and furnished a most meticulous and exacting description of the disease, which he termed "dermatitis exfoliativa neonatorum" (89). This astounding accumulation of cases probably appeared partly because of poor hygienic conditions in his institution, and accompanied an endemic of staphylococcal omphalitis. However, after Ritter's retirement the disease seemingly vanished in his institution, presumably due to implementation of preventive hygiene measures. Ritter quite clearly stated that "dermatitis exfoliativa" is characterized by erythema and exfoliation, and that

blistering occurs only as a secondary feature (89,90). However, he also claimed that his disease was strictly limited to the newborn period, a conclusion which has subsequently proved to be less valid.

While Ritter considered the disease non-infectious, though of "pyemic" etiology, an association with staphylococcal infection was noted soon thereafter (49,103). Nevertheless, until fairly recently, doubts have been advanced concerning the role of staphylococci (21,63). Ritter's disease was classified as one extreme of the staphylococcal pyodermas -- "pemphigus benignus neonatorum" -- with impetigo at the opposite extreme (49,85,103). The characteristic histologic changes of SSSS however were described, though not interpreted correctly, at the end of the nineteenth century (12,24,98, 109).

Despite a trickling of case reports (e.g., 54,60,85,104), a puzzling forty year lapse ensued in our understanding of this disease. The most plausible explanations are: (1) general unawareness of Ritter's disease among dermatologists and pediatricians due to "lumping" such cases with bullous impetigo; (2) natural fluctuation in the incidence of the causative phage group 2 organisms; and (3) improved hygiene with a concommitant decrease in staphylococcal infection in infants. Eradication of staphylococci from perinatal and neonatal hospital units may have resulted in a relative increase in cases outside the neonatal period where, by definition, Ritter's disease does not occur.

Major progress was made by introduction of phage typing. Vast evidence showed that bullous impetigo and Ritter's disease were specifically caused by staphylococci of phage group 2, whereas such organisms were only rarely found in other types of staphylococcal skin infection (13,14,27,45,48,63,82, 96,99).

As early as 1952 Lee et al. advanced the hypothesis that a specific toxin was responsible (60), a view echoed by several workers prior to identification of the responsible toxin in the 1970's.

While awareness of toxic epidermal necrolysis was awakened by the simultaneous reports of toxic epidermal necrolysis by Lyell (65), and by Lang and Walker in 1956 (59), progress in understanding the staphylococcal scalded skin syndrome was dealt a setback by the erroneous "lumping" of Ritter's Disease with TEN. In fact, general unawareness of the distinctive features of staphylococcal scalded skin syndrome continues to stimulate both terminological confusion as well as advocation of inappropriate and inadequate therapy. The first case of staphylococcal scalded skin syndrome incorrectly classified with toxic epidermal necrolysis was probably Case #1 of Lyell himself (65), followed by Catto's first report of "TEN" in a young child (20) and many others (1,11.22.28,86,105). Only after another ten years did the differences between the "infantile" and "adult" form of TEN become generally accepted on the basis of detailed clinical (6,47,50,64,91) and histological studies (56,68). Unfortunately, Lyell's group (4-7) continues to link SSSS with other forms of the toxic epidermal necrolysis syndrome. This view still predominates in some current editorials and review articles (19,30,31,79,102), though the distinction has been clear since Melish and Glasgow described the mouse model of staphylococcal scalded skin syndrome (74). Their study and numerous others which followed proved conclusively the strict causal relationship between an extracellular toxin of staphylococcal origin and the varied manifestations of the staphylococcal scalded skin syndrome (5,33,51,57,75). Continued lumping of SSSS with other forms of TEN can only lead to further diagnostic confusion and therapeutic errors for both entities.

Since 1970 considerable progress has been made in isolation and chemical characterization of the responsible exfoliating substance (variously termed exfoliative toxin, epidermolytic toxin, or epidermolysin) (6,51,57,77, 111). Koch's postulates are largely fulfilled -- exfoliatin elaborated by staphylococci isolated from patients with SSSS produced a similar syndrome first in neonatal mice (5,51,77) and, most definitively, in human volunteers (33,108). Thus, all the diverse clinical forms of the scalded skin syndrome are now firmly linked to the elaboration of an exfoliatin by resident staphylococci. Finally, a plethora of recent reports of SSSS in human adults has focused new attention on the pathogenesis of the disease in both children and adults.

IV. Clinical Aspects

A. Clinical Expressions of Staphylococcal Scalded Skin Syndrome (Table I)

The staphylococcal scalded skin syndrome comprises a spectrum of disease ranging from a purely localized form - bullous impetigo - to generalized exfoliation. Most cases fall into either the purely localized or generalized form but important intermediate forms exist between these two extremes.

Lyell reported a slight preponderance of males over females in his review (67), and Rasmussen noted a relatively high incidence in Caucasians relative to Blacks (87). The reasons for these propensities are unclear. Children, particularly neonates and infants (Table 2), are responsible for almost all cases of SSSS. No evidence of antecedent or subsequent immunologic or metabolic compromise has been noted in children with either localized or generalized forms of the disease, though the few adult cases have usually occurred with overwhelming infection (37,94), immunologic incompetence (46,61,83), and/or metabolic compromise (61,88). Recent evidence (44) suggests that the age incidence of SSSS may be a direct reflection of postnatal renal development.

(1) Generalized Forms: Dermatitis exfoliative neonatorum (Ritter von Rittershain) and other forms of gernalized childhood scalded skin syndrome are not considered part of the same nosologic entity (76). Cases of generalized SSSS usually occur in children under five years of age (Table 2) (Figs. 4,6) (reviewed in 20,47,50,64,70,76,81,87,92,97). Initially, a distinctive, faint, macular, yellow-orange-brick-red colored rash erupts following purulent conjunctivities or an upper respiratory infection. Even at this time the presence of cutaneous tenderness, the characteristic distribution (the central portion of the face, neck, axillae, and groin) (Fig. 4,6), and often, a positive Nikolsky's sign, permit early diagnosis even prior to spontaneous wrinkling. In addition, affected children often exhibit a typical facies - a sad, lacrimose expression with impetiginous crusts around facial orifices (Fig. 4,8). Within 24-28 hours the disease usually progresses from a scarlatiniform eruption to spontaneous wrinkling and the appearance of large, flaccid bullae (Fig. 5). As the upper part of the epidermis separates in sheets and ribbons a moist erythematous base is revealed. This dries quickly and recovery, accompanied by post-inflammatory desquamation, is usually complete within five to seven days, especially with prompt institution of antibiotic therapy. But even without appropriate treatment recovery is the rule (see below). While the initial denuding process may involve the lips, severe mucosal involvement usually does not occur.

Since the fluid from intact bullae is usually sterile (76), it is generally assumed that the disease is caused by elaboration of a staphylococcal exotoxin from a distant site of infection (usually purulent conjunctivitis, pharyngitis, or otitis media). Reports of positive skin cultures from these patients are probably due to secondary seeding of denuded skin from the primary focus of infection.

(2) Localized Forms: An association between phage group 2 staphylococci and bullous impetigo has been noted for years (27,99). Although patients with bullous impetigo seldom receive the diagnostic consideration accorded patients with generalized SSSS, the majority of patients harbor phage group 2 staphylococci (27,76). As with generalized SSSS, bullous impetigo is primarily a childhood disease though cases do occur in older patients (32). Bullous impetigo may spread and become generalized so that some of these distinctions can blend. Presumably important host differences account for the manifestation of localized vs. generalized staphylococcal scalded skin syndrome in a given patient (see Section V.C. below).

(3) Abortive Forms: There have been several case reports of a scarlatiniform eruption accompanying staphylococcal infections (3,29,41,70,73,76, 101), but Melish and Glasgow were the first to document that the condition may halt spontaneously at the scarlatiniform stage without advancement to frank exfoliation (76). They suspect that this clinical presentation is probably fairly common, but correct diagnosis depends upon a high index of suspicion. The presence of skin tenderness, a positive Nikolsky's sign, and occult staphylococcal infection should permit differentiation from scarlet fever, drug eruptions, and viral exanthems. Whether diagnostic histopathologic or ultrastructural changes (see Section VI) are present at this stage is unknown.

(4) Adult Forms: In the last two years many reports of staphylococcal scalded skin syndrome in adults have been published (32,37,46,61,65,88,94). To date all of these cases have been bullous impetigo, i.e., localized disease, with varying degrees of extension beyond the site of infection, since in all except one case (46), phage group 2 staphylococci were cultured from intact bullae. An impaired host response or overwhelming infection may have been responsible for spread of the infection. In contrast, adult cases of generalized scalded skin syndrome, characterized by sterile bullae, such as

those which develop in children with occult infection have not yet been reported. The explanation for this is suggested by experimental studies which indicate that adults possess an enhanced capacity to metabolize the staphylococcal exfoliating toxin (34,44). A dramatic example of the differences in the capacity of adults and children to handle the disease are cases of bullous impetigo occurring on the breasts of mothers breast-feeding neonates with Ritter's Disease (e.g., 53,81). Whether such cases will yet occur against a background of metabolic, i.e., hepatic and/or renal, insufficiency alone remains speculative.

Whether specific host factors play a role in the rarity of bullous impetigo in adults, or whether adults simply possess a lesser tendency to develop bullae is not known. The ease with which injections of staphylococcal exfoliating toxin produce bullae in adult humans and mice (34) strongly suggests that the latter is probably not correct (vide infra).

B. Differential Diagnosis.

(1) Differentiation from Other Dermatoses: Although the diagnosis of advanced disease is immediately apparent, TEN and SSSS in their pre-exfoliative stages may mimic a variety of other dermatoses. A review of our own material reveals that the initial diagnoses have been as varied as sunburn, eczematous dermatitis, Leiner's Disease, erythema multiforme, scarlet fever, and exfoliative erythroderm. Unfortunately for the clinician, the gross appearance of the rash in its early stages is non-diagnostic, though even at these times cutaneous tenderness and a positive Nikolsky's sign may herald the approaching exfoliative stage.

In advanced cases, SSSS can be differentiated from other bullous diseases with relative ease. First, SSSS and TEN are characterized by cutaneous tenderness. Second, in contrast to other bullous diseases, lesions are not focal, but coalesce to involve broad portions of the cutaneous surface.

Third, the tendency to exfoliate is so marked that intact bullae are encountered relatively infrequently. Nevertheless, despite the distinctive features of both TEN and SSSS, many authorities, including Lyell (69), consider TEN a form of erythema multiforme.

(2) Differentiation of TEN from SSSS (Table 3): The differential diagnosis of toxic epidermal necrolysis vs. staphylococcal scalded skin syndrome can no longer be assumed on a basis of age alone: adults contract SSSS, and children non-staphylococcal TEN (e.g., 14). While the clinical features, cultures, and biopsy material allow eventual differentiation of the two entities (Table 3), considerable time may elapse before the necessary laboratory data are available to the clinician, who may feel compelled to initiate therapy quickly. Since the prognosis and morbidity may be affected by the initial choice of therapy (95), the clinician needs rapid diagnostic methods so that appropriate therapy can be quickly instituted. Two such methods are available (28,43): first, frozen sections of bullae roofs will reveal the level of cleavage; second, exfoliative cytology of lesions will reveal the affected cell population, i.e., acantholytic keratinocytes in SSSS (Fig. 10,11) (28,33,43) or inflammatory cells, cellular debris, and basal keratinocytes in non-staphylococcal TEN (Fig. 12).

C. Management.

Since the large majority of Group 2 staphylococcal isolates from patients with SSSS have been penicillin-resistant, it is now generally accepted practice to administer semi-synthetic, penicillinase-resistant penicillin analogues to afflicted patients (76,95). Although all these drugs are comparably effective, methicillin has been the most popular for parenteral usage (74), while dicloxacillin is the most logical choice for oral therapy. Studies on the effectiveness of alternate drugs in the penicillin-allergic patient have not been reported, though our choice would be

a bacteriocidal agent such as cephaloridin (some cross-reaction with penicillin may occur), with the bacteriostatic agents, erythromycin or minocycline, as less satisfactory alternates. Fortunately, rapid recovery is the rule regardless of the initial drug chosen (87), although complications and deaths still occur (Table 4).

