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Experimental Infections with Group A Streptococci in Humans

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Experimental inoculation of 7 strains of Group A streptococci failed to result in either colonization or infection of normal intact skin of human volunteers. All strains rapidly died on normal skin; suppression of the resident microflora did not affect survival and no difference in survival was seen between inoculation on lipid-rich and lipid-poor body areas. Inoculation on skin damaged by superficial scarification resulted in localized infections when 1×10^4 or more organisms were inoculated into the wound by rubbing and covered with an impermeable plastic film. Intradermal inoculation resulted in localized cellulitis, regional lymphadenopathy, and fever. All strains were equally effective in producing localized infections in scarified skin.

Group A streptococci are a major cause of skin infections. In recent years, a great deal has been learned about the epidemiology of streptococcal pyoderma and the subsequent glomerulonephritis induced by nephritogenic strains. Personal hygiene and environmental factors are particularly important. In an intensive survey in a tropical region with the population distributed at different altitudes, Taplin *et al* found that the attack rate was much lower in the cold higher altitudes and greatest in the steaming hot lowlands [1]. At each level the prevalence of infection was highest among the poor who lived in crowded, unhygienic conditions. One important epidemiological question remains unanswered. What is the source of the streptococci? The bite of the Hippelates fly, which harbors streptococci in its intestine and feeds on wounds constitutes one mode of transmission [2], but other reservoirs are unknown. Insect bites and scabies are common pre-infection events.

In epidemic areas, intensive skin sampling among children at risk has shown that Group A streptococci are frequently recoverable from clinically normal skin prior to the appearance of lesions. This happens so regularly that Ferrieri et al and Dudding and co-workers believe that colonization of normal skin may be antecedent to frank infection [3,4]. With semiquantitative sampling methods, Dudding et al found 10 to 50 colonies of Group A streptococci in a third of the subjects, and more than 50 colonies in 10%. Approximately 18% of wrist and ankle sites contained 10 to 50 colonies, and 14% and 17% respectively contained 50 to 500 colonies. While these are small numbers, these investigators thought their presence on normal skin was the first step in the development of infection. These investigators viewed different possibilities for the high prevalence of streptococci on normal skin. One possibility was dispersal from infected lesions with repeated deposition on the skin of uninfected contacts without survival and colonization. This is particularly possible since poor hygiene, especially inadequate washing and uncleanliness, is commonplace on the Indian reservations where these studies were carried out. Other possibilities include survival of organisms for protracted periods, perhaps days or they could multiply and establish a self-sustaining population (colonization). Although the organisms were regularly recovered from various body regions, the great preponderance of pyoderma (ecthyma) on the lower legs implicated additional factors in pathogenesis. Practically all workers believe that trauma in the form of cuts, abrasions, and insect bites are important factors. Such injuries which breach the horny layer barrier are common in bare-legged children.

On the other hand, other investigators have concluded that streptococci cannot colonize normal skin. It has long been realized that Group A streptococci die rapidly when placed on normal skin, a finding usually explained by the high susceptibility of this organism to the free fatty acids contained in surface lipids [5,6]. In extensive studies designed to investigate the factors involved in streptococcal infection, Duncan et al produced only 1 infection in 78 inoculations of Group A streptococci on normal skin. Despite a large inoculum (8 to 45 million organisms), streptococci were recovered in only 47 of 78 sites. They then damaged the skin in a variety of ways. The most effective tactic involved stabbing the skin with a lancet through a droplet of a bacterial suspension, followed by covering the site with an agar disc to promote growth. Pustules resulted in 13% of forearm inoculations and in 38% of lower leg inoculations. This study is compromised by the fact that the suspension consisted of a mixture of Group II phage type 71 *Staphylococcus aureus* and Group A streptococci. Since the lesions were not cultured, it is impossible to know which organism was responsible or whether both were collaborating. The clinical description of "pustules" suggests that the lesions were of staphylococcal origin; streptococcal lesions are typically ulcerated with a thick, purulent crust. This possibility is strengthened by Dajani's finding that type 71 *S. aureus* strains can produce a substance which is inhibitory to Group A streptococci [8].

