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STAPHYLOCOCCAL TOXIC EPIDERMAL NECROLYSIS (TEN): THE EXPANDED MOUSE MODEL*

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ABSTRACT

Strains of phage Group 2 staphylococci and cell-free fractions isolated from the same strains induced toxic epidermal necrolysis (TEN) when injected into neonatal mice. Furthermore, adult mice developed TEN in both hairy and glabrous skin following intracutaneous (but not systemic) administration of cell-free fractions. While normal adult mice did not develop TEN after inoculation of cocci, generalized TEN could be produced in adult mice pretreated with systemically injected corticosteriods for 3 weeks. Mice which had survived injections of cell-free fractions as neonates remained susceptible to intracutaneously administered fractions in adulthood. Regardless of the age of the animal and the cause of TEN (cocci or cell-free filtrates), the pathogenesis appeared identical both histologically and ultrastructurally, i.e., via intercellular cleavage without evidence of cell necrosis. These studies demonstrate that neither maturation nor hair development, as previously proposed, confer cutaneous resistance to TEN. Instead, an extracutaneous factor(s) probably confers resistance to the normal adult mouse. Since similar factors lead to naturally occurring and experimental staphylococcal TEN in adult humans and adult mice, the adult mouse may prove to be a valuable model for the study of factors resulting in staphylococcal TEN in adult humans.

In 1970, Melish and Glasgow [1] reported that injection of newborn mice with staphylococci isolated from children with toxic epidermal necrolysis (TEN) resulted in a syndrome similar to TEN. Several investigators [2–5] have confirmed the susceptibility of newborn mice to phage Group 2 staphylococci. More recently, TEN has been induced in neonatal mice by inoculation of cell-free filtrates elaborated by Group 2 staphylococci in vitro, and the "exfoliating" or "epidermolytic" toxin (ET) from these filtrates has been isolated, purified, and partially characterized [2,4–7]. Reportedly, after mice became hirsute (at about 5–7 days of age), neither injections of cocci nor of cell-free fractions produced TEN [1–4]. Hair development or maturational changes were thought to explain the resistance of older humans and mice to staphylococcal TEN. Recently, Arbuthnott et al [8] produced TEN in adult hairless mice, intimating that hairiness rather than maturational changes may be responsible for normal adult resistance. Furthermore, staphylococcal TEN is now being recognized with increasing frequency in human adults [9–14].

Since the question of adult resistance seemed far from settled, in this study we have evaluated not only age, but several other factors including: hairiness, a history of previous TEN, the route of bacterial and ET administration, the topographical site of intracutaneous injection, and host resistance factors in susceptibility to staphylococcal TEN in the murine model.

MATERIALS AND METHODS

Staphylococci

Two strains of phage Group 2 staphylococci, which elaborated large amounts of ET, were utilized: Strain A was obtained from the Cross-Infection Reference Laboratory, London. Strain B was isolated from the throat of a 4-year-old male patient (Vienna Allgemeines Krankanhaus) with typical staphylococcal TEN. Control strains consisted of non-phage Group 2, coagulase-positive staphylococci. Strain A produced small amounts of delta hemolysin but negligible amounts of alpha and beta toxin; strain B was an alpha and delta hemolysin producer; and the non-phage Group 2 control (C) yielded alpha, beta, and delta hemolysin. Assessment of nonepidermolytic toxins was accomplished according to the method of Kapral and Miller [4]. All organisms were cultured in standard media at 37°C. Immediately before the beginning of experiments, organisms were transferred to peptone water by means of wire loops, and the concentration of cocci to be injected was adjusted first by turbidity [3] and later by cell count.

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Animals

Wild-type albino mice were used in all experiments. Standard bioassays were performed on randomly selected 1- to 3-day old suckling mice. In addition to newborns, juvenile (3–4 weeks) and adult (2–3 months) animals were included for assessment of the following variables in toxin-induced TEN:

1. *Age:* The susceptibilities of newborn (1–3 days old), juvenile (10–25 days old), and adult mice weighing up to 35 gm were compared.

2. *Previous TEN:* Newborn, juvenile, and adult mice (as above) surviving TEN during the first 3 days of life were rechallenged with ET at later intervals.

3. *Hairiness:* Hairy and glabrous regions (ears) of adult animals were compared. Because the thick mantle of hair could mask a spontaneous wrinkling or a positive Nikolsky's sign and/or interfere with skin stroking, in some experiments hairy areas were epilated with commercially available thioglycollate-containing depilatories before injection.

4. *Topography:* The relative susceptibilities of various regions (abdomen, neck, ear) to near-threshold doses of toxin were compared.

5. *Mode of administration:* The type and extent of reactions in adult mice following intradermal, subcutaneous, intramuscular, and intraperitoneal injections were compared.