On the other hand, administration of steroids alone to patients with SSSS is contraindicated on strong experimental grounds (34,75,108). Steroids aggravate SSSS in neonatal mice, permitting exfoliation at 1/100-1/1000 the dose of organisms required to produce the syndrome in untreated mice (75,108). Furthermore, in adult mice, prolonged, massive steroid administration resulted in generalized SSSS in animals who, if untreated, did not develop either localized or generalized disease (34). Finally, immunological incompetence has been considered the pivotal factor in most of the adult patients with SSSS (88). Taken together, these data provide a cogent argument against the administration of steroids alone to patients with SSSS. Whether steroids administered in conjunction with antibiotics ameliorate or worsen the disease is still not known.

Skin Care: Patients with generalized SSSS have temporarily lost their barrier against percutaneous water loss. Therapy should include careful attention to the patient's fluid, electrolyte, and colloid status, with appropriate replacement as needed. Topically, the patients should be treated as for an extensive, infected second-degree burn wound until both their infection is treated and their barrier function is restored. We have employed cool, continuous wet dressings with 0.5% silver nitrate alternating with applications of Sulfamylor^{R} or silver sulfadiazine cream. In addition, utilization of a rotating Striker^{R} frame may facilitate application of topical medications to all cutaneous surfaces as well as hastening re-epithelialization.

Fortunately, because of the high cleavage plane in SSSS, the water barrier is usually quickly regenerated so that topical therapy can generally be appropriately modified within a few days of admission. During the post-inflammatory, desquamative phase application of a bland cream, such as Eucerin^{R} or hydrated petrolatum usually suffices.

D. Prognosis and Sequelae.

Although recovery is the rule in SSSS, fatalities still occur, particularly in newborns with generalized SSSS (Table 4). Despite current controversy about the influence of the administration of antibiotics on the outcome of SSSS (87), the appreciable morbidity and mortality which still attend this disease makes such therapy advisable. Reported complications include sepsis, cellulitis, and pneumonia (these seem to occur more frequently in children mistakenly treated with steroids, e.g., 41), while fatal outcomes have been due to overwhelming sepsis and fluid-electrolyte imbalance.

In contrast to TEN, which often leaves scarring, milia, atrophy, and pigmentary residua, SSSS usually heals without scarring (Table 3). The differences in sequelae reflect the entirely different pathogenesis of the disease, i.e., the level of cleavage with respect to the basement membrane (vide infra).

V. Microbiology, Animal Models, and Experimental Staphylococcal Scalded Skin Syndrome

A. In vitro and In vivo Conditions for Exfoliatin Elaboration.

In vitro techniques: While phage group 2 staphylococci, like other staphylococcal strains, can be readily cultured on standard media, the requirements for exfoliatin elaboration are more stringent. Generation of the toxin in vitro requires culture in a semi-solid, enriched nutrient medium in a 10-20% CO_2 atmosphere at 37^{o} C (5,51). Under these conditions exfoliatin appears in culture supernatants within 8-10 hours, and maximum concentrations are attained in 24-48 hours. Exfoliatin-rich supernatants can then be easily harvested and concentrated by dialysis, ammonium sulfate precipitation, and

column chromatography (Table 5).

In vivo method: Melish et al and subsequent workers found that phage group 2 staphylococci elaborate exfoliatin if cultured in dialysis bags implanted in the peritoneal cavity of rabbits or guinea pigs (75,110). After 24-28 hours, the dialysis bags contain large quantities of exfoliatin. Since supernatants obtained in this manner do not contain broth constituents, subsequent purification is easier than with the in vitro method (vide supra).

B. Genetics of Exfoliatin Elaboration.

Extensive studies on the genetics of exfoliatin synthesis are underway in Glasgow's laboratory: Exfoliatin elaboration can be eliminated by incubation with ethidium bromide or sodium dodecyl sulfate, indicating that elaboration of the staphylococcal exfoliatin is primarily under the genetic control of a gene located on a plasmid (93,106). Furthermore, as with other staphylococcal plasmids, toxin production was sensitive to culture at 44° C. Recently, the extrachromosomal DNA which codes for exfoliatin has been isolated and characterized (107). But phage group 2 staphylococci that have lost their plasmid still can elaborate small amounts of exfoliatin (57). This may explain the occasional outbreak of SSSS from non-phage group 2 organisms (40,60). Finally, we have observed a marked tendency for organisms to cease the elaboration of exfoliatin following serial passage, even at 37°C (Elias, P., Fritsch, P., Mittermayer, H., unpublished observations).

C. Experimental Production in Man and Animal Models.

1. Newborn and Adult Mouse Models: Thorough study of the staphylococcal scalded skin syndrome requires the availability of animal model(s) both for studies on pathogenesis and for bioassay of isolated toxin fractions. Thus the entire field gained tremendous impetus with the description of the neonatal mouse model by Melish and Glasgow in 1970 (74). Injection of group 2 organisms isolated from patients with staphylococcal scalded skin syndrome

into neonatal mice produced exfoliation which clinically and histologically resembled that found in human disease (Table 6) (Fig. 13,14). Susceptibility to injected organisms is greatly dependent both on the initial inoculum (Table 7) and on the animal's age (26,34,74,111): Doses of less than 10^5 organisms generally fail to produce exfoliation; furthermore, at five to seven days of age mice suddenly become refractory regardless of the initial inoculum. A minimum incubation period of 9-12 hours is required following inoculation, regardless of the numbers of administered organisms (Table 7) (34). The neonatal mouse soon was found to be highly susceptible to the isolated exfoliating substance as well (Table 6) (5,51,77), but in this case the incubation period following toxin administration is much shorter (average two hours), and dose-dependent, occurring in as little as 20-30 minutes with more potent preparations (Table 8).

Since SSSS is primarily a disease of young children, the resistance of older animals to both organisms and exfoliatin seems to correlate nicely. The assumed cause for adult resistance was thought to be either the development of hair or undefined maturational factors (77). However, more recent documented SSSS in both "normal" and immunologically compromised human adults (vide supra). On re-examination of the mouse model, we found that although normal adult mice resisted injected organisms, animals pretreated with massive doses of steroids developed a mild form of generalized SSSS (Table 9) (Fig. 15,17) (34). Furthermore, while adult mice resisted <u>systemically</u> administered toxin, normal (34) and hairless (7,52) adult mouse skin overlying sites of intracutaneous inoculation exfoliated readily (Table 10) (Fig. 16).

2. Exfoliatin Metabolism: While it is now clear that an intact immune status protects adult patients from getting SSSS, the rarity of the syndrome over the age of five may also reflect an enhanced metabolic capacity of older individuals. Adult resistance is probably not due to syn-

thesis of protective antibodies, since prior treatment of mice with exfoliatin does not render them less susceptible to localized disease production later in life (Table 11) (35). Although direct evidence for this is lacking, the adult human is presumably able to metabolize the toxin since systemically administered exfoliatin even in large doses does not produce exfoliatin in normal adult mice (Table 10) (34). Furthermore, distribution of iodinated, purified exfoliatin indicated that the toxin is excreted rapidly by adults but not newborns (Fig. 29) (44). Finally, either nephrectomized or carbon tetrachloride-poisoned adult mice develop generalized SSSS following systemic administration of exfoliatin (44). It seems likely that cases of SSSS will eventually be reported not only in immunologically compromised patients, but also in humans with metabolic derangements as well.

Experimental Production in Human Skin: Human skin is exquisitely sensitive to exfoliatin: Both injections in adult human volunteers (Table 12) (Fig. 18,19), as well as quantitative studies utilizing as in vitro system (Table 13) (33). Again, these studies indicated that neither age nor hairiness provide a significant barrier to SSSS production.

Other Animal Models: Using both in vivo injections and the in vitro system (see below) we have screened numerous other mammalian and two non-mammalian species for susceptibility to the exfoliatin. Among species tested to date - hamester, monkey, and human skin are susceptible (36) (hamsters are less susceptible than mice, however) (Table 16). On the other hand, rat, guinea pig, rabbit, dog, frog, and chicken skin are resistant (Table 15) (36,51). Despite the close phylogenetic similarities of the rat and mouse, for example, they have opposite sensitivities to toxin. Thus, exfoliatin activity appears to be highly species specific. The basis for both susceptibility and resistance are discussed in Section VII.

D. In Vitro Models of Staphylococcal Scalded Skin Syndrome

The phenomenon of exfoliation accompanying the scalded skin syndrome in vivo is mimicked when skin from susceptible species is exposed to comparable doses of exfoliatin in vitro (Table 14) (Fig. 20) (33,72). The in vitro system has provided valuable information about the relative susceptibility of various age groups, species, as well as hairy vs. glabrous skin (all are comparable) (33,36). Furthermore, the relative susceptibility of keratinizing cutaneous, keratinizing extracutaneous, and non-keratinizing epithelia have been screened (Table 17) and the sources of resistance localized to the epithelia itself (Table 18) (36). Most recently, we have tried to modify in vitro exfoliation with various metabolic inhibitors in order to discern the mechanism of action (see Section VII below).

VI. Biochemical Characterization of the Staphylococcal Exfoliatin

Exfoliatin has been purified in five laboratories from phage group 2 staphylococci grown in vitro (6,51,57,77,111). All these preparations cause splitting of mouse epidermis beneath the stratum granulosum identical to that caused by inoculation with living bacteria. Kapral and Miller purified the exfoliatin by passage of crude culture supernatants through membranes that allowed passage of material of molecular weight 10,000 to 50,000 followed by repeated chromatography on DEAE Sephadex (51).

Subsequent investigators have first fractioned the crude culture supernatant by ammonium sulfate precipitation (at 40% to 80% saturation) (Table 5). This results in an approximately three-fold purification. This material was then further purified either by column isoelectric focussing or by column chromatography on Sephadex G-75 and DEAE-cellulose followed by preparative polyacrylamide disc gel electrophoresis (57). The former technique produces material that gives a single band on sodium dodecyl sulfate (SDS)-polyacrylamide disc gel electrophoresis but separates into two fractions of differing pI, and each such fraction contains two immunologically distinct

components. When the two components are re-focussed by thin layer gel isoelectric focussing, multiple components are separated but much of this heterogeneity disappears when urea is added to these gels. The authors concluded that only two components of different covalent structures were present (8). The latter technique separates four bands of differing electrophoretic mobility, all of which have exfoliating activity and all of which are inactivated by antibody produced by immunization with one of them. Similar results have been reported by Wuepper et al using Pevikon block electrophoresis at pH 9, followed by carboxymethylcellulose chromatography. The most highly purified preparation cause newborn mouse skin to exfoliate when 0.25μg of protein is injected subcutaneously and represent 150 to 800 fold purification over the crude culture supernatant (77,111).

The staphylococcal exfoliatin is antigenic (34,58,78,111), and pretreatment of toxin fractions with specific antibody protects newborn mice and humans from exfoliation (33,34,78) (Table 19) (Fig. 17).

The properties of the exfoliatin isolated from each of the five laboratories are similar (6,51,57,77,111). Exfoliatin has a molecular weight on Sephadex chromatography of approximately 24,000 (53,59), on SDS-polyacrylamide gel electrophoresis of 25,000 or 28,600 (8,111), and on ultracentrifugation of 32,000 (75,111). It is precipitated and inactivated at pH 4, is stable to heating at 60^{o} for one hour, but inactivated by heating at 100^{o} for prolonged periods (51,57,77,111), and is inactivated 80-100% following exposure to the proteases pronase, trypsin, or pepsin (77). The pI on isoelectric focussing is 7.1, although a second, minor peak of activity is found at 6.0-6.2 (8), and these two fractions differ on polyacrylamide gel electrophoresis even in the presence of high concentrations of urea.

More recently, an exfoliative toxin has been isolated from non-group 2 staphylococci (58). This differs in being precipitated at a lower ammonium

sulfate concentration, being less stable to storage at -30° (group 2 exfoliatin is stable for a year at -30°) and to heating (inactivated at 60° for 30 minutes). In addition, antibodies directed against phage group 2 exfoliatin or non-group 2 exfoliatin do not cross-react. Both, however, have a molecular weight of 24,000 by Sephadex chromatography in the same laboratory and are purified by similar techniques.

Purified exfoliatin precipitates with concanavalin A (Con A), this being evidence that it contains branched carbohydrates, and exposure to exfoliatin enhances the agglutinability of mouse embryo fibroblasts when treated with Con A in vitro (99). In this study, 9% of the weight of purified exfoliatin was carbohydrate, and no lipid was found. In addition, this preparation had no proteolytic activity toward denatured casein (99). No amino acid analysis of the toxin has been published.

