

previous study had shown that Trafuril also elicits a skin response in healthy individuals resulting in increased PG concentrations (17). The skin reaction caused by KI or Trafuril in DH therefore seems to be unspecific although it may be mediated by PGs.

In DH a severe burning itch usually precedes the rash (1). Under experimental conditions PGs can potentiate itch by lowering the threshold tolerance of human skin to histamine (8, 9). Although the PG concentrations are high in blister fluid it seems unlikely that PGs are of primary importance in producing itch and skin lesions in DH. Acetylsalicylic acid and other anti-inflammatory agents inhibiting PG synthetase are neither clinically effective nor do they control experimental skin lesions in DH (1, 15). However, the demonstration of increased concentrations of PGE and PGF_{2α} in suction blister fluid from patients with DH is compatible with the role PGs seem to play as chemical mediators in skin inflammation and blister formation.

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Quantitation of Skin Bacteria: Lethality of the Wash Solution Used to Remove Bacteria

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Abstract. A widely used technique for the quantitative removal of bacteria from the skin uses a detergent, Triton X-100 (*p*, *t*-octylphenoxynonaethoxyethanol), to remove and suspend the bacteria. We determined the half-life for the survival of five common skin bacteria suspended in the solution. The shortest-lived was *Streptococcus pyogenes*

Table 1. Average half-lives for survival of bacteria suspended in wash or control solution

P. acnes and *S. epidermidis* were removed from the forehead and suspended with the indicated solutions. The other species were grown from clones in broth solutions

Species	Triton X-100 Wash-solution Half-life (hours)	Buffer-only Control-solution Half-life (hours)	<i>p</i>
<i>P. acnes</i>	1.5 (0.89–2.3) ^b <i>n</i> =3	1.0 (0.32–1.7) <i>n</i> =2	<0.65
<i>S. epidermidis</i>	4.5 (3.8–5.3) <i>n</i> =5	6.6 (2.7–12.5) <i>n</i> =4	<0.21
<i>S. aureus</i>	2.2 (1.8–2.6) <i>n</i> =2	7.3 (5.6–8.9) <i>n</i> =2	<0.09
<i>S. pyogenes</i>	0.9 (0.8–1.0) <i>n</i> =2	30 (25–35) <i>n</i> =2	<0.04
<i>K. pneumoniae</i>	>30 <i>n</i> =2	>30 <i>n</i> =2	–

^a *p*=probability. Half-life Triton ≥ Half-life Control.

^b Range.

with a half-life of 0.9 hours. All of the others (*Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*) had half-lives of 1.5 hours or longer. *K. pneumoniae*, the only Gram-negative species tested, had a half-life of more than 30 hours. Thus, a one hour delay in plating and incubation of samples suspended in this detergent solution inhibits quantitation of most species tested.

The widely used technique for quantitative sampling of skin bacteria utilizes a detergent, Triton X-100, in a phosphate buffer solution, to remove and suspend the bacteria (8). If investigators using this technique must delay plating, it is important to know how many bacteria die as a function of time in this solution. The present study determined the half-life for survival of five common skin bacteria suspended in the wash solution.

MATERIALS AND METHODS

Wash solution. Triton X-100 (0.1% in 0.75 M phosphate buffer (pH 7.9)) was used as the wash solution (8). As a control a buffer-only solution was used (see below).

Sources of Bacteria. Five species of bacteria were tested: *propionibacterium acnes*, *Staphylococcus epidermidis* (Biotype 1, Baird-Parker), *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*.

P. acnes and *S. epidermidis* were removed from the foreheads of two adult subjects using a detergent-scrub technique (8). A sterile metal cylinder 2.4 cm in diameter was placed firmly on the forehead of the subject. One ml

of sterile detergent wash-solution was placed inside the cylinder. The skin exposed to the wash-solution was rubbed with moderate pressure for one minute with a sterile Teflon scraper. The wash-solution, containing the two species of bacteria, was aspirated for culture. As a control, an area of the skin was scrubbed using a buffer-only solution.