Streptococcal pyodermal has been induced in hamsters by deep intradermal injection [9]. A human model does not exist. The object of this paper is to describe a method for producing streptococcal infection in humans and to present an analysis of the factors involved in the development of infection.

MATERIALS AND METHODS

Subjects

The subjects were the investigators, laboratory personnel, and young, healthy, male, college student volunteers. The subjects were carefully screened. Normal values were found on urinalyses, complete blood counts, and SMA 12. None had a history of recurrent streptococcal infections. Written informed consent was obtained from each subject. Protocols were reviewed and approved by the institutional review board to the Simon Greenberg Foundation.

Group A. Streptococcal Strains

Seven strains of Group A streptococci were used: (1) American Type Culture Strain 13450 (T-type 14/49); (2) a strain (T-type 13/B3264) isolated by Taplin in an epidemic in New Mexico; the 5 other strains were T-types 5/27/44, 8/25/Imp. 19, 3/13/B28, 11/12, and 12. Attempts to M-type these strains were unsuccessful. The first 2 strains had T-types previously shown to be associated with "nephritogenic" M-serotypes, while the latter five T-types were not known to be associated with nephritogenic M-types. Postinfection nephritis occurs principally in preschool children and exempts adults. According to Taplin, there were no documented cases of streptococcal-induced nephritis in the American troops during the Viet Nam war despite widespread lesions containing nephritogenic strains of streptococci (Taplin D, personal

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communication). For these reasons, we undertook cautious inoculation of "nephritogenic" streptococci in ourselves and senior members of our research staff. Each subject was evaluated by urinalysis before, immediately at the end of the experiment and 2 weeks later by urinalysis.

Preparation of Inoculum

Based on our previous experiences with *S. aureus* and *Candida albicans*, streptococcal strains were subcultured at least 2 times on trypticase soy agar with 6% sheep's blood. This procedure maintains virulence. When this was not done, stock cultures largely failed to induce infections.

The inoculum was prepared from a fresh overnight culture on sheep's blood agar by suspending the organism in equal parts of normal saline and Todd-Hewitt broth containing 1.0% Tween-80. In saline alone, viable streptococcal organisms decreased substantially within 4 hr.

Culture Technique

The standard detergent-scrub technique of Williamson and Kligman was utilized [10]. Since the non-ionic detergent (Triton X-100) can be inhibitory to some strains of streptococci, we investigated the survival of these strains in the scrub wash fluid. With all strains we found a 1 to 2 log decrease within 1 hr. The addition of 1.0% Tween-80 to the regular wash fluid completely prevented this. After sampling the skin, the fluid was diluted in 10-fold steps in 0.05% Triton and immediately plated on Trypticase Soy Agar with 6% sheep's blood, Trypticase Soy Agar and T.S.A. with 6% sheep's blood and 0.8 mg/ml crystal violet. The plates were incubated aerobically for 48 hr at 37°C.

1. Inoculation of Group A Streptococci on normal skin: Twenty volunteers were inoculated with 1×10^6 organisms of each strain on the volar forearms. Each site was covered with 25 cm^2 of impermeable plastic film (Saran Wrap) and sealed under overlapping strips of adhesive tape. Quantitative cultures were obtained from 10 subjects after 24 hr and from the other 10 after 48 hr of occlusion.

2. Inoculation of Group A Streptococci on lipid-rich and lipid-poor body areas: Numerous investigators have suggested that the susceptibility of Group A streptococci to various lipids may account for the rarity of isolation of these organisms from intact human skin. To test the role of skin surface lipids in preventing colonization, we inoculated 1×10^6 organisms of strain T-13/B3264 on three 25 cm^2 areas on each forearm, and on three 25 cm^2 areas on each side of the upper back, of 10 subjects. A similar inoculum of strain T-14/49 was applied to the forearms and back of another 10 volunteers. The design was to compare survival of streptococci on an area comparatively rich in surface lipids (back) with a lipid-poor area (forearm). Each site was covered with impermeable plastic film and then sealed with overlapping strips of adhesive tape. Quantitative cultures were obtained from 1 arm and 1 side of the back after 24 hr, and from the other arm and side of the back after 48 hr.