6. *Corticosteroid administration:* Two separate experiments were performed: Adult mice, weighing 25 to 35 gm, were divided into three groups. Mice in group 1 (20 animals) each received 1 or 5 mg of prednisolone intramuscularly daily for 3 weeks. Half the animals in group 1 received intravenous injections (0.2–0.3 ml) of India ink immediately prior to injection of organisms in order to produce reticuloendothelial blockade. Group 2 (4 animals) received India ink alone. Group 3 (4 animals) served as sham-injected controls. At the end of 3 weeks, the abdominal and back skin of each mouse was epilated and each animal received 5×10^{10} strain 1 cocci intracutaneously in the abdominal skin. Mice were checked for the presence of TEN 12, 24, and 48 hr after injection.

Toxin Isolation and Purification

To isolate the toxin, we cultured TEN-producing and control strains according to the method of Arbuthnott et al [3, 7]. Both phage Group 2 (strains A and B) and control (strain C) staphylococci and the resultant filtrates were subsequently isolated and partially purified by a modification of the methods of Melish et al [6] and Arbuthnott et al [7]. The organism-containing medium was initially centrifuged at 3,000 rpm for 45 min. The supernatant fraction was then passed through a 0.45-μm Millipore filter, dialyzed extensively against distilled water, and concentrated about 1:50 in a vacuum evaporator (Heidolph, Kelheim, West Germany). Next, we precipitated the filtrate by bringing the solution to 40% saturation with ammonium sulfate, washed the precipitate 3 times with 40% ammonium sulfate, and discarded it. The washings were pooled with the supernatant fraction and brought to 80% saturation with ammonium sulfate. Subsequently, the precipitate was dissolved in distilled water and dialyzed extensively against distilled water. Each liter of culture fluid resulted in a yield of approximately 0.2 gm protein in the 80% $(NH_4)_2SO_4$ precipitate. Fractions were either lyophilized or stored at 4°C until used. There was no evidence of diminished activity in specimens stored up to 7 months at this temperature.

Ion-Exchange Chromatography

Aliquots (100 mg) of the above precipitates were run on 15- to 30-cm columns of CM-Sephadex C-50, equilibrated with 0.01 M sodium acetate (pH 5.8) containing 0.026 M NaCl. With this method, the ET was adsorbed, while impurities were eluted. The ET was eluted with 1 M NaCl. Filtered fractions were dialyzed against distilled water, concentrated as above, and bioassayed (see below). The protein concentration was assayed by the biuret method. Experiments were performed either with 80% ammonium sulfate precipitates or with the Sephadex column fractions.

Immunological Methods

Antibodies were produced by subcutaneously injecting adult rabbits with 5 mg/ml of the 80% ammonium sulfate precipitate combined with 1 ml of 0.9% NaCl in 1 ml complete Freund's adjuvant. Subsequent injections (containing the same amount of toxin in complete Freund's adjuvant) were made at weekly intervals. Ten days after the last injection, the animals were bled and globulins were prepared from fresh, unfrozen serum by precipitation with 40% ammonium sulfate. The precipitate was washed 3 times with 40% ammonium sulfate, brought to half the initial serum volume with distilled water, dialyzed against several changes of distilled water at 4°C for 24 hr, and then against several changes of phosphate-buffered saline (pH 7.4, 4°C) for 48 hr. The term antiserum refers to these globulin fractions.

Absorptions were accomplished by mixing 0.09 ml of antiserum or normal rabbit serum with 0.02 ml of the 80% ammonium sulfate precipitate containing ET (this removed all demonstrable anti-ET activity), or against equal protein concentrations of control supernatants containing alpha, beta, and delta toxin. The latter procedure resulted in antisera with predominantly anti-ET activity (Fig. 1). Absorptions were preformed for ½ hr at 37°C, then for ½ hr at 4°C, followed by removal of precipitates by centrifugation at 35,000 $\times$ *g* for 30 min at 4°C. The supernatants were brought to 0.5 ml, and serial dilutions were used for in vivo testing in the "blocking" experiments described below. Antisera developed precipitin lines in barbitone buffer (pH 8.2) against ET fractions, but not against control supernatants, at a dilution of 1:4 before adsorption and 1:2 after adsorption (Fig. 1) on standard Ouchterlony double immunodiffusion and on immunoelectrophoresis in barbitone buffer at pH 8.2.

Antisera were used: (1) to test for the presence of ET in various cell-free fractions; (2) to ascertain the specificity of anti-ET antibody by in vivo blockade of TEN in neonatal and adult mice; and (3) to further implicate a single substance, i.e., ET, in the pathogenesis of TEN.