VII. Pathogenesis and Mechanism of Exfoliation

A. Light Microscopic and Ultrastructural Pathology

Because of the tendency to lump SSSS with TEN the distinctive histopathologic features of each escaped attention until fairly recently (56). In localized or generalized SSSS cleavage occurs at the level of the lower stratum granulosum (Fig. 7). Splitting is accompanied by minimal necrosis, and acantholytic cells float in the cleavage space (Fig. 7,10). By electron microscopy the cleavage plane of lesions in humans or mice is at the spinous-granular layer interface (33,34,34,62,111), with some apical extension into the lower granular layer, and splitting appears to occur without damage to adjacent, acantholytic keratinocytes (Fig. 21,22). In some studies there is disagreement whether the primary sites of attack are desmosomes (62), interdesmosomal regions (35,111), or both simultaneously (33,35).

B. Pathogenesis and Subcellular Site of Action

Until recently, information about the pathogenesis of the staphylococcal scalded skin syndrome was limited to purely descriptive light and

electron microscopic studies of the disease in man and the newborn mouse. As we shall show, knowledge has progressed from recognition of the distinctive intraepidermal cleavage plane (56), to proof of the essentially intercellular nature of the cleavage process (35), and finally to a search for the subcellular cell surface target utilizing cytochemical, immunological, and biochemical techniques.

Although to be suspected from the unperturbed appearance of acantholytic cells lying within and along cleavage space, the non-toxic nature of the exfoliating process to keratinocytes has been demonstrated in several ways (35): (1) Perfused, water-soluble tracers [horseradish peroxidase, thorium dioxide (Thorotrast^{R})] remain segregated within intercellular domains during all stages of acantholysis; (2) Several cultured cell lines of keratinocyte and non-keratinocyte origin remain unperturbed even after prelonged exposure to high concentrations of exfoliatin (Fig. 23,24); and (3) Intraepidermal cell surfaces, exposed during exfoliation and examined both by scanning electron microscopy and by transmission electron microsocpy of surface replicas reveal smooth, unbroken membrane surfaces. Therefore, all available evidence (35) suggests that exfoliation is primarily an extracellular, non-cytotoxic process.

Despite several studies, the subcellular site of attack of the exfoliatin remains an enigma. Currently, we know that this substance does <u>not</u> significantly remove binding sites for (35): (1) ruthenium red (presumably acid mocopolysaccharides) (Table 20) (Fig. 25); (2) Con A (presumably neutral, branched polysaccharides); (3) pemphigus vulgaris antibody (Table 21); and (4) certain HL-A antigenic sites shared by lymphocytes and keratinocytes. However, the inability to detect a morphologically altered site may only reflect the insensitivity of the various assays employed, and future studies might still demonstrate selective (or secondary) removal of some of these constituents. Alternatively, it is also possible that the exfoliatin: (a)

may attack still other chemical substrates in the intercellular space; or (b) attach to species and tissue-specific binding sites on the cell membrane surface in a manner analogous to several other cytotoxins, bacterial exotoxins, and protein hormones.

Recent studies on the susceptibility of several different species and extracutaneous epithelia tend to support the concept of specific binding sites: Responsiveness is remarkably species (see above) and tissue-specific (36). These studies have been greatly facilitated by an in vitro system which permits rapid, accurate evaluation of susceptibility to exfoliatin (33). Furthermore, both susceptibility and resistance seem to be characteristics of the epidermal keratinocyte itself, since dermis and plasma contain no inhibitory (or facilitating) substances (36). These studies suggest that sensitivity to the staphylococcal exfoliatin is an inherent, genetically-determined characteristic endowed to certain species. Moreover, among these species, the epidermis is the only keratinizing epithelia which can be reproducibly and unequivocably cleaved (Table 17), indicating remarkable tissue specificity as well (Fig. 26-28) (36).

C. Mechanism of Action

As indicated above, a substantial number of studies point to an intercellular target, or possibly a specific receptor-toxin interaction as the basic mode of exfoliatin action. Since dead murine epidermis (frozen-thawed, heat-treated, formalin-fixed) does not respond to the exfoliatin, we deduced that intracellular metabolic processes must be mobilized as part of the exfoliative process. We have defined the requirements for in vitro exfoliation and attempted to interfere with exfoliation by utilizing specific metabolic inhibitors (Elias, P., Epstein, E., Jr., unpublished observations). In vitro exfoliation occurs somewhat more rapidly at 37^{o} C than at room temperature and effectively ceases at 4^{o} C. Cleavage occurs over a wide pH range (pH 5 to 9, but not at or below pH 4), and in a 100% nitrogen atmosphere.

Though treatment with cycloheximide or puromycin produced a 95 to 90% decrease in epidermal protein synthesis, they did not block in vitro exfoliation. In addition, pretreatment of susceptible tissues with sodium azide, dinithrophenol, 2-deoxyglucose, vinblastine, and soybean trypsin inhibitor did not prevent cleavage. Furthermore, trypsin inhibitors reportedly likewise do not block exfoliation in vivo (111). These negative studies do not rule out metabolic intracellular participation since other mechanisms (or additive mechanisms) may be involved: or our assay system may not detect changes which are physiologically relevant.

VIII. Summary

In this review the essential clinical features of the staphylococcal scalded skin syndrome and other forms of toxic epidermal necrolysis have been contrasted. Whereas TEN is a devastating disease of multiple etiologies and high fatality affecting all age groups, SSSS comprises a spectrum of clinical entities, occurring primarily in early childhood, caused by certain phage group 2 staphylococci. These organisms elaborate an exfoliating substance which cleaves the epidermis beneath the stratum granulosum, producing localized or generalized cutaneous exfoliation. Because of this high level of cleavage the water barrier is only transiently perturbed and rapid recovery is the rule. Although the early stages of SSSS may resembly other widespread dermatoses clinically, the correct diagnosis is suggested, even prior to frank exfoliation, by the presence of cutaneous tenderness and a positive Nikolsky's sign. While the definitive diagnosis must await culture and biopsy results, rapid bedside confirmation can be made with exfoliative cytology and/or frozen sections of desquamating sheets, which reveal individual squamous cells and the subgranular split, respectively.

Recent availability of in vivo and in vitro animal models of the disease have advanced knowledge of SSSS. The responsible exfoliatin has been extensively characterized and the purely extracellular pathogenesis of the

disease process are now appreciated. The exfoliatin is strikingly species and tissue specific. Of species studied, thus far, only the mouse, hamster, monkey, and man are susceptible, and in these species all non-keratinizing and most extracutaneous keratinizing epithelia are spared. The lower incidence of SSSS in adults is not due to maturational changes, hairiness, or immunity, but rather to superior host capability for catabolism and excretion of the exfoliatin, as well as more efficient immune capabilities. The mechanisms of exfoliatin action and the molecular site of action are still the focus of active investigation.

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Table 1: Clinical Forms of Scalded Skin Syndrome

Disease	Synonyms	Cultures from Intact Bullae	Age Distribution
Bullous impetigo	Pemphigus neonatorum	+	All ages
Bullous impetigo w. generalization	Pemphigus neonatorum	+	Biphasic (neonates and immune-compromised adults)
Scarlatiniform rash	None	-	Neonates and young children
Generalized scalded skin syndrome	Toxic epidermal necrolysis, Ritter's Disease, Lyell's Disease	-	Neonates and young children

Table 2: Age Incidence (Years) of Staphylococcal Scalded Skin Syndrome*

	First+	Second	Third to Fifth	Fifth to Eleventh
Number of Patients	77	58	71	8

Total: 312

Average Age: 21 months

*Totals are drawn from Jefferson (52), Melish and Glasgow (81, 82), Rudolph et al (102), Margileth (75), and Rasmussen (92).

+Approximately 1/3 of the cases in the first year occur in the first month of life.

Table 3: Differentiation of Toxic Epidermal Necrolysis and Staphylococcal Scalded Skin Syndrome*

	Toxic Epidermal Necrolysis	Staphylococcal Scalded Skin Syndrome
History	Drug intake Often milder episodes preceding	No drug intake First episode
Family history	Non-contributory	Members of family often have impetigo or harbor staphylococci
Epidemiology	Cases sporadic	Sometimes linked to epidemics of impetigo
Age predilection	Over forty	Under five
Exanthemata	Generalized without clear distribution pattern	Typical distribution pattern and succession of development (face, neck, axillae, groin first)
Cutaneous tenderness	Mild to moderate	Marked
Nikolsky's Sign	Positive only in lesions	Positive also in apparently uninvolved skin
Mucous membranes	Severely afflicted	Uninvolved
Course	Protracted (1-3 weeks)	Brief (2-4 days)
Mortality	High (25-50%)	Very low; high incidence of spontaneous recovery
Systemic therapy	High corticosteroids, water, electrolyte and blood volume maintenance	Penicillinase-resistant penicillins; corticosteroids alone contradindicated
Histology+	Necrosis of epidermis, starting in basal layer	Acantholysis; subgranular cleavage plane
Exfoliative cytology+	Necrotic epidermal cells, polymorphs, debris	Normal-appearing acantholytic cells

*This table contains only points where differences exist between the two diseases, and only considers the rules not the exceptions.

+Particularly useful for rapid bedside differential diagnosis.

Table 4: Mortality of Staphylococcal Scalded Skin Syndrome in the Antibiotic Era

	Cases	Deaths	Percent
Holzel and Jacobs (1966)	6	-	-
Lowney et al (1967)	10	1	10
Jefferson (1967)	31	1	3
Lyell (1967)	30	-	-
Samuels (1967)	42	2	5
Melish and Glasgow (1971)	28	2	7
Rudolph et al (1974)	18	-	-
Margileth (1975)	42	1	2
Rasmussen (1975)	95	1	1
Totals:	312	7	2.5%

Table 5: Toxin Isolation and Preliminary Purification

1. Cultured in semisolid agar medium containing 5 g Trypticase soy broth and 25 g nutrient broth per liter at 37°C under 20% CO_2 for 48 hours.
2. Centrifuged at 3,000 RPM at 0-4°C for 45 minutes.
3. Supernatant passed through millipore filter (pore size 0.45μ).
4. Supernatant purified by 40% and 80% $(NH_4)_2SO_4$ precipitation and dialysis.
5. 80% $(NH_4)_2SO_4$ precipitate purified further by ion exchange chromatography on CM-Sephadex C50.

Table 6: Activity of Staphylococci and Sterile Cell-Free Filtrates

Strain	Phage type	Live Organisms	Sterile filtrates
A	3A/3C/55/71(Gr.II)*	+	+
B	3C/55/71 (Gr.II)	+	+
C	29/80/42E/54(Gr.I)	-	-

*Obtained from Cross Infection Reference Laboratory, London, England

Table 7: Time/Dose Response of Newborn Mice to ET-Producing Cocci In Vivo

Group 2 (strains A & B)	Time Hours				
	3	6	12*	24+	(# with TEN)
10^6 - 10^9 cocci	-	-	10/10	10/10	
10^5	-	-	0/10	4/10	
10^4	-	-	0/10	0/10	
Non-Group 2 (Strain C)					
10^7 - 10^9	-	-	0/15✤	0/15+✤	

*TEN became evident between 9-10 hours

+several animals died or were cannibalized

✤intense erythema appeared without a positive Nikolsky sign

Table 8: Time/Dose Response of Neonatal Mice to ET

Source	Concentration (mg/ml)	Approx. % Animals with TEN[a]			
		30 min	60 min	2 hrs	4 hrs
Strain A or B (Group 2)	> 10	100	100	100	100
	5-10	-	75	100	100
	1-5	-	25	100	100
	0.5-1.0	-	-	100	100
	0.1-0.5	-	-	50	100
	0.05-0.1	-	-	25	75
	0.01-0.05	-	-	-	25
	< 0.01	-	-	-	-
Strain C[b] (non-Group 2)	> 10	-	-	-	-

[a]number of animals tested within each concentration range varied from 10 to 200.

[b]intense erythema developed within 2 hrs, but a positive Nikolsky sign was never demonstrable.

Table 9: TEN in normal and steroid-treated adult mice. All animals injected with 5 x 10^{10} cocci (strain 1) into chemically depilated skin. Tested for TEN in distant sites 24 hrs after injection.

	Animals with TEN		
	1 wk	2 wk	3 wk
Prednisolone (1-5 mg/day)	0/20*	0/20*+	6/6+
India ink alone	0/4	0/4	0/4
Sham injected	0/4	0/4	0/4

*Half the animals in each group received 2-3 ml india ink IV 30 min prior to injection of cocci.

+Several animals died after the second week, either of unknown causes, or following third inoculation of cocci prior to development of TEN.