The other species tested were not available on the skin in sufficient numbers for our purposes. From clones, we grew stock cultures of *S. aureus*, *S. pyogenes*, and *K. pneumoniae*. The growth medium was brain-heart infusion broth with 1% glucose added. Concentrations of log-phase bacteria at harvest varied from 100 000 to 10 000 000 organisms/ml.

Dilution procedures. The following steps were accompanied by 30-sec mixings on a Vortex Jr mixer.

P. acnes and *S. epidermidis* (mixed together) were left in the solutions that they were aspirated in. At timed intervals aliquots were taken, serially diluted in half-strength wash-solution, and cultured on appropriate media.

For the other three species, stock broth solutions were serially diluted 10² to 10⁵ fold (depending on the organism) in full strength wash-solution. These formed our test solutions from which aliquots were taken at timed intervals, diluted appropriately in half-strength wash-solution and cultured.

As a control, the bacteria were diluted into 0.075 M buffer-only solution. Otherwise the same procedure was followed as with the full-strength wash-solution.

Length of exposure. The bacteria in the detergent solution (except for *K. pneumoniae*) were followed for 10–15 hours. (Although *K. pneumoniae* was followed for 20 hours, its population density fell by less than half in that time.) Four to six points of data were taken for each half-life determination. Each determination was repeated two or more times.

Table II. Summary of data from the literature analogous to that presented in Table I

One study found that highly dilute tryptose phosphate broth protected the organisms for up to 2 weeks (6). The detergent-free controls of *P. caudatum* were unaffected by the control solution; they had a half-life greater than 10 hours (3)

Amount Triton X-100	Organism	Buffer	Half-life	Ref.
None	<i>S. aureus</i>	Half-strength Ringer's solution (mainly NaCl)	2 h	(1)
None	<i>S. aureus</i>	"Physiological saline"	0.33 h	(6)
None	<i>Bacterium lactis aerogenes</i>	Phosphate buffer, pH 7.1	0.5 h	(4)
1.5 mM (our strength)	Human red blood cells	Phosphate buffer, pH 7.0 0.067 M NaCl	The Triton caused lysis of the red blood cells under these conditions	(7)
No Triton. 29 mM sodium dodecylbenzene-sulphonate ^a	<i>Klebsiella</i> strains	"Synthetic growth medium"	The detergent promoted growth of the organisms	(5)
1.5 mM (our strength)	<i>Paramecium caudatum</i>	Salt solution + Tris buffer pH 7.3	Highly lethal in 1 min compared to control	(3)

^a An anionic detergent considered to be more lethal than Triton X-100 (1).

For the control solutions the same procedure was followed except that both *K. pneumoniae* and *S. pyogenes* were followed for 20 hours (during which their population densities dropped by less than half).

Bacteriological methods. *P. acnes*-*S. epidermidis* (mixed together) and *K. pneumoniae*, were grown on plates containing BHI agar with 1% glucose added.

S. pyogenes and *S. aureus* were grown on TSA agar plates.

The *P. acnes*-*S. epidermidis* plates were done in duplicate; one was incubated anaerobically and one aerobically. (Primarily *S. epidermidis* grew on the aerobic plates with relatively few micrococci and no *P. acnes*.) All other plates were grown aerobically in a standard 37°C incubator. Aerobic plates were incubated for 1-2 days and anaerobic plates for 7 days. The organisms were identified by the appropriate biochemical methods (2).

RESULTS

The data are summarized in Table I. *S. pyogenes* with a half-life of 0.9 hours in the wash-solution was the shortest lived bacterium studied. *S. aureus* and *S. pyogenes* had notably lower survival rates in the detergent solution than in the control solution. *P. acnes* had a slightly lower survival rate in the control solution than in the detergent solution, 1.0 versus 1.5 hours.