Assays for ET

Fractions to be tested in newborn mice were first dialyzed against distilled water overnight, then diluted stepwise with normal saline, and subsequently injected in 0.05- to 0.1-ml aliquots into the napes of newborn mice. In order to prevent back-leakage, we pushed the needle caudally toward the lumbar region. Since the potency of different batches varied widely, the activity of each fraction was defined as the maximum dilution in mg protein per ml which induced TEN in neonatal mice.

Clinical and Microscopical Evaluation of TEN

After the injection of ET preparations, the animals were inspected at half-hourly intervals up to 4 hr. After

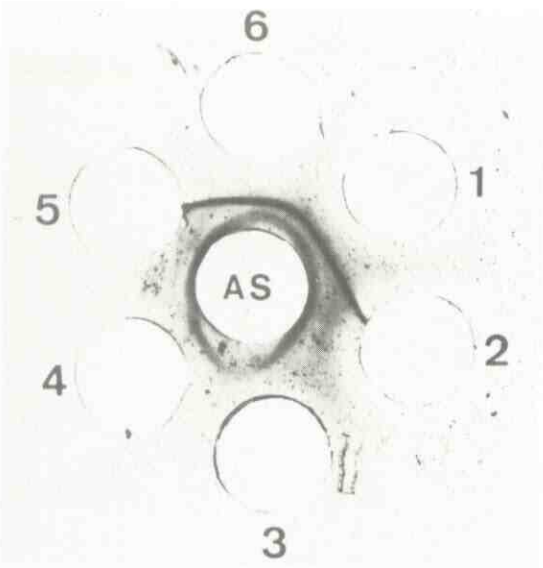

FIG. 1. Ouchterlony immunodiffusion pattern in agar. Adsorbed specific rabbit antiserum (*AS*) in center well. Antigen (ET) is diluted clockwise from well 6 to well 5. Precipitation lines form opposite antigen wells at 1:1 and 1:2 dilutions. Diffuse precipitin lines around center well probably represent disaggregated antigen–antibody complexes.

inoculation of cocci, animals were tested hourly from 9 hr onward. TEN was observable macroscopically and by stroking, which elicited the Nikolsky phenomenon. Several animals were biopsied sequentially early and late in the course of TEN, and the specimens were subjected to histologic and electron microscopical examination. Samples for electron microscopy were fixed in a 1:1 dilution of Karnovsky's fixative for 1 hr [15], then minced carefully to avoid artifactual cleavage, and reimmersed in fixative for a total of 5 hr at room temperature. After 3 washes and overnight rinsing in 0.1 M cacodylate buffer, specimens were postfixed in unbuffered 3% osmium tetroxide for 1½ hr at 0–4°C, dehydrated, and finally embedded in Epon [16]. Thin sections were mounted on naked copper grids, stained with uranyl acetate and alkaline lead citrate [17], and examined in a Zeiss EM 9S electron microscope.

RESULTS

In these studies, we have used the term TEN to denote spontaneous wrinkling or a demonstrable Nikolsky's sign. Histologically, TEN indicates intraepidermal cleavage limited to the upper spinous and/or granular layer in the absence of a dermal inflammatory infiltrate or evidence of cellular necrosis.

TEN in Newborn Mice

Response to injected organisms. The wild-type neonatal mice used in our experiments were uniformly responsive to injected Group 2 organisms, with no variance in sensitivity from that of imbred strains employed in previous studies [1–4]. After 9 to 10 hr, most animals were diffusely erythematous, and a positive Nikolsky's sign could then be elicited (Tab. I). To produce TEN in 100% of injected animals, a dose of 10^6 cocci was required. In a smaller percentage of animals, TEN developed after the injection of 10^5 cocci, while 10^4 induced no signs of the disease. Nine to 10 hr elapsed before TEN was observed, regardless of the intitial dose of injected organisms (Tab. I).

Histologic analysis disclosed typical intraepidermal cleavage at the level of the lower granular layer (Fig. 2). Viewed by electron microscopy, cleavage was more precisely localized to the lower granular layer and spinous–granular layer interface. Cell separation occurred suddenly, along the entire affected surface, without antecedent or concurrent cytolysis or intercellular edema (Fig. 3). Both desmosomes and nonjunctional sites of cell-to-cell attachment appeared to be simultaneously affected.

Injection of strain 3 organisms into newborns resulted in diffuse erythema without TEN (Tab. I).

TABLE I

Time/dose response of newborn mice to ET-producing cocci in vivo

	Time (hr)			
	3	6	12[a]	24[b] (Number with TEN)
Group 2 (strains A and B)				
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[c]	0/15[b, c]

[a] TEN became evident between 9 and 10 hr.

[b] Several animals died or were cannibalized.

[c] Intense erythema appeared without a positive Nikolsky's sign.