Table 10: Influence of Route of ET Administration on Subsequent TEN in Adult Mice

Route	Dose(mg)	Sites	No. +	Type of Reaction	Comment
Intracutaneous	1	back, abdomen (epilated) ear (non-treated)	14/14	Localized	Both chemically epilated and non-treated skin are susceptible
Systemic	10-25	intraperitoneal, intramuscular	0/9	None	Local reactions sometimes occurred due to leakage over injection sites

Table 11: Re-administration of ET to Mice Surviving Previous ET-Induced TEN

Group	Ages	Doses (mg protein)	# animals with TEN
-	NBM (3-5 days)	1	12/12
1	21-28 days	1-10	6/6
2	2-3 months	1-10	9/9*

*3 animals from Group 1 were included in Group 2 for later testing.

Table 12: TEN Production in Human Thigh Skin In Vivo. Summary of patients injected with ET; varying doses reflect differences in batch potency and total fluid injected. Patient 2 was the only male tested.

Patient	Age	Dose(mg/protein)	Clinical Observations	Histology
1	76	9	spontaneous wrinkling; + Nikolsky - 2 hrs	cleavage
2	26	10	spontaneous wrinkling; no inflammation	not done (n.d.)
3	32	1.0-10	spontaneous wrinkling moderate erythema	cleavage
4	73	5.0	spontaneous wrinkling; erysipelas-like plaque	not done (n.d.)
5	29	0.3-1.0	spontaneous wrinkling; erysipelas-like plaque	n.d.
6	79	0.05-0.5	spontaneous wrinkling; frank bulla; moderate erythema	cleavage
7	54	0.05-0.5	frank bulla	n.d.
8	86	0.002-0.2	spontaneous wrinkling; frank bulla, moderate erythema	cleavage
9	70	0.002-0.005	spontaneous wrinkling	n.d.
10	43	0.005	spontaneous wrinkling	n.d.
11	67	0.02-0.2	spontaneous wrinkling	n.d.
12	68	0.02-0.05	spontaneous wrinkling	n.d.

Table 13: TEN Production in Human Skin in Organ Culture. Uninvolved skin surrounding lesions removed at surgery served as explants. ET doses varied from 1 to 5 mg protein/ml culture fluid. Age, sex, anatomical site, and underlying diagnosis did not appear to influence TEN production.

Patient	Age	Sex	Diagnosis	Origin of Skin Sample	Basis for Judged Positive Response*
1	10	M	erythropoietic protoporphyria	dorsal forearm	2
2	44	F	familial melanoma	buttocks	1,2,3
3	46	F	contact dermatitis	buttocks	1,2,3
4	21	M	sebaceous cyst	scalp	1,2,3
5	70	F	sebaceous cyst	labia majora	1,2
6	17	F	nevus	breast	2
7	54	F	sebaceous cyst	back	2
8	73	F	stasis ulcer	buttocks	1

*Peeling = 1; Histologic mid-epidermal cleavage = 2; Exfoliative Cytology = 3

Table 14: Adult human and mouse skin of various ages: quantitative comparison of response to exfoliatin in vitro[a]

Tissue	Exfoliatin concentration	Time exposed to exfoliatin 1 Hr	2 Hr	4 Hr
Human (adult)[b]	3 mg/ml[b]	+	+	+
	3×10^{-1}	+/-	+	+
	3×10^{-2}	-	+/-	+
	3×10^{-3}	-	-	-
Mouse (adult)	3 mg/ml	-	+/-	+
	3×10^{-1}	-	-	+
	3×10^{-2}	-	-	-
	3×10^{-3}	-	-	-
Mouse (juvenile)	3 mg/ml	-	+	+
	3×10^{-1}	-	+/-	+
	3×10^{-2}	-	-	+/-
	3×10^{-3}	-	-	-
Mouse (newborn)	1.5 mg/ml	-	+	+
	1.5×10^{-1}	-	-	+
	1.5×10^{-2}	-	-	+/-
	1.5×10^{-3}	-	-	-

[a] Response was assessed by the following manifestations: Peeling, exfoliative cytology, cleavage in histologic sections, and, in some cases, ultrastructural modifications.

[b] The same batch of exfoliatin was used in determining all dose-response curves.

Table 15: Mammalian Species Susceptible to Scalded Skin Syndrome

Susceptible	Resistant*
Man	Rat
Monkey	Guinea Pig
Mouse	Rabbit
Hamster	Dog

*In addition, one amphibian and one avian species tested to date have been unresponsive.

Table 16: Comparative responses of newborn mice and newborn hamsters to exfoliatin administered in vivo[a]

Time after injection (hr)	Toxic epidermal necrolysis	
	Newborn mouse (% responding)	Newborn hamster (% responding)
1	25-75	0
2	100	25
4	100	50
6	100	75

[a]At least 6 animals from 3 separate litters were tested at each time. The dose of toxin was adjusted to 1 mg/gm body weight.

Table 17: Susceptibility of various human, monkey, and murine epithelia to exfoliatin[a,b]

Species	Epithelium	In vivo	In vitro
Mouse	Glabrous skin (N,A)	Positive (1,2,3,4)	Positive (1,2,3,4)
	Hairy skin (A)	Positive (1,3,4)	Positive (1,2,3)
	Esophagus (N)	Positive (3,4)	Positive (3,4)
	Vagina (A)	Not tested	Positive (1,2,3)
	Cervix (A)	Not tested	Negative (1,2,3)
	Endometrium (A)	Not tested	Negative (1,2,3)
	Bladder (N)	Negative (3)	Not tested
	Ureter (N)	Negative (3)	Not tested
	Stomach (N)	Negative (3)	Not tested
	Adrenal gland (N)	Not tested	Negative (3,4)
	Epididymis (N)	Not tested	Negative (3,4)
Human	Glabrous skin (A)	Positive (1,2,3,4)	Positive (1,2,3,4)
	Hairy skin (A)	Positive (1,3)	Positive (1,3,4)
	Genital mucosa (A)	Not tested	Positive (1,3)
Monkey	Glabrous skin (A)	Not tested	Positive (1,2,3)
	Mucous membrane (A) (oral mucosa)	Not tested	Positive (1,2,3)

[a] N = neonatal; A = adult,

[b] Assessed by: 1 = peeling; 2 = exfoliative cytology; 3 = history; 4 = electron microscopy.

Table 18: In vitro comparison of newborn mouse and newborn rat skin sensitivity to exfoliatin[a]

Sensitive	Resistant
Mouse skin (full-thickness)	Rat skin (full-thickness)
Mouse skin in mixed mouse and rat organ-cultures	Rat skin in mixed mouse and rat organ-cultures
Mouse skin in rat plasma	Rat skin in mouse plasma
Mouse epidermal sheets	Rat epidermal sheets
Mouse epidermis recombined with rat dermis	Rat epidermis recombined with mouse dermis

[a]Concentration of exfoliatin = 5 mg/ml media.

Table 19: Antibody Blockade of TEN in Neonatal Mice

	Dilution[a]	# animals with TEN	Severity[b]
Control	undiluted	6/6	4+
	1:1	6/6	4+
	1:3	6/6	3+
Antibody Pre-Incubated	undiluted	-	-
	1:1	0/6	-
	1:3	4/6	1+

[a]Undiluted fraction adjusted to 2.5 mg protein after incubation with either normal rabbit serum (control) or antitoxin antibody. Further dilutions made with normal saline.

[b]4+ = spontaneous wrinkling; 3+ = easily elicited Nikolsky at 2 hrs; 2+ = easily elicited Nikolsky at 4 hrs; and 1+ = Nikolsky elicited with difficulty at 4 hrs.

Table 20: Cells tested for Susceptibility of Surface Coat to Exfoliative Toxin

	Mouse	Guinea Pig
Keratinocytes	1° cultures	1° & 2° cultures
Fibroblasts	dermis, lung - 1° & 2° cultures	dermis - 2° cultures
Epididymis	1 mm^3 pieces in suspension	----------
Sperm	suspension	suspension

Tissue	First Incubation	Substrate	Second Incubation	Substrate	Intercellular Antibody	Titer
Monkey exophagus	NS	Frozen tissue section (in vitro)	NS	Frozen tissue section	-	0
	EF		NS		-	0
	NS		PS		+	1:160
	EF		PS		+	1:160
Newborn mouse skin	NS	Live newborn mouse (injection) (in vivo)	NS	Frozen tissue section	-	0
	EF		NS		-	0
	NS		PS		-	0
	EF		PS		-	0
Normal human skin	NS	Organ culture (in vitro)	NS	Frozen tissue section	-	0
	EF		NS		-	0
	NS		PS		+	1:160
	EF		PS		+	1:160
TEN staph skin	EF	Patient with TEN (in vivo)	NS	Frozen tissue section	-	0
	EF		PS		PS	1:160
Pemphigus skin	PS	Patient with pemphigus (in vivo)	NS	Frozen tissue section	+	NA
	PS		EF		+	NA
Pemphigus skin	PS	Patient with pemphigus (in vivo)	NS	Organ Culture	+	NA
	PS		EF		+	NA

Specimens exposed to EF, NS or PS either in vivo or in vitro (Incubation I) were overlayed with EF, NS or PS in forzen tissue section or organ culture (Incubation II). Specimens were then stained with fluorescein-labeled antihuman or antimouse IgG and examined for intercellular fluorescence (reaction).

Studies were undertaken to see if exposure of skin to exfoliative toxin could in any way affect pemphigus antigen, or whether pemphigus antibody could block or otherwise affect the action of exfoliative toxin.

Key: NS - Normal saline
EF - Exfoliative fraction TEN toxoid
PS - Pemphigus serum
NA - Not applicable (direct immunofluorescence)

FIGURES TO LEGENDS

Figure 1 — Drug-induced TEN. Exfoliation occurs almost simultaneously over entire body surface. Because entire thickness of epidermis is involved, effect is that of second degree burn.

Figures 2 & 3 — Graft vs host-induced TEN. Initial assault is directed against basal layer (Fig. 2), but quickly the entire epidermis is destroyed and detaches from underlying dermis (Fig. 3). This sequence is indistinquishable from drug-induced TEN. Fig. 2 X 650. Fig. 3 X 100.

Figure 4 — Early stages of generalized staphylococcal scalded skin syndrome. Note peri-orificial and centripetal exfoliation.

Figure 5 — Close-up view of early generalized scalded skin syndrome. Flaccid bullae coalesce and rupture easily leaving a denuded base.

Figure 6 — Late stages of staphylococcal scalded skin syndrome (Ritter's Disease). Groin involvement is typical of tendency for axial involvement in staphylococcal-induced disease. Healing is almost complete here.

Figure 7 — Generalized staphylococcal scalded skin syndrome. Note mid-to-upper epidermal cleavage plane with individual, acantholytic cells adjoining the cleavage plane. The dermis contains no inflammatory cells. Fig. 7 X 400.

Figure 8 — Localized scalded skin syndrome (bullous impetigo). Note bullae arising on normal-appearing skin. In some areas lesions and erosions become confluent.

FIGURES TO LEGENDS (con't)

Figure 9 Histopathology of bullous impetigo. Again, there is an intra-epidermal cleavage plane, and individual acantholytic cells within the space. But, in contrast to generalized scalded skin syndrome, in this form of the disease the dermis contains many inflammatory cells, and phage group 2 organisms were cultured from intact bullae. Fig. 9 X 150.

Figures 10 & 11 Exfoliative cytology in early and late stages of staphylococcal scalded skin syndrome. Nucleated squames predominate early (Fig. 10), and anucleate; cornified squames late (Fig. 11). Regardless of stage, these cells are easily distinquished from those found in cytological preparations of drug-induced TEN. (c.f., Fig. 12 Below). Fig. 10 X 160. Fig. 11 X 1,000.

Figure 12 Exfoliative cytology from case of drug-induced TEN. Inflammatory cells, a few cuboidal epithelial cells, and cellular debris predominate because of the low cleavage plane (c.f., Fig. 10, 11 above). Figures 10-12 demonstrate potential application of exfoliative cytology in the rapid differential diagnosis of non-staphylococcal scalded skin syndrome. Fig. 12 X 1,000.

Figure 13 Neonatal mouse injected 24 hours earlier with exfoliatin-elaborating phage group 2 staphylococci. Rubbing produces exfoliation and denudation (positive Nikolsky's Sign).

Figure 14 Histopathology of generalized scalded skin syndrome in neonatal mouse injected 24 hours earlier with phage group 2 organisms. Note intra- and subgranular layer intraepidermal cleavage plane and absence of inflammatory cells in the dermis. Appearance is comparable to human disease (c.f., Fig. 7). Fig. 14 X 400.