When the buffer-only control solution was used to wash the forehead it was visibly less cloudy than the detergent wash-solution; possibly less debris was removed from the skin with this solution.

DISCUSSION

The present study provides data on the survival of bacteria suspended in buffered solution of Triton X-100. The solutions contained little or no nutrients for growth, and, as expected, the organisms either remained at about the same density or declined during the periods studied.

Our basic premise is this: Bacteria require energy and nutrients to maintain cellular integrity (e.g., the correct internal ionic composition) and particularly to maintain membrane integrity. The primary reason for the decline in all cases was probably lack of nutrients—"starvation". Triton X-100 is known to disrupt cell membranes at the strength used; this imposes a stress on the bacteria in addition to "starvation". Most bacteria showed a decline in their detergent-free control solutions, which was in most cases similar to the decline in the detergent wash-solution.

In Table II, we have compiled analogous data from the literature. Few of the data are from experiments with conditions directly comparable to ours. The better of the two studies in regard to half-lives for survival on *S. aureus* (1) gives a half-life comparable to our test value. Nikodemus et al. (5) used presumably *Klebsiella* strains which were acclimatized to the detergent studied; their data imply that their bacteria could break down the detergent and use it as a nutrient source. The addition

of glucose (6) did not prolong the life of the bacteria; however, the authors found that highly dilute (0.003%) Tryptose Phosphate Broth, although unable to support growth, protected the bacteria from death for about 2 weeks (6). It is conceivable that many bacteria, given adequate nutrients, could survive the stress of being suspended in Triton X-100 at the strength used.

A word on the *P. acnes* and *S. epidermidis* data is in order. No simple comparison between the experimental and the control data can be made. Fewer bacteria and possibly less "debris" were removed from the skin with the detergent-free solution than with the detergent solution. The difference in the numbers of bacteria removed was probably not such as to make any difference in the survival rate. High densities of bacteria protect each other in "starvation" solutions, presumably because those bacteria that die first lyse out nutrients to protect the remaining bacteria (4). Of greater importance perhaps is the "debris" removed from the skin; it may have protected the bacteria either by supplying nutrients or, more likely, by adsorbing Triton X-100 and lowering its activity (effective concentration). We were not primarily concerned with measuring the theoretical contribution of Triton X-100 to the death of the bacteria. Our main concern was the *in use* situation in order to help investigators know how many bacteria are lost in the wash-solution if delay is introduced between sampling and plating. The organisms died at the same rate whether observed in the first 2 hours of incubation or observed over a 10–12 hour incubation period.

In conclusion, except for *S. pyogenes* which seems to be particularly sensitive to the detergent, the species of bacteria tested can be left in the wash-solution for one half-hour with only about a 20% loss of the *P. acnes*—less for other species. Due to the variability of the data, our estimated half-lives cannot be used as correction factors when longer delays occur between sampling and plating.

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Linear Dermo-Epidermal IgA Deposition in Bullous Pemphigoid

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Abstract. A case is reported of a patient with a bullous eruption with *in vivo* linear deposition of IgA and complement (C3) along the basement membrane zone of perilesional skin (DIF method). Some data presented here are in favour of the concept that cases of linear *in vivo* deposition of IgA alone, or in combination with other IF findings, might be classified as bullous pemphigoid (BP). Clearing of the lesions due to oral prednisone therapy was accompanied by disappearance of complement deposition, while IgA deposition remained unchanged. Some aspects of tissue injury in this case are briefly discussed.

From several reports in the literature it appears that, even with the aid of immunofluorescence (IF) techniques, in some so-called indeterminable cases of bullous pemphigoid (BP) and dermatitis herpetiformis (DH) a definite classification may be difficult or impossible (1, 3, 6). In view of the characteristic occurrence of IgA deposition in DH, several authors stated that even cases of strictly linear deposition of this class of immunoglobulin along the basement membrane (BM) of the skin and in the absence of circulating BM antibodies should be included in the diagnostic category DH (3,9).