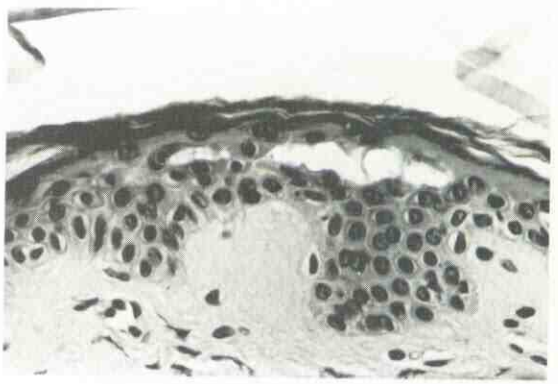

FIG. 2. Neonatal mouse skin 12 hr after injection of cocci. Note intraepidermal microvesiculation localized to lower granular layer and absence of dermal inflammatory infiltrate (× 240).

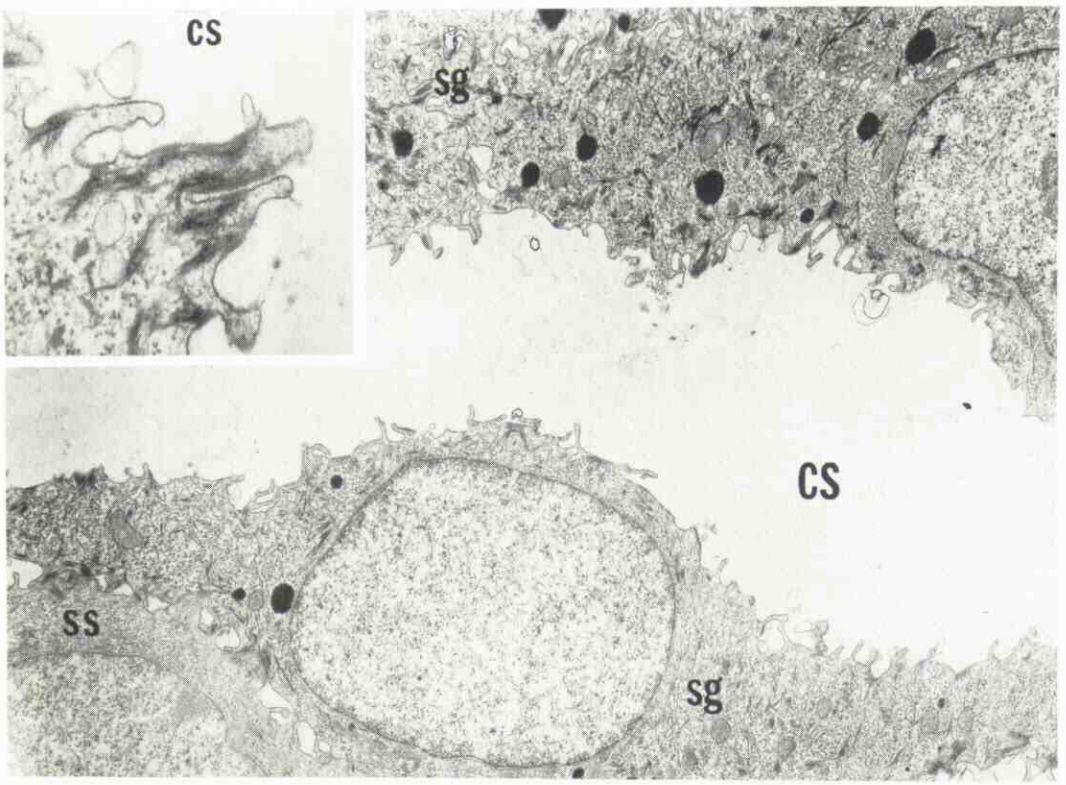

FIG. 3. Electron micrograph of TEN in neonatal mouse. Cleavage space (*CS*) lies between cells in lower stratum granulosum (*SG*), but may reach upper stratum spinosum (*SS*). Cells seem to separate without evidence of cytolysis. Cleaved desmosomes are seen along separated surfaces; some appear to be in various stages of internalization within phagocytic vacuoles (× 3,600; inset, × 17,000).

Response to injected cell-free filtrates. Newborn mice injected with fractions prepared from Group 2 organisms developed TEN with doses as low as .01 mg protein, depending on the potency of the preparation (Tab. II, Fig. 4). With large doses (1–10 mg), erythema and spontaneous wrinkling of the epidermis emerged 30 to 90 min after injection; but with lower doses, a positive Nikolsky's sign was present only after 3 to 4 hr (Table II). Animals often survived generalized wrinkling, appearing essentially normal by 48 hr after injection. Again, histologic and electron microscopical examination revealed subgranular cleavage and acantholysis without cytolysis. Cell-free filtrates from strain C organisms produced intense erythema, but no TEN developed (Tab. II). Incubation of ET-containing fractions with specific antisera before injection into neonatal mice prevented the production of TEN in 2 of 6 neonatal mice and significantly reduced the severity of disease in the remaining 4 mice (Tab. III).