FIGURES TO LEGENDS (con't)

Figure 15 Adult mouse pretreated with steroids for three weeks, then inoculated with phage group 2 staphylococci 24 hrs earlier. Epidermis is so thin in adult mice that exfoliation is less evident than in newborns. But intraepidermal cleavage occurs here as well.

Figure 16 Normal adult mouse injected with purified staphylococcal exfoliatin 2 hours earlier. Rubbing produces positive Nikolsky's Sign: exfoliation and denudation.

Figure 17 Summary of animal and in vitro models of scalded skin syndrome. Note situations where specific anti-exfoliatin antibody has been shown to block disease production (arrows).

Figures 18 & 19 Experimental production of staphylococcal scalded skin syndrome in human volunteers 2 hours after intradermal injection of purified staphylococcal exfoliatin. Fig. 18 illustrates an experimental bulla, and Fig. 19 an area of spontaneous wrinkling accentuated by rubbing.

Figure 20 Production of exfoliation in viable human skin incubated for 2 hours with media containing staphylococcal exfoliatin. Intraepidermal cleavage occurs in vitro just as in vivo and reveals skin markings of exfoliated upper epidermis.

Figure 21 Adult mouse epidermis 2 hours after incubation in exfoliatin-containing media. Note intercellular cleavage and free-floating acantholytic cells in cleavage space (cs). Fig. 21a depicts adult human skin incubated as above. Again, note intercellular cleavage and prominent half-desmosomes (D). Fig. 21 X 4,000. 21a X 12,500.

FIGURES TO LEGENDS (con't)

Figure 22 Adult human epidermis 2 hours after incubation in exfoliatin-containing media. Cell adjacent to cleavage space (CS) is early in process of detachment and exhibits clumping of tonofilaments (tf) characteristic of acantholytic cells. Fig. 22 X 11,000.

Figures 23a & b Mouse keratinocytes, primary culture. Culture in Fig. 23a has been exposed to high concentration of exfoliatin in media (10 mg/ml), while cells in 23b are controls. After 6 hours of exposure there is no cytotoxicity apparent. Culture media retained its full biological potency when serial dilutions were injected into neonatal mice at termination of experiments. Fig 23 a & 23b X 1,250.

Figure 24 Primary culture of neonatal mouse keratinocytes exposed to exfoliatin for 6 hours. Note desmosomal cleavage (arrows) in vitro mimicking such separation in vivo (c.f., Fig. 21 & 22). Fig. 24 X 22,000.

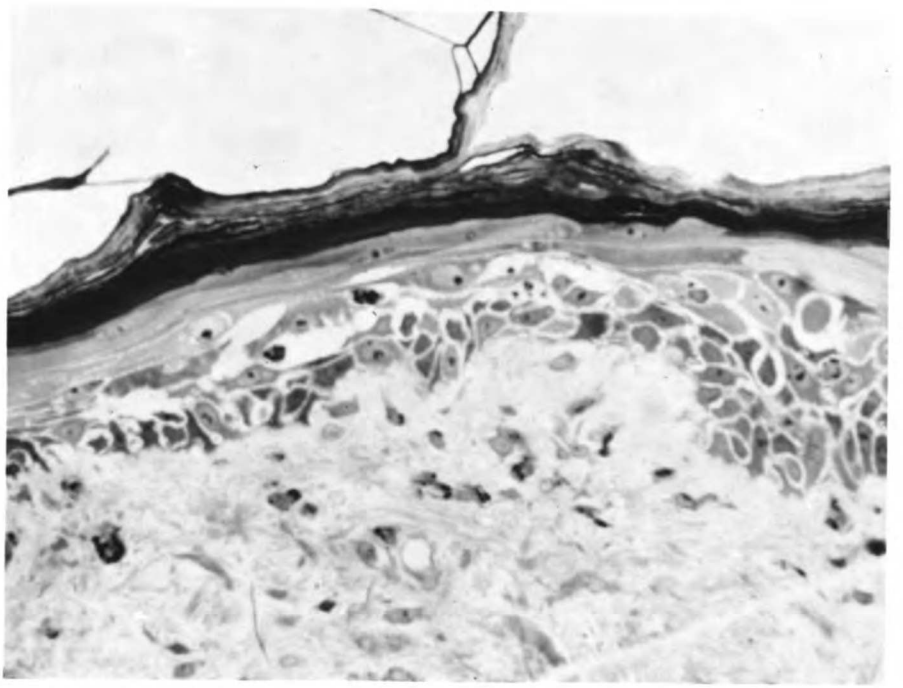
Figure 25 Primary culture of neonatal mouse keratinocytes treated as above, then stained for cell surface acid mucopolysaccharides with ruthenium red. Despite cell separation, stainable surface coat is not removed. Similar results follow staining with concanavalin A (36). Fig. 23 X 75,000.

Figures 26 - 28 Newborn mouse esophagus 2 hours after administration of exfoliatin (Figs. 26 & 27) or control supernatants (Fig. 28) in vivo. Note variable location of cleavage plane - relatively superficial in Fig. 26 and in the lower stratum granulosum in Fig. 27. Con-

FIGURES TO LEGENDS (con't)

trol solutions produce no cleavage (Fig. 28). However, responsiveness of esophageal and other extracutaneous keratinizing epithelia, even in susceptible species is variable. (see Ref. 36)

Fig. 26 X 8,500. Fig. 27 X 15,000. Fig. 28 X 4,500.



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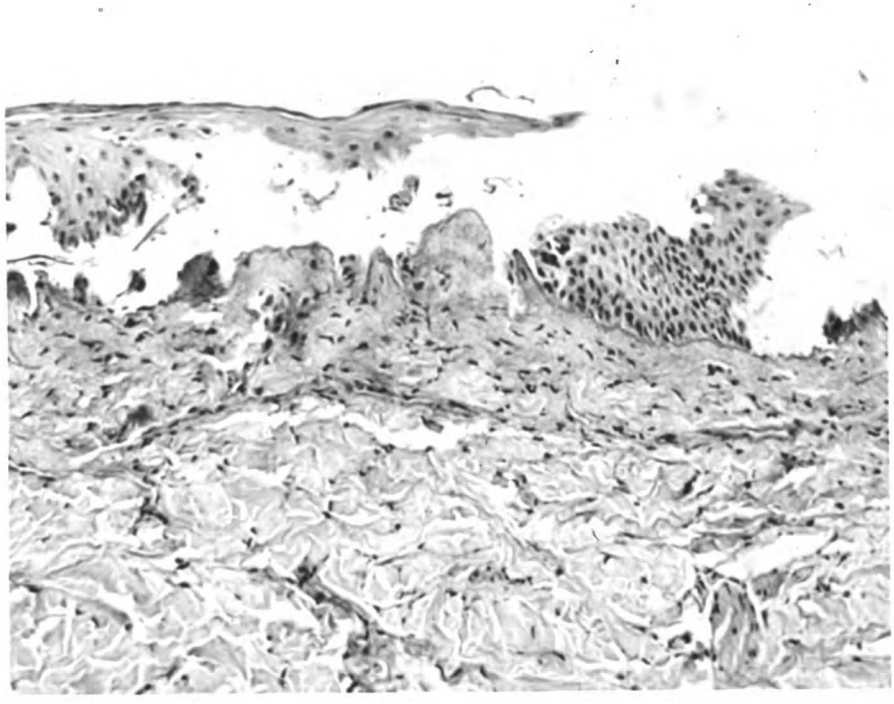
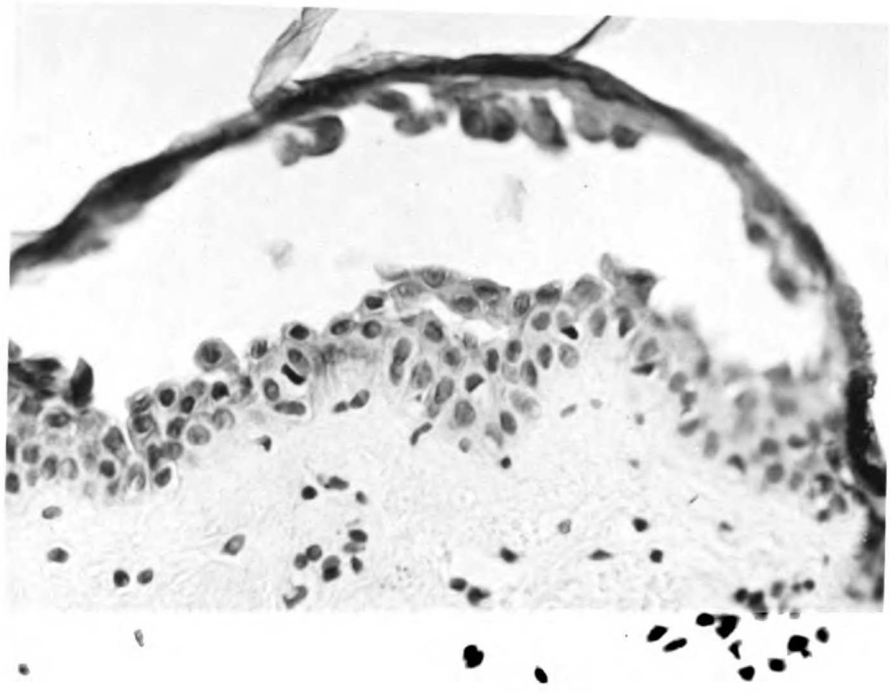
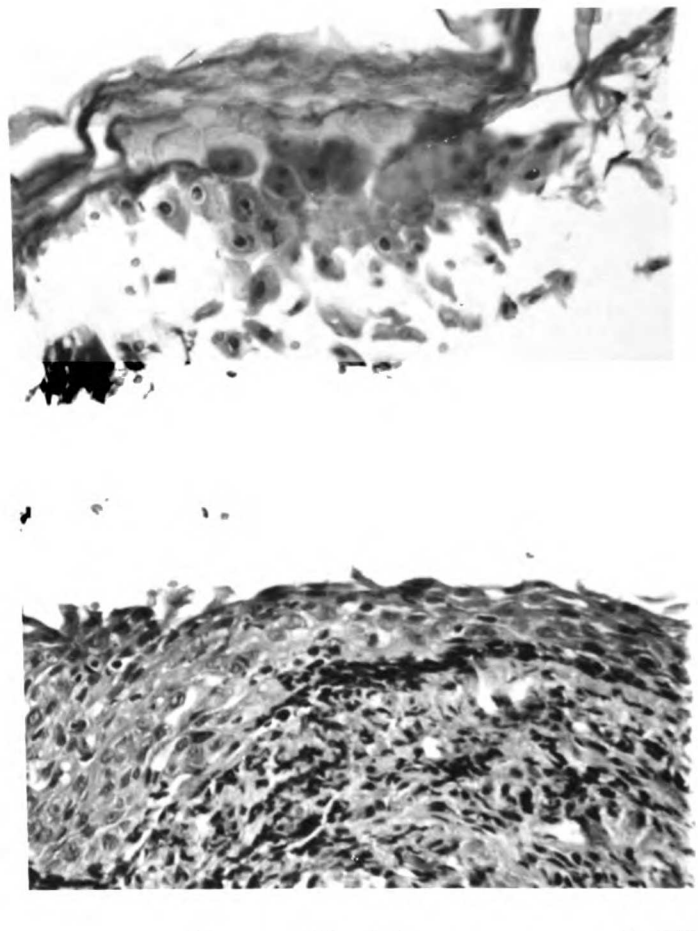
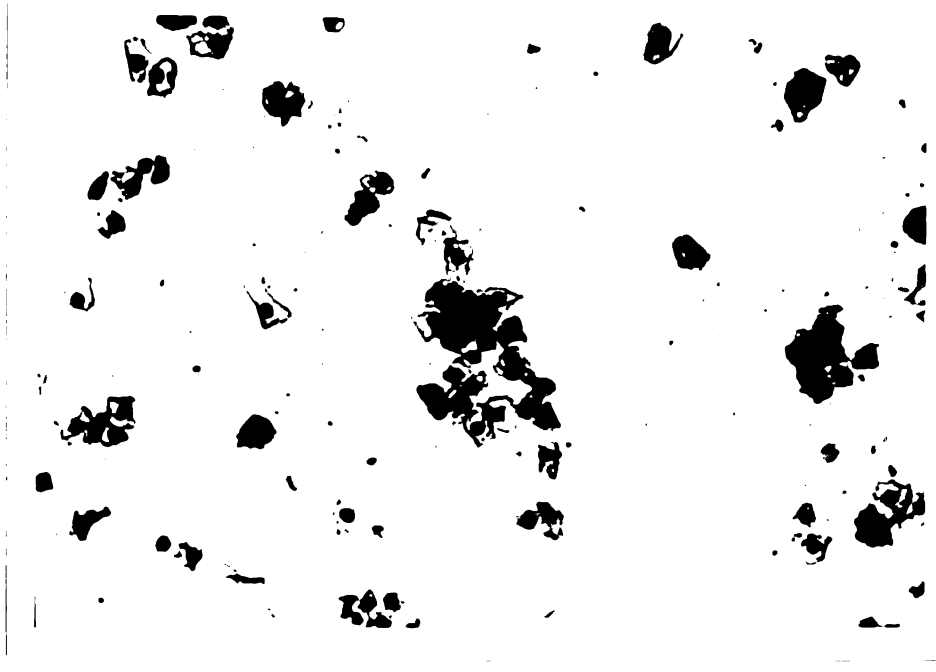
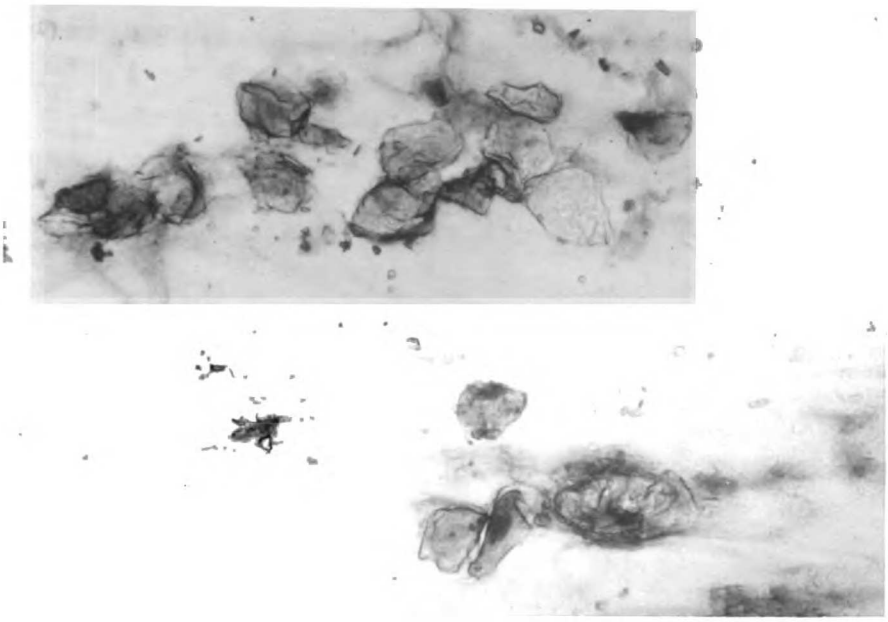