3. Effect of removing the normal flora: Previously Singh et al of our laboratory demonstrated that the resident aerobic flora deters infection by a virulent organism such as *S.aureus* [11]. Degerming the skin prior to inoculation enhanced survival of the inoculum and subsequent development of localized infection. The failure of previous investigators to create experimental infections with *S.aureus* was largely due to competition by normal skin residents.

The skin surface of 5 volunteers was "degermed" by application of 70% ethyl alcohol for 2 min. Strain T-13/B3264 was applied as above, after complete evaporation of the alcohol. Each subject was inoculated on three 25 cm^2 sites on the forearm and three similar sized areas on the upper back with 2×10^6 organisms. Quantitative cultures were obtained after 24 hr of occlusion.

Following Dajani's finding with phage type 71 *S.aureus*, we sought to determine whether the organisms comprising the normal skin flora were capable of inhibiting Group A streptococci *in vitro*. Multiple strains of *S.epidermidis* and lipophilic diphtheroids (at least 2 of each morphological variant obtained from normal skin of the back) were crossed-streaked on blood agar plates with each strain of Group A streptococci.

4. Inoculation of Group A streptococci on damage skin: Superficial scarifications to pin-point bleeding were made in a criss-cross fashion (2 parallel scratches in one direction and another 2 at right angles) on the forearms of 15 volunteers. Three sites on 1 arm were inoculated with 7.2×10^5 organisms of strain T-14/49 and three sites on the other arm with T-13/B3264. The suspensions were rubbed into the site with a sterile Teflon rod and occluded with plastic tape as above. An additional site on each arm served as an uninoculated control. One site on each arm was quantitatively cultured after 6, 24, and 48 hr. Clinical reactions were graded on a 0 to 4 scale with: 0 = no difference from control; 1 = erythema along the scarification line; 2 = erythema and edema confined to the scarification line; 3 = erythema and edema spreading more than 3 mm form the scarification line; and 4 = localized cellulitis with lymphangitis.

In an additional panel of 10 subjects, 6 superficial scarifications were made on the forearm as described above, and 5×10^5 organisms of strains T-5/27/44, 8/25/Imp. 19, 3/13/B28, 11/12, and 12 were inoculated into one site each, rubbed in with a Teflon rod, and covered as described above. The 6th site served as an uninoculated control. Quantitative cultures and clinical assessment were made after 24 hr of occlusion.

5. Effect of inoculum size, environment of wound, and trauma: Further studies on scarified skin took into consideration the following variables: (1) the size of the inoculum was varied from 1×10^6 to 1×10^2 organisms; (2) varying inocula with and without rubbing the inocula into the wound; and (3) occlusion versus open application. In 2 groups of 4 volunteers, 4 scarifications were made on each arm. One site was inoculated with 1×10^6 organisms of strain T-13/B3264, a second with 1×10^4 and a third with 1×10^2, while the 4th served as an uninoculated control. On one arm the inocula were rubbed in, and on the opposite side the suspension was gently spread over the site. In one panel the sites were occluded as above, while no dressings were used in the second panel. The inoculum was allowed to dry completely before the subject was released.

6. Effect of wound depth: Three different types of wounds were compared: (1) very superficial, nonbleeding scratches, cleaving the stratum corneum barrier but probably not reaching the papillary dermis; (2) deeper scarification as described above; and (3) deep intradermal injection. In a group of 8 volunteers, 2 superficial scarifications (no bleeding) were made in one forearm and inoculated with 1.2×10^7 organisms of strain T-13/B3264, while a third site served as an inoculated control site. On the opposite arm, 2 deeper scarifications were similarly inoculated, while a third site served as an uninoculated control site. In one subject (JJL) 1×10^7 organisms of strain T-13/B3264 were inoculated intradermally into 2 deep scarifications.