TABLE II

Time/dose response of neonatal mice to ET

Source	Concentration (mg/ml)	Approximate percent animals with TEN[a]			
		30 min	60 min	2 hr	4 hr
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 hr, but a positive Nikolsky's sign was never demonstrable.

TEN in Adult Mice

Injection of cocci into immunologically competent hosts. When chemically epilated back or abdominal skin of adult mice was injected intracutaneously with up to 5×10^{10} organisms of strain A or B phage (Group 2) cocci, abscesses, which often drained spontaneously, usually developed. However, neither spontaneous wrinkling nor a positive Nikolsky's sign was noted in epilated skin directly over injection sites during the 24 hr after injection.

Injection of cocci into steroid-treated host. Injection of 5×10^{10} strain A cocci into animals treated for 1 to 2 weeks with 1 or 5 mg of prednisolone per day also failed to produce TEN. However, after 3 weeks, the 6 surviving animals developed a mild generalized form of TEN after the injection of similar doses. There was no spontaneous wrinkling or erythema, but a positive Nikolsky's sign could be elicited both over injection sites and at foci distant from areas of inoculation (Fig. 5). The histopathology of skin from such regions consisted of typical intraepidermal cleavage. Sham-injected animals and animals injected with India ink alone never developed TEN. Additional intravenous injections of India ink administered 30 to 60 min before the introduction of cocci did not enhance susceptibility to TEN at any interval.

Systemically administered ET fractions did not produce TEN in steroid-treated animals.

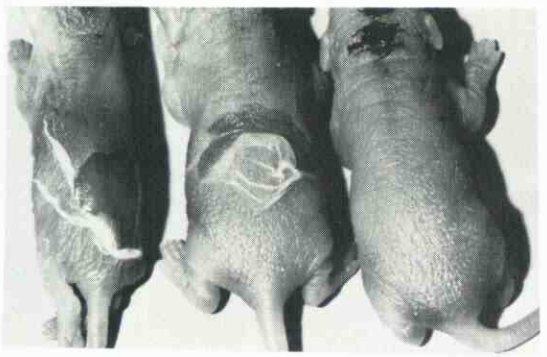

FIG. 4. Neonatal mice injected with stepwise dilutions of ET 2 hr earlier. Mouse on left received most concentrated fraction and demonstrates spontaneous wrinkling as well as a positive Nikolsky's sign. Mouse in center received 1:10 dilution and has positive Nikolsky's sign only, while animal on right received 1:100 dilution and demonstrates neither spontaneous wrinkling nor a positive Nikolsky's sign.

TABLE III

Antibody blockade of TEN in neonatal mice

	Dilution[a]	No. 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 were made with normal saline.

[b] 4+ = spontaneous wrinkling; 3+ = easily elicited Nikolsky's sign at 2 hr; 2+ = easily elicited Nikolsky's sign at 4 hr; 1+ = Nikolsky's sign elicited with difficulty at 4 hr.

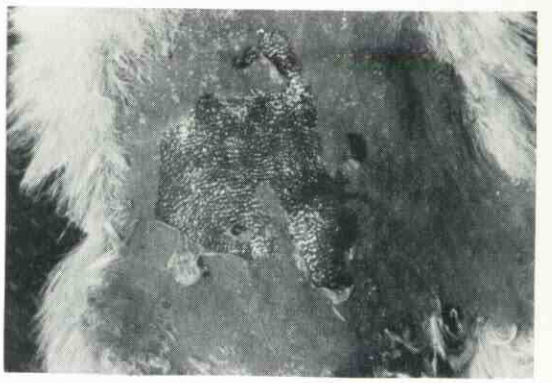

FIG. 5. Epilated back skin of steroid-treated adult mouse. Positive Nikolsky's sign is observed 24 hr after Group 2 organisms were injected into abdominal skin (see text).

Response to injected cell-free filtrates. Hairy vs non-hairy skin: In both hairy and relatively hairless adult mouse skin, TEN developed following the injection of as little as 0.1 mg of ET. Spontaneous wrinkling and erythema were absent, but light rubbing produced extensive denudation (see Fig. 5) and, as in neonates, microscopical intraepidermal vesiculation occurred in nontraumatized skin (Fig. 6). While TEN could not be appreciated grossly in hairy areas, typical intraepidermal cleavage was observed microscopically (Fig. 7). Since TEN could be elicited with equal ease in either chemically epilated or glabrous ear skin (Fig. 8), chemical pretreatment was apparently not a decisive factor in responsiveness.