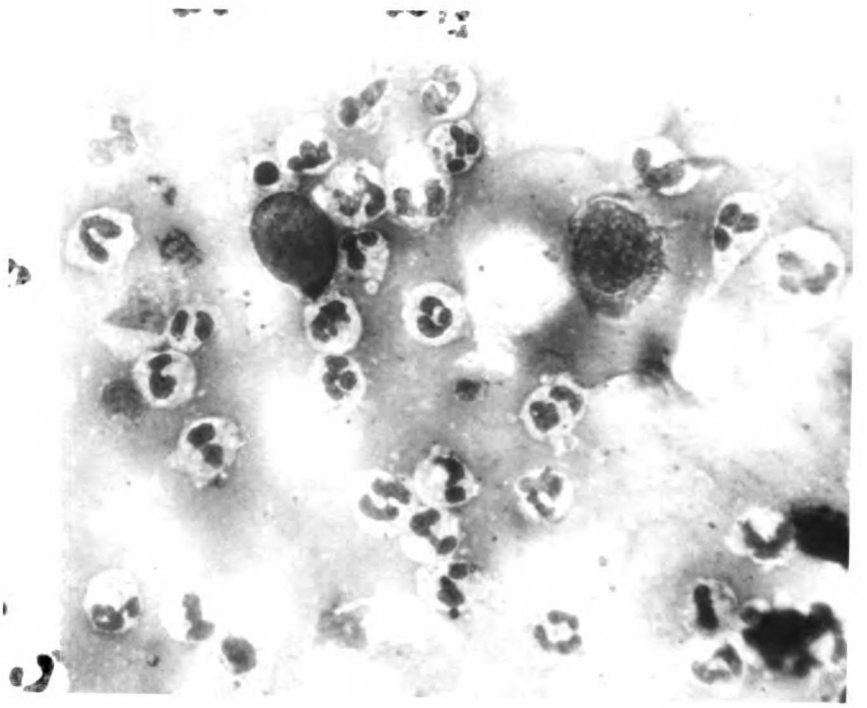
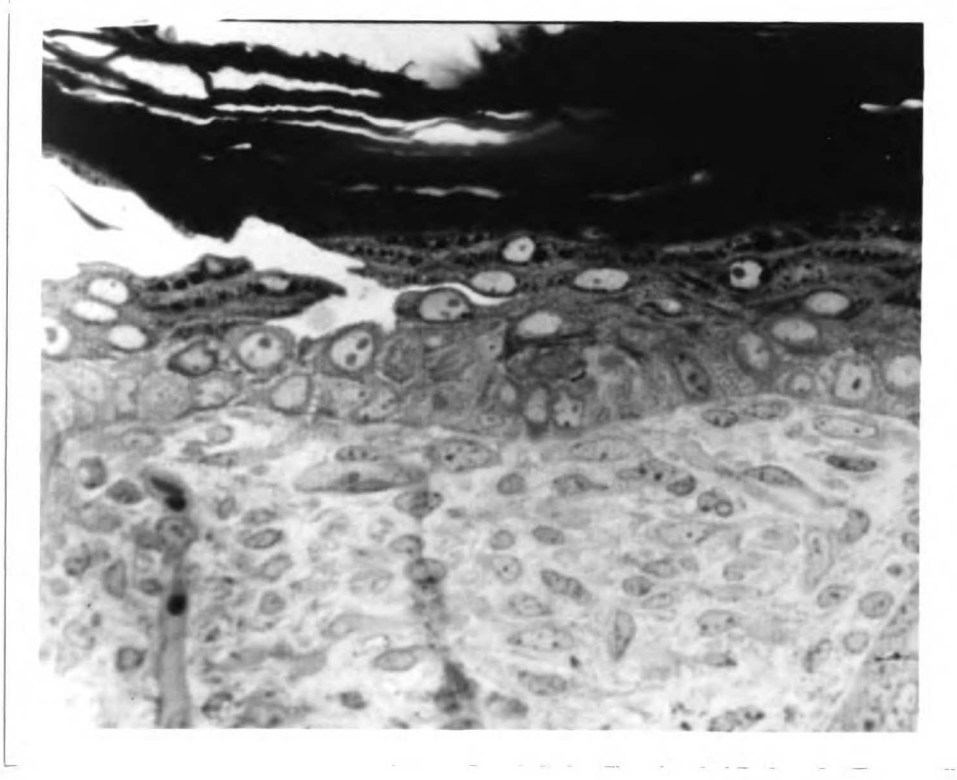


FIGURE 13



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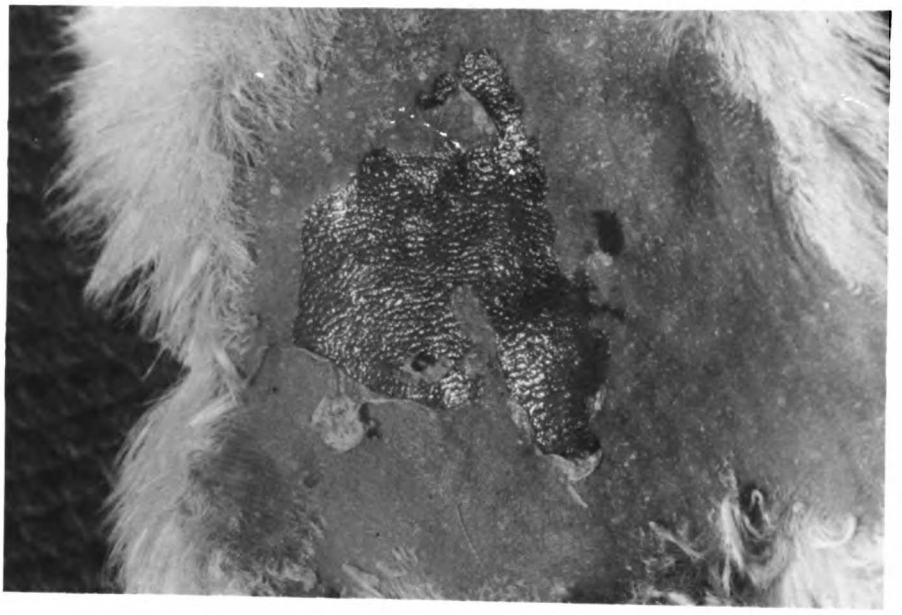


FIGURE 16

FIGURE 17

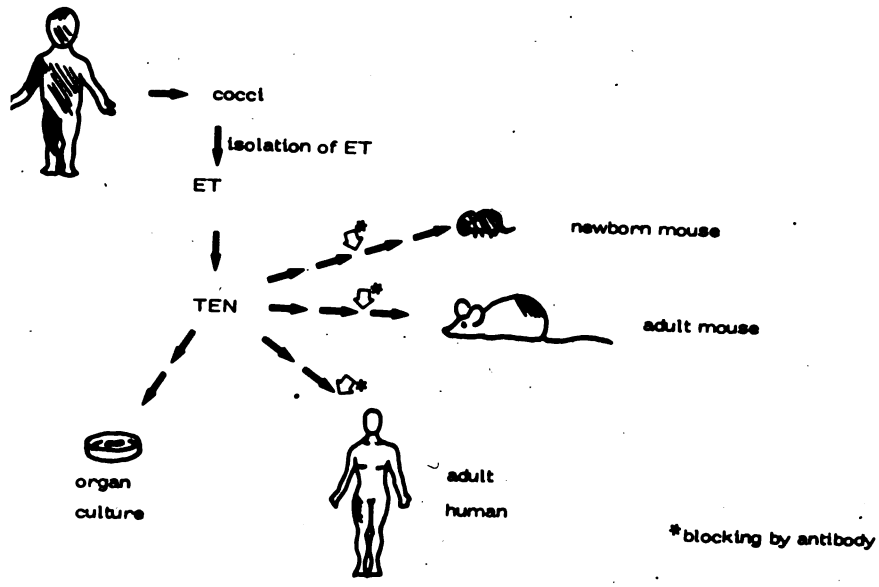
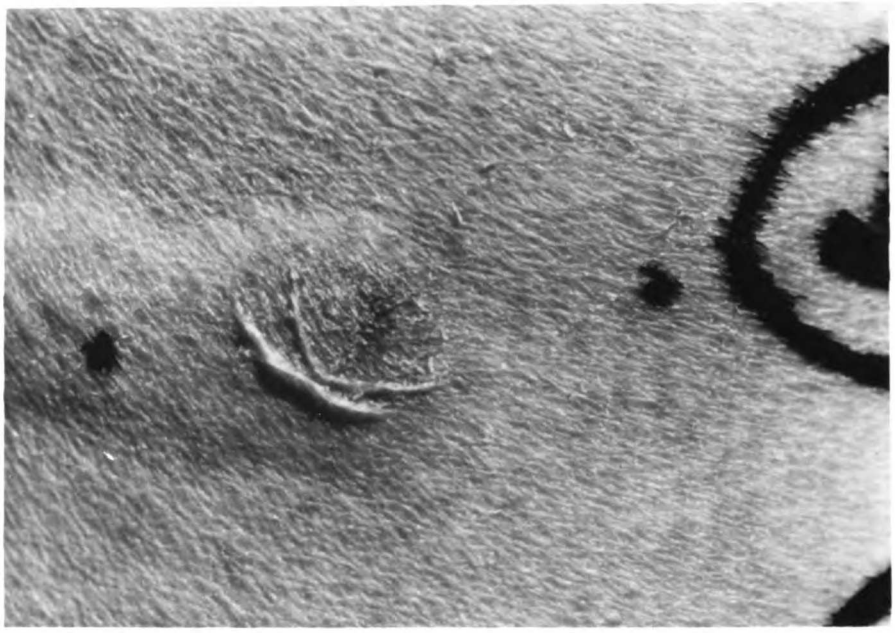

cocci
isolation of ET
ET
TEN
newborn mouse
adult mouse
organ
culture
adult
human
*blocking by antibody