7. Recovery of S.aureus after inoculation by streptococci: For many years there was heated debate regarding the relative importance of *S.aureus* and *S.pyogenes* in the etiology of pyodermas. In ecthyma both organisms are recoverable in the majority of well developed cases, although it is clear that streptococci initiate ecthymatous lesions which then become colonized by *S.aureus*. We wondered whether *S.aureus* would colonize lesions experimentally induced with Group A streptococci. Each culture from a lesion resulting from streptococcal inoculation was also plated on agar selection for *S.aureus*.

RESULTS

Inoculation on Normal Skin

The results are summarized in Table I. After 24 hr, the geometric mean count for all strains had sharply declined from the 1×10^6 inoculum to a range of 740 to 1200 organisms per cm^2. Streptococci were recovered from 36 of 70 forearm sites. Only one clinical infection developed: it consisted of a nonfollicular pustule at 24 hr which became crusted and surrounded by intense erythema after 48 hr. Group A streptococci were recovered after unroofing the crust but none were recovered from the surrounding normal skin. By 48 hr streptococci had disappeared.

The normal aerobic microflora exceeded the density of strep-

TABLE I. *Survival of Group A streptococci on normal skin*

Strain	24 hr occlusion—10 subjects		48 hr occlusion—10 subjects	
	S.pyogenes per cm^2[a]	Total aerobic count[b]	*S.pyogenes* per cm^2	Total aerobic count[b]
T-14/49	860 (4/10)	1,200,000	0	2,100,000
T-13/B3264	1120 (6/10)	2,100,000	0	3,000,000
T-5/27/44	820 (6/10)	1,800,000	0	4,120,000
T-8/25/Imp 19	740 (4/10)	1,000,000	0	3,200,000
T-3/13/B28	680 (5/10)	1,320,000	0	2,800,000
T-11/12	1200 (5/10)	1,130,000	0	2,200,000
T-12	830 (6/10)	1,400,000	0	1,400,000

[a] Geometric mean of positive site. Proportion of positive sites in parentheses.

[b] Geometric mean.

tococci by several orders of magnitude. This serves as a check on occlusion, for the resident population on uncovered skin usually numbers only a few hundred organisms per cm^2.

2. Survival of streptococci on lipid-rich and lipid-poor areas: Table II summarizes the survival of 2 strains of streptococci on a lipid-poor area such as the forearm and on a lipid-rich area such as the upper back. After 24 hr of occlusion both strains of Group A streptococci had fallen off from the original inocula with streptococci recovered from 14 of 30 forearm sites for strain T-14/49, and 16 of 30 forearm sites for strain T-13/B3264. Similar results were seen on the back with low numbers of streptococci found in 24 of 30 sites for the first strain and 20 of 30 sites for the second strain. There were no significant differences in either the recovery rate of streptococci with respect to body site nor any differences in the total number of organisms recovered. Again by 48 hr, all strains were no longer recoverable.

3. Survival of Group A streptococci after removal of the resident flora: After 24 hr of occlusion Group A streptococci were recovered from 6 of 15 forearm sites with a geometric mean count of 400 organisms per cm^2, and from 9 of 15 back sites with a geometric mean of 750 organisms per cm^2. The total aerobic flora on the forearm was 2,800,000, and a geometric mean of 900,000 per cm^2 was found on the back, assuring that adequate occlusion had been achieved. By 48 hr, the total aerobic flora geometric mean was 3,200,000 for the forearm and 2,600,000 for the back, but no Group A streptococci were recovered. These results were not significantly different from those obtained by inoculating the same strains on skin without removal of the resident microflora.

In-Vitro Studies

No zones of inhibition of any strain of Group A streptococci were associated with any strain of *Staphylococcus epidermidis* or lipophilic diphtheroids.