The ultrastructure of ET-injected sites in adult mice revealed a subgranular, acantholytic process similar to that observed in the neonatal mice (Fig. 9). In constrast to neonatal epidermis, adult interfollicular epidermis is very thin, often lacking a granular layer (see Fig. 3). Where the interfollicular epidermis thins to three cell layers, a pemphigus-vulgaris-like pattern of suprabasilar cleavage may be present (Fig. 10 *bottom*).

Effect of previous TEN. Several mice surviving toxin injections as newborns were divided into two groups and rechallenged at age 3 weeks and 4 months, respectively (Tab. IV). TEN was easily evoked, even with relatively low doses of toxin (1.0 mg protein), suggesting that earlier exposure to ET did not confer protection.

Localized vs generalized TEN. Regardless of the total dose or route of administration, generalized TEN never developed in adult mice. Occasionally, localized TEN appeared over parenteral or intraperitoneal injection sites, but this was attributed to back-leakage. An apparent spreading of TEN to adjacent skin following intracutaneous injections of large amounts of ET seemed attributable to local diffusion, since TEN could not be elicited at still more distant sites.

Influence of route and site of administration. TEN could be evoked only after intracutaneous

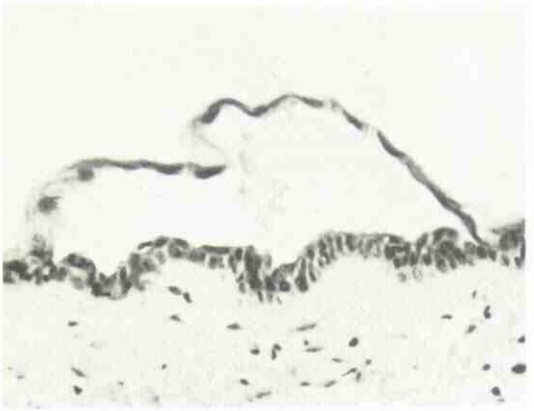

FIG. 6. Adult mouse skin 2 hr after injection of ET. Epidermis is much thinner than that of neonates (see Fig. 2). Note intact subgranular bulla and, again, the absence of a dermal inflammatory infiltrate (× 250).

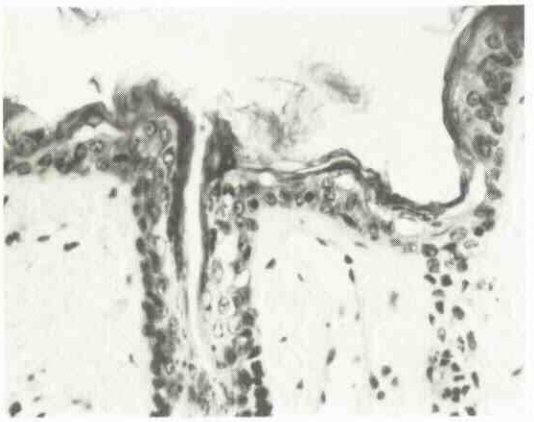

FIG. 7. Hairy skin of adult mouse 2 hr after ET injection. Although TEN was not noticeable grossly, the process microscopically involves hairy areas as evidenced by extensive subgranular microvesiculation (× 250).

injection of ET (14/14 animals). Abdominal skin epidermis appeared to be more sensitive to ET than back skin, but this might have reflected differences in dermal thickness in these regions rather than intrinsic differences in epidermal responsiveness. When ET was injected intramuscularly or intraperitoneally (9 animals), TEN did not result, even when doses in excess of the amount required to produce TEN in newborn mice were administered, e.g., 1 to 2 mg protein per gm body weight. Data from intravenously administered ET are not included because leakage was unavoidable, making interpretation difficult (see [8]).

DISCUSSION

Our results confirm the fact that a syndrome resembling staphylococcal TEN in humans can be induced in newborn mice by injecting either phage Group 2 staphylococci or cell-free filtrates from the same organisms (Fig. 10 *top*) [1–4]. However, the data presented here dispel the idea [3,6] that adult mouse skin is resistant to cell-free filtrates of Group 2 bacteria. We found that normal adult mice were quite susceptible to intracutaneously administered cell-free coccal filtrates. The fact that preincubation with specific antibody abolished or reduced the TEN-evoking capacity of cell-free filtrates provides additional proof of the relationship between staphylococcal TEN and ET. Complete prevention of TEN by antibody preincubation might have been possible if either unabsorbed antisera (more potent) had been used, or if longer incubations with ET fractions had been performed prior to injection. Furthermore, since TEN could be readily induced in either hairy or non-hairy skin, the available data suggest that neither age nor hairiness constitutes a significant barrier to production of localized TEN.