FIGURE 18

FIGURE 19



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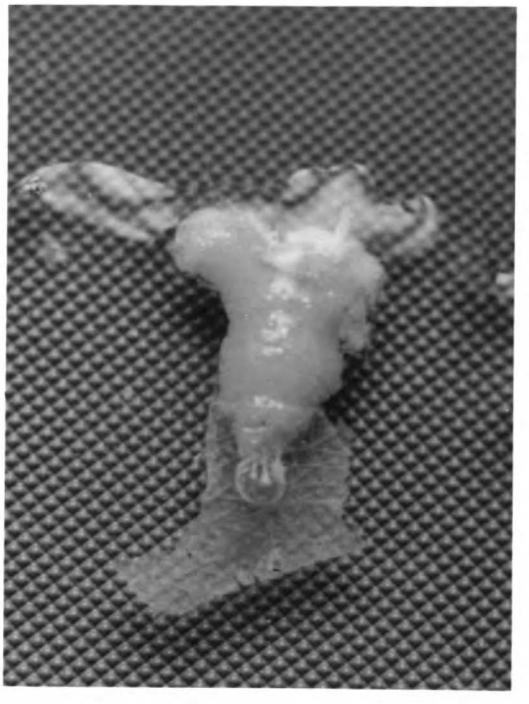

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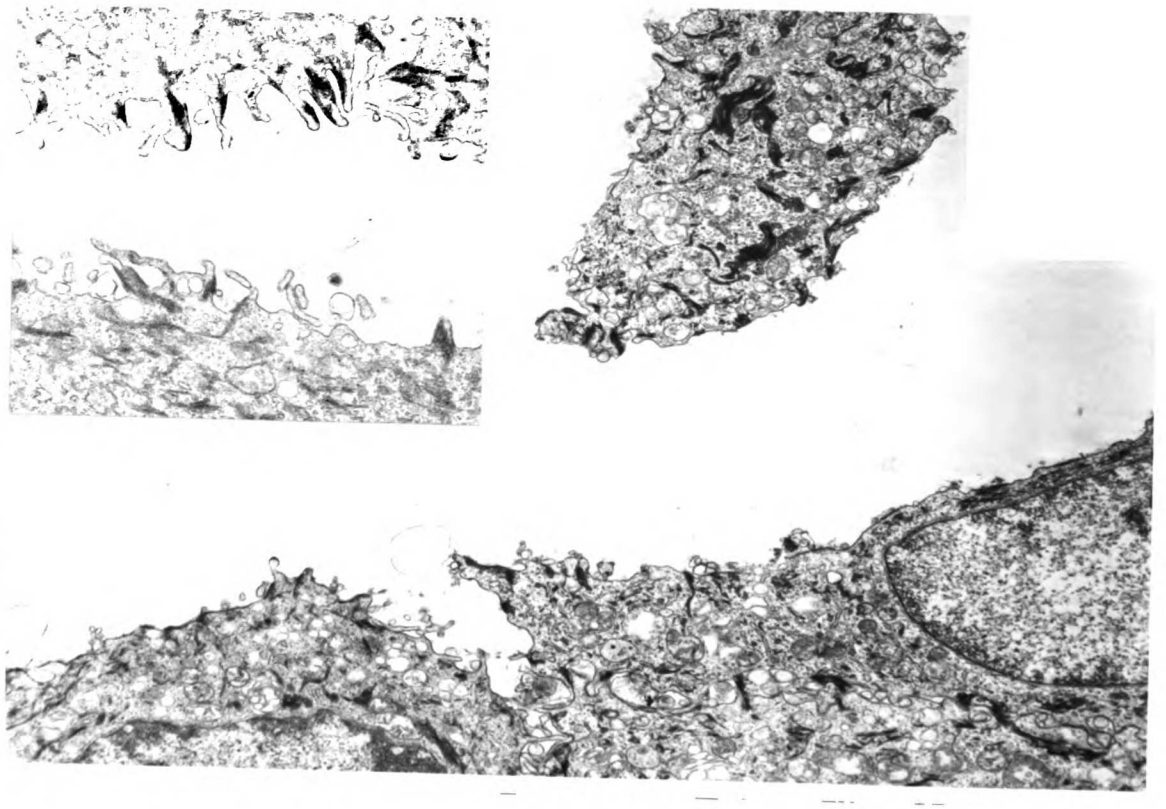

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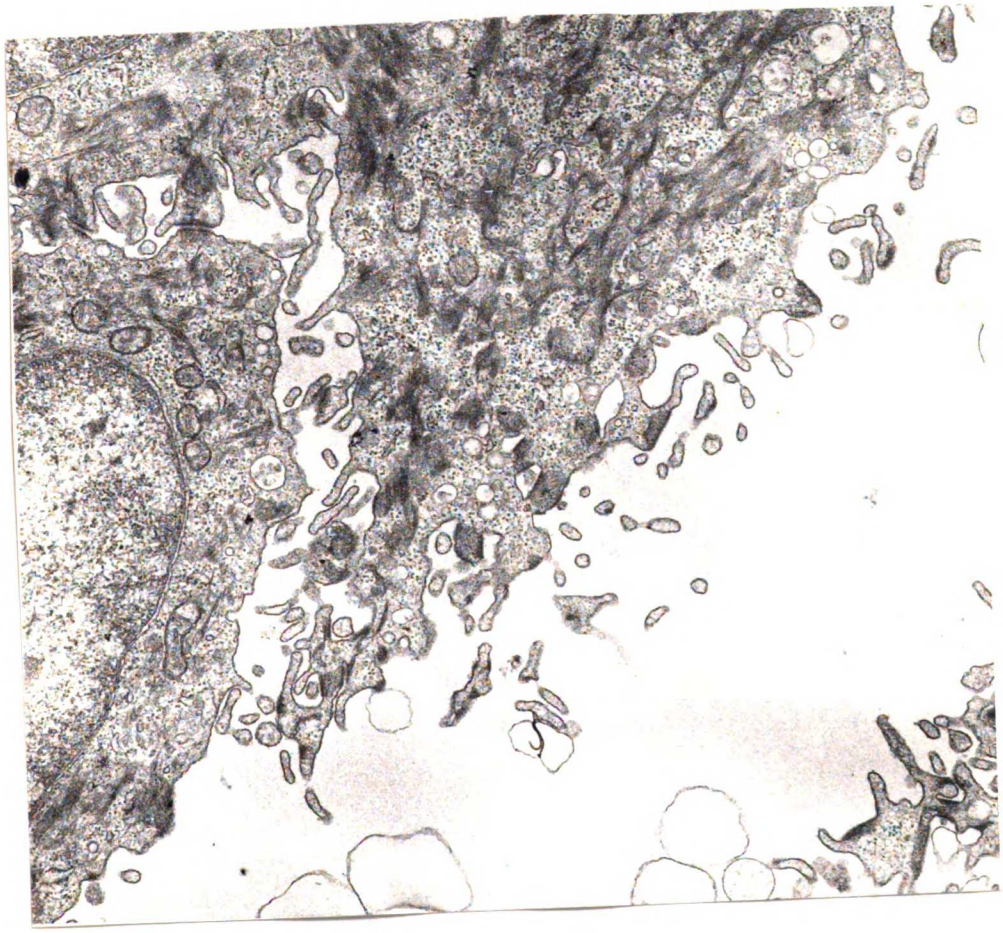