4. Inoculation on scarified skin: The results are summarized in Table III. Group A streptococci were recovered from every site. After 6 hr, the geometric mean count was 5,129 for Strain T-14/49 and 7,435 for Strain T-13/B3264, i.e., a significant fall-off occurred from the 7.2×10^5 inoculum. By 24 hr the mean streptococcal count had risen to 103,896 for the strain T-13/B3264 and to 154,610 for the other. Strain T-14/49 produced an average clinical reaction of 1.5 compared to 2.2 for Strain T-13/B3264. By 48 hr, the streptococcal count was beginning to decline with a mean count of 21,544 for Strain T-13/B3264 and 138,000 for Strain T-14/49 and a corresponding decline in the severity of the clinical reaction in most subjects (Table III). In the panel of 10 subjects, inoculated with the other 5 strains, localized infections developed at all sites. The results of quantitative cultures and clinical reactions after 24 hr of occlusion are summarised in Table IV. There were no significant differences in the total number of organisms or severity of infection. The number of streptococci recovered was slightly lower than that found with strains T-14/49 and T-13/B3264 but there was no significant difference in the clinical expression of the experimental infection.

The evolution of the experimental infection is illustrated in Figs 1 to 4. Initially a row of vesicles forms along the scarification lines (Fig 1). These quickly turn into pustules (Fig 2), which soon become crusted. An areola of indurated erythema forms (Fig 3), which in some instances progresses to a localized cellulitis (Fig 4). While there is some variability in the severity of individual infections, the vast majority consist of superficial lesions of palpable erythema with crusting. Upon removal of the dressings and twice daily application of a neomycin-bacitracin-polymyxin ointment, the infection healed within 7 days. The deep, intradermal inoculation of 1×10^7 cells of T-13/B3264 in one author resulted, within 72 hr, in deep, punched-out ulcerations with a localized cellulitis, low-grade fever, and regional lymphadenopathy (Fig 5). This infection promptly responded to systemic erythromycin.

Effect of Inoculum Size, Occlusion, and Trauma

The results are summarized in Table V. In both the occluded and unoccluded scarifications, applying the inoculum with rubbing was essential. Without rubbing, even a high inoculum (1×10^6) produced only a minimal reaction of palpable erythema limited to the scarification line in 1 subject. An inoculum of 1×10^4 organisms which was rubbed into the wound produced clinical infections in 24 hr, but they were not as severe as those with a 1×10^6 inoculum. A 1×10^2 inoculum was insufficient to produce a clinical infection. Wounds covered for 24 hr had higher numbers of organisms and more severe reactions than uncovered wounds at all inoculum levels.

6. Effect of depth of wound: Group A streptococci became equally well established in both the very superficial scarifications and those accompanied by pin-point bleeding. The density of organisms was the same and the clinical responses were similar (Table VI). Evidently it is only necessary to breach the horny layer barrier. Streptococci were recovered from all superficial scarifications and ranged from 10,400 to 474,000 organisms per site. The clinical reactions consisted of palpable, indurated erythema spreading beyond the scarification line (+3) to produce localized cellulitis. The sites scarified to the

TABLE II. *Survival of Group A streptococci on lipid-rich and lipid-poor body areas*

Body site	24 hr occlusion		48 hr occlusion	
	Total aerobic count/cm^{2a}	*S.pyogenes*/cm^{2b}	Total aerobic count/cm^{2a}	*S.pyogenes*/cm^{2b}
	Strain T-14/49			
Forearm	3,600,000	755 (14/30)[c]	2,100,000	0
Back	1,200,000	706 (24/30)	3,000,000	0
	Strain T-13/B3264			
Forearm	2,800,000	1,020 (16/30)	3,200,000	0
Back	1,400,000	1,130 (20/30)	4,120,000	0

[a] Geometric mean count.
[b] Geometric mean count of positive sites.
[c] Ratios in parentheses are proportions of inoculated sites from which streptococci were recovered.

TABLE IV. *24-hr occlusion—Inoculation on scarified skin*

Strain	*S.pyogenes*/cm^{2a}	Clinical Reaction[b]
T-3/13	68,200	3.0
T-5/27/44	52,600	2.9
T-11/12	82,000	3.1
T-8/25/Imp. 19	48,200	2.7
T-12	63,100	3.2
T-3/13/B28	72,000	3.4

[a] Geometric mean count of 10 inoculations.
[b] 0 to 4 scale with 4 = cellulitis.