Yet certain distinctions between the susceptibilities of newborn and adult mice emerged in this study. First, although localized TEN could be easily induced in both neonates and adult animals, generalized TEN could not be elicited in adult animals even with doses (mg protein/gm body weight) exceeding those required to produce TEN in newborn mice. Whereas adult epidermis remains unchanged in its capacity to respond to ET, the adult animal manifests *extracutaneous* resistance to cell-free filtrates, presumable because of its enhanced metabolic capabilities. This possibility could be tested in the adult mouse by experimentally inducing diminished hepatic and/or renal function.

A second important distinction is that whereas Group 2 cocci caused generalized TEN in newborn mice, injected organisms did not produce even localized TEN in normal adult mice. Possible explanations for the failure in injected Group 2 organisms to produce TEN in adult mice include (1) enhanced host metabolism of (?gradually released) ET; (2) the presence of a less favorable milieu for bacterial growth and/or ET elaboration in the normal adult mouse; or (3) the acquisition of resistance to ET because of previous subclinical

FIG. 8. Relatively hairless ear skin of adult mouse. Positive Nikolsky's sign is easily elicited 2 hr after ET injection.

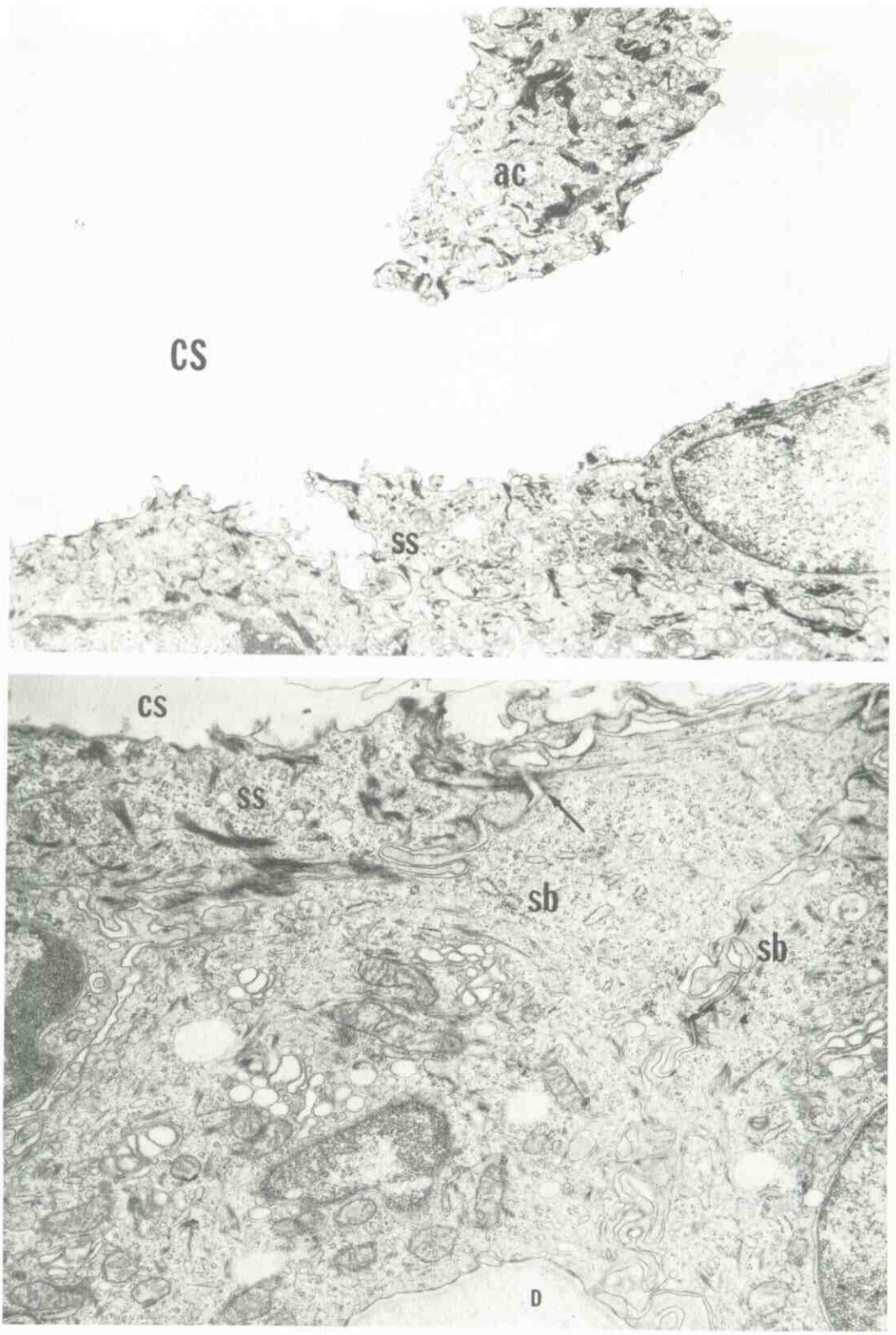

FIG. 9. Electron micrographs of adult mouse skin 2 hr after ET injection. *Top*: an acantholytic cell (*AC*) lies free in the cleavage space (*CS*). Where epidermis thins to three cell layers (*bottom*), the cleavage space may adjoin the stratum basale (*SB*) or stratum spinosum (*SS*). Note the absence of keratohyaline granules and the presence of numerous cleaved or cleaving (*arrow*) desomosomes along separated surfaces. (*Top*, × 260; *bottom*, × 7,800)

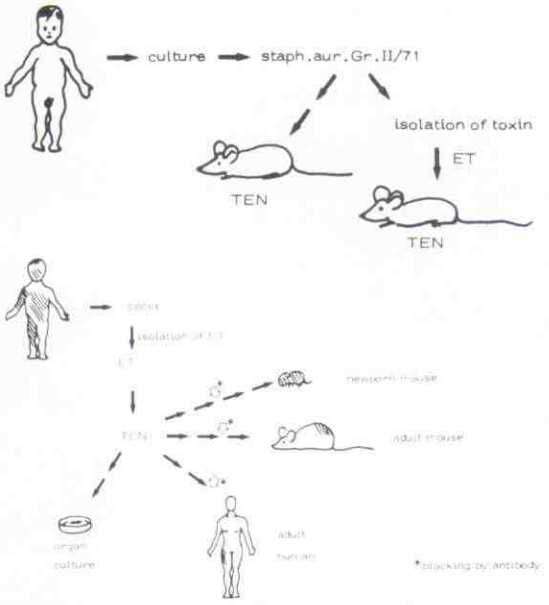

FIG. 10. *Top* diagrammatically represents the previous model of staphylococcal TEN limited to children and neonatal mice. *Bottom* incorporates additional information, i.e., extension of the model to the adult mouse as described here, to adult human volunteers [5], to both mouse and human skin in organ culture [5], and blockade of clinical TEN with specific antibody.

TABLE IV

Readministration of ET to mice surviving previous ET-induced TEN

Group	Age	Dose (mg protein)	No. of animals with TEN
—	Newborn (3–5 days)	1	12/12
1	21–28 days	1–10	6/6
2	2–3 months	1–10	9/9[a]