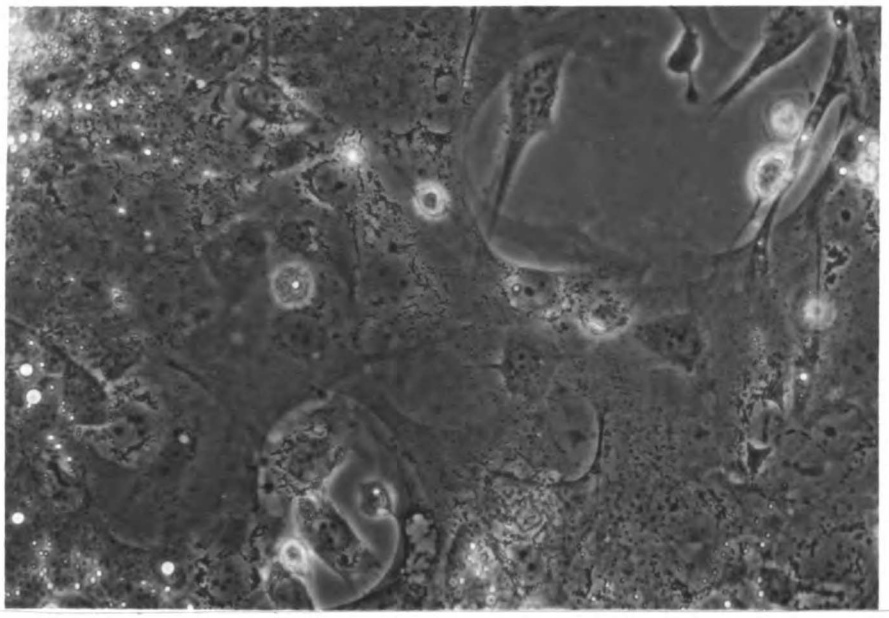

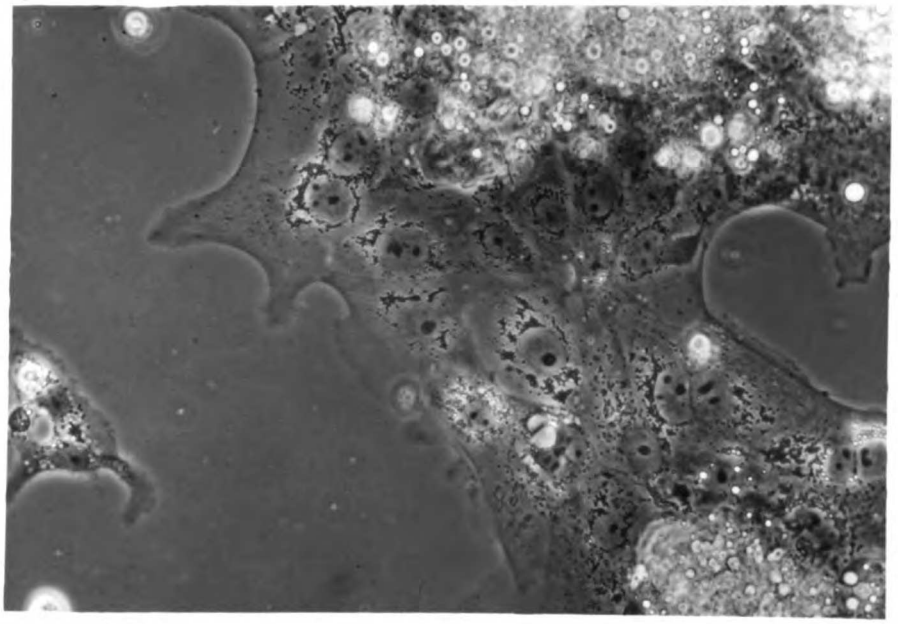

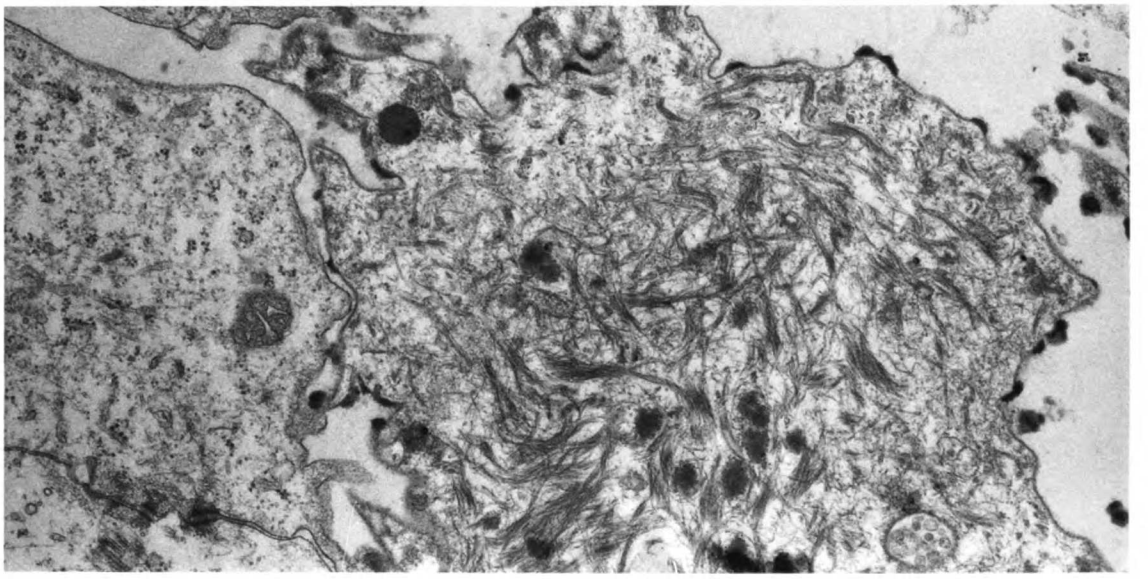

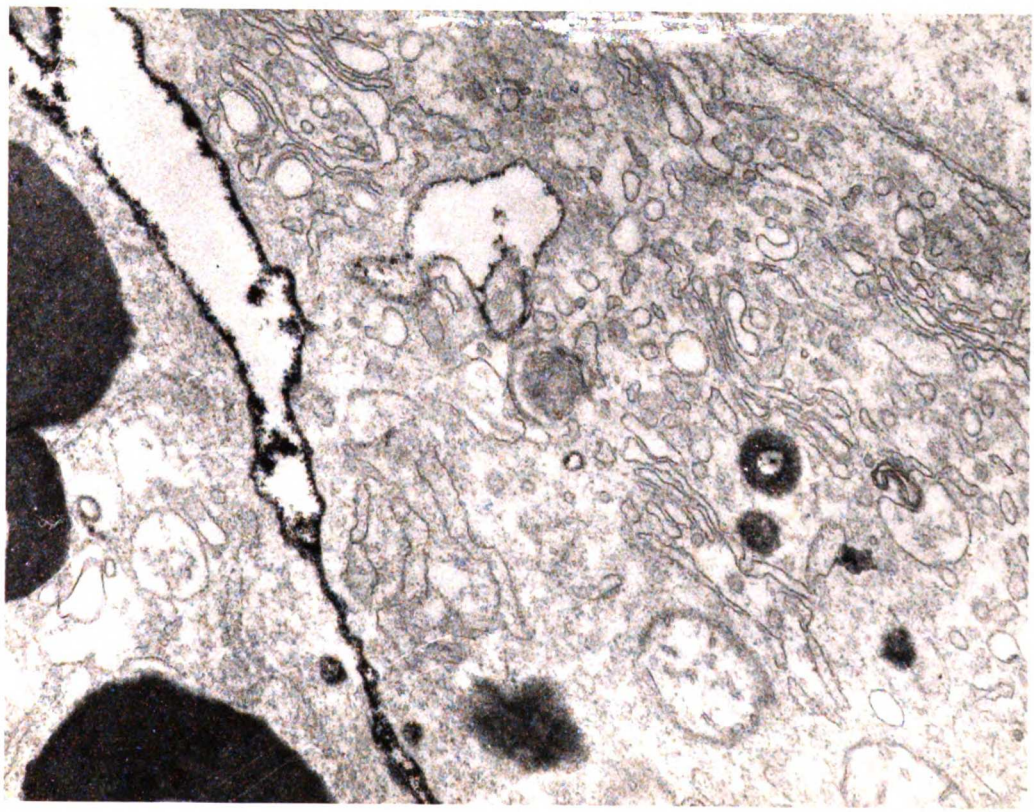

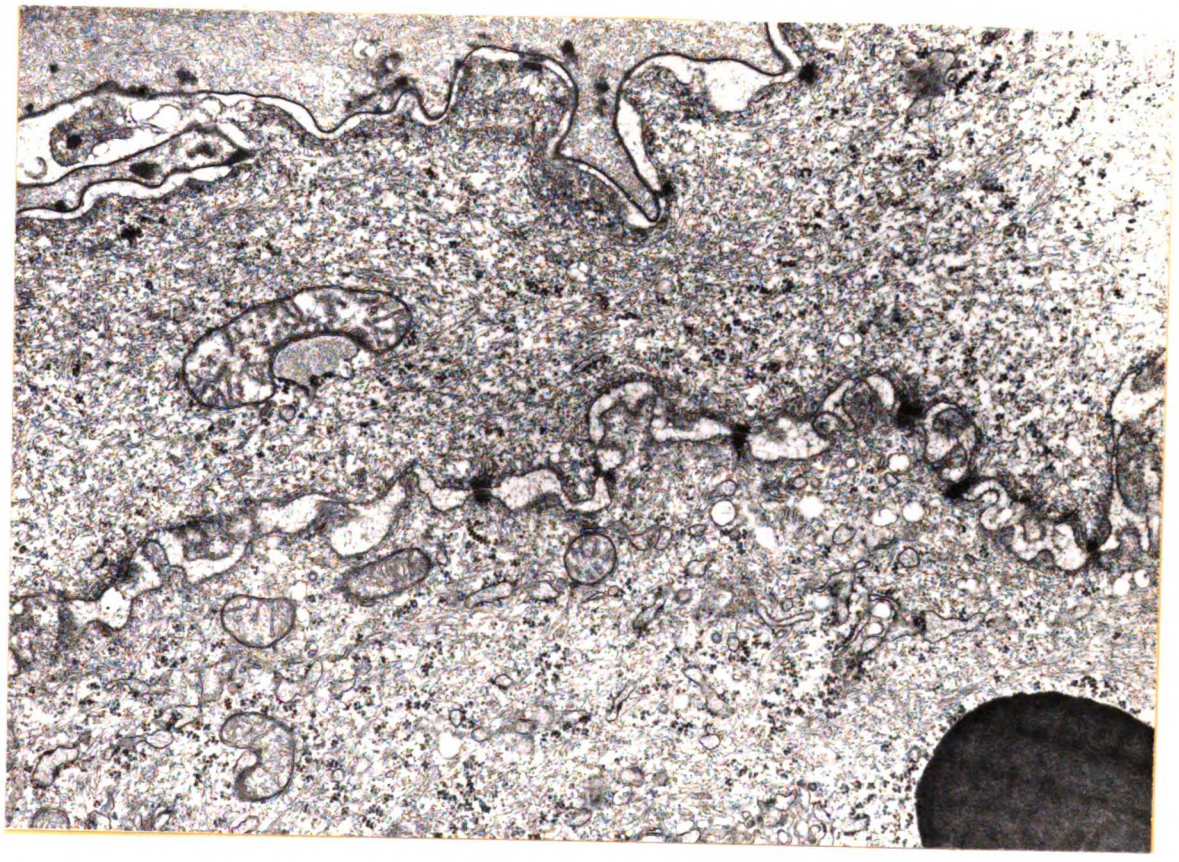

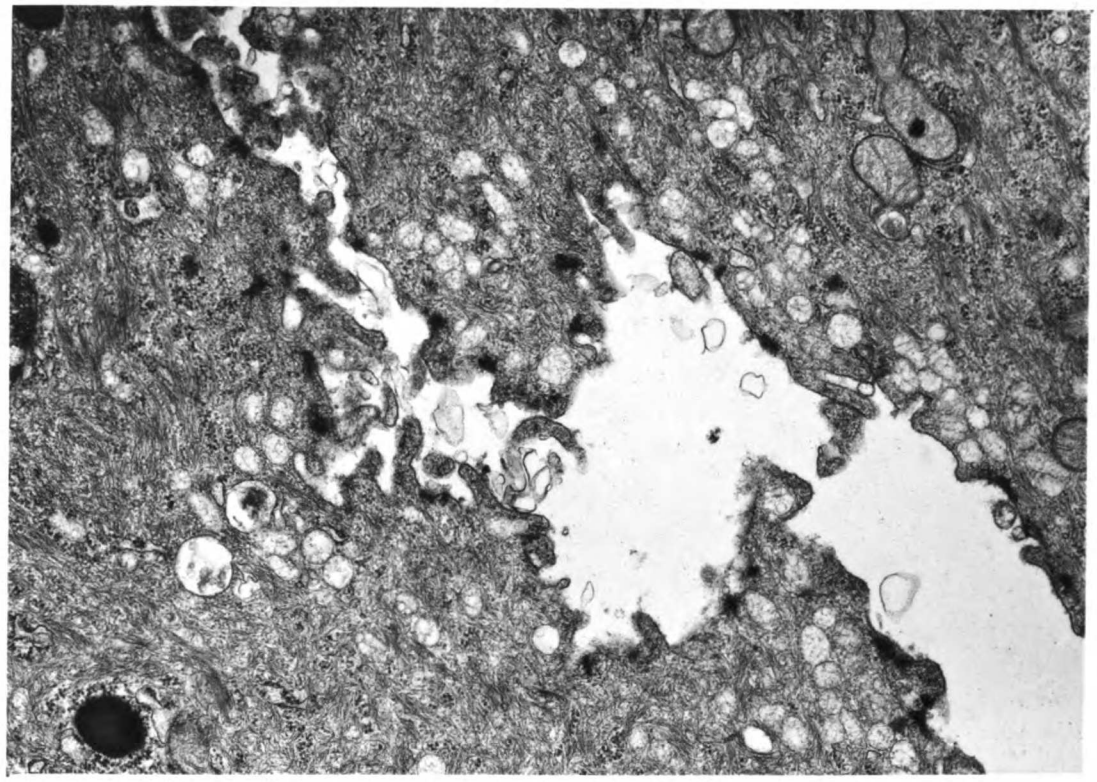

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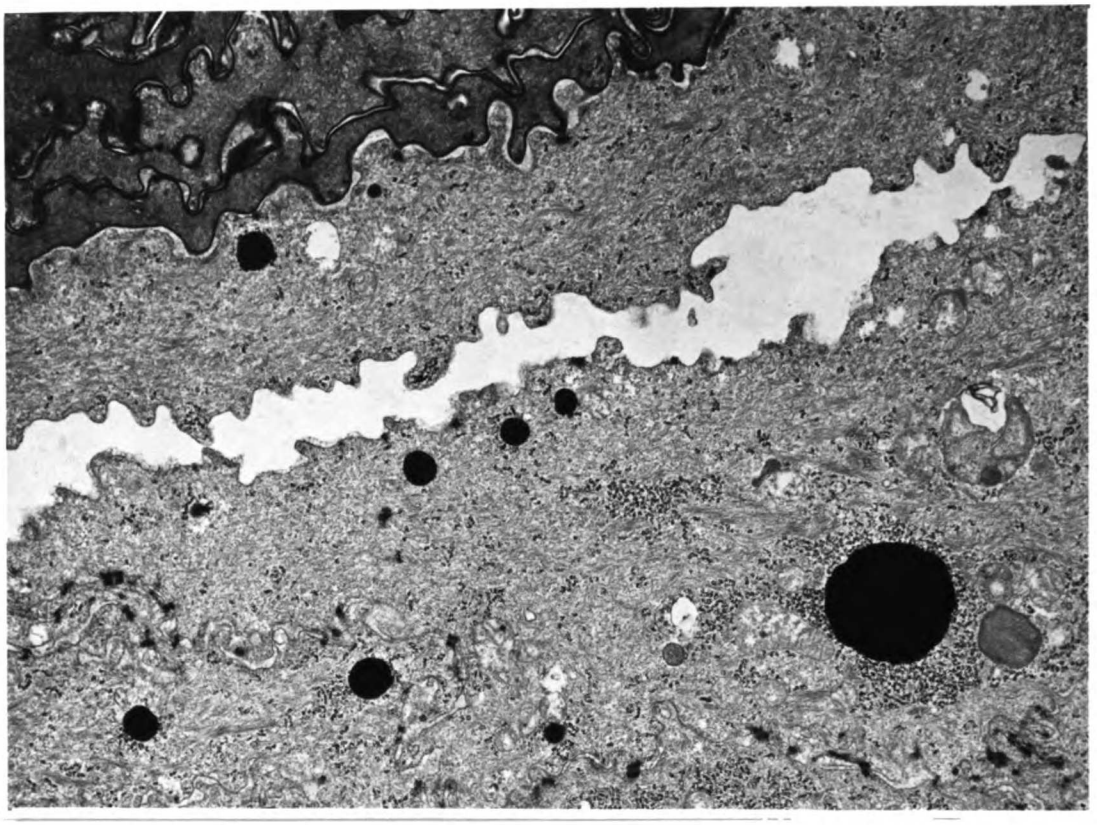

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