TABLE III. *Comparison of infections produced by 2 strains of Group A streptococci (scarified skin)*

Strain	6 hr		24 hr		48 hr	
	S.pyogenes count[a]	Clinical reaction	*S.pyogenes* count[a]	Clinical reaction	*S.pyogenes* count[a]	Clinical reaction
T-14/49	5,129	0.60	154,610	2.2	138,000	1.3
T-13/B3264	7,435	0.35	103,896	2.5	21,544	0.75

[a] Geometric mean count.

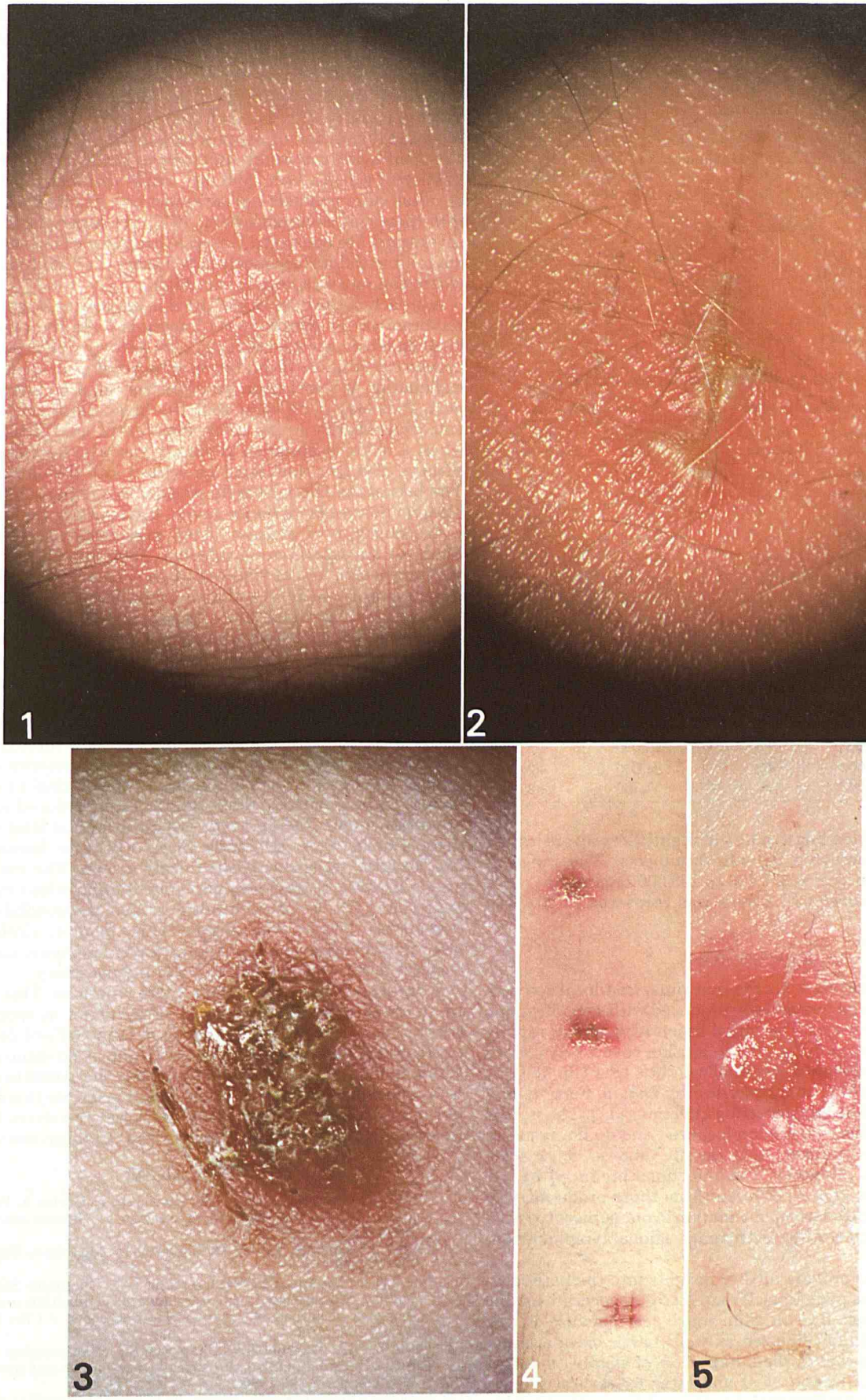

FIG 1. Vesicles along scarification lines 12 hr after inoculation of Group A streptococci.