[a] Three animals from Group 1 were included in Group 2 for later testing.

exposure to resident staphylococci. Two experiments in this study dealt with these potential explanations. Conditions more conducive to the development of TEN followed high-dose steroid administration, since these animals acquired a mild form of generalized TEN after 3 weeks of continual treatment. In a relevant recent report, Wiley et al (18) observed increased susceptibility of immunosuppressed neonatal mice to phage Group 2 organisms. Whether the effect in these experiments is due to immunosuppression, permitting unrestrained bacterial multiplication, or whether some other effect of systemically administered steroids is responsible for enhanced susceptibility, is not clear. Steroid treatment did not seem to affect ET metabolism, since systemically injected toxin did not produce TEN. Since animals which had survived TEN as newborns remained susceptible when rechallenged with cell-free filtrates as adults, adult resistance may not be attributable to prior, inapparent exposure to phage Group 2 staphylococci followed by elaboration of neutralizing antibodies, as suggested by Wiley and his co-workers.

The rather special requirements for TEN induction in the adult mouse may explain the apparent rarity of generalized staphylococcal TEN in normal human adults [9–14]. Most cases have occurred in debilitated or immunosuppressed patients [10, 12,13]. Moreover, to our knowledge, no report of adults with bullous impetigo (a form of localized TEN ascribed to Group 2 staphylococci) have appeared in the literature. Thus a parallel situation seems to exist here in the adult mouse and in man: both are resistant to generalized and localized TEN resulting from inoculated phage Group 2 staphylococci. Is adult human skin, like that of the adult mouse, sensitive to cell-free filtrates? In a separate report, we have detailed the experimental induction of TEN in the skin of adult human volunteers in vivo and in vitro (Fig. 10 *bottom*) [5]. Furthermore, a report of localized TEN following intracutaneous injection of cell-free fractions recently appeared [18]. In every parameter studied to date, the adult mouse and the adult human have responded analogously. The adult mouse, therefore, would appear to be an appropriate animal model for further investigations of conditions favorable to the development of staphylococcal TEN in adult humans.

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