FIG 2. Eighteen hours after inoculation pustules form along scarification lines.

FIG 3. Erythema developing around site of inoculation and exudation crust beginning to form.

FIG 4. Upper 2 sites show localized cellulitis surrounding pustules along scarification lines. Bottom site is an uninoculated control.

FIG 5. Punched out ulcer with surrounding palpable erythema (ecthyma) resulting from deep intradermal inoculation.

TABLE V. *Effect of Inoculum size and method of inoculation*

Inoculum	Rubbing		No rubbing	
	S.pyogenes count[a]	Clinical reaction	*S.pyogenes* count	Clinical reaction
	Occluded			
1×10^6	160,000 (4/4)	3.50	2,060 (1/4)	1.0
1×10^4	38,400 (4/4)	2.25	0 (0/4)	0
1×10^2	1,200 (1/4)	0.50	0 (0/4)	0
Control	0 (0/4)	0	0 (0/4)	0
	Unoccluded			
1×10^6	96,000 (4/4)	3.25	560 (1/4)	0.025
1×10^4	6,940 (1/4)	1.75	0 (0/4)	0
1×10^2	120 (0/4)	0.50	0 (0/4)	0
Control	0 (0/4)	0	0 (0/4)	0

[a] Geometric mean count.

TABLE VI. *S. Pyogenes inoculation on scarified skin*

Subject #	Superficial scarification		Deep scarification	
	S.pyogenes count	Clinical reaction	*S.pyogenes* count	Clinical reaction
1	304,000	+4	15,200	+2
	328,000	+4	28,000	+2
2	24,000	+2	17,120	+2
	10,400	+2	24,800	+2
3	112,000	+2	207,200	+2
	86,000	+2	223,000	+2
4	247,000	+3	79,840	+2
	71,600	+2	34,400	+2
5	215,200	+3	3,200	+1
	474,400	+3	2,400	+1
6	240,000	+3	20,600	+2
	29,600	+2	24,000	+2
7	191,000	+3	135,000	+3
	176,000	+3	223,000	+3
8	80,000	+2	24,000	+2
	128,000	+3	7,760	+2

point of pin-point bleeding resulted in slightly less severe lesions overall but this occurred in only 2 subjects. The range of recovery of streptococci was 2,400 to 223,000 organisms.

7. S. *aureus recovery:* S. aureus was recovered in 20.6% of lesions.

DISCUSSION

We have demonstrated, for the first time, that localized self-healing infections can be regularly produced with several strains of Group A streptococci. The initial lesion is vesicular, rapidly becoming pustular, evolving into an erosion covered by a thick crust and surrounded by a localized cellulitis (Fig 1 to 4). This sequence is identical to that described by Dajani, Ferrieri, and Wannamaker in naturally occurring infections [12]. The lesions produced by scarification remain localized and do not result in lymphadenopathy or fever, heal rapidly, and so cause little discomfort to the volunteer. The infections produced by nephritogenic strains were no different from those produced with ordinary strains. Intradermal inoculation in one subject resulted in a deep dermal ulceration (ecthyma), regional lymphadenopathy and fever.

Our experiments provide further insights into the pathogenesis of streptococcal pyoderma. Group A streptococci are unable to survive on intact human skin, much less colonize it. We presume this holds for children as well. Large inocula under occlusive dressings rapidly died. Rapid loss of viability was first demonstrated by Ricketts et al and later by several groups, including a recent confirmation by Aly and his co-workers [5–7,13]. The strains we tested died just as quickly on lipid-rich and lipid-poor body areas, suggesting that the fatty acids in surface lipids are not very important in destroying the organism or alternatively that streptococci are so susceptible to lipids that they are unable to survive in the presence of even a minimal amount of lipid. We interpret the frequent recovery of small numbers of streptococci from the normal skin of children in close contact with cases of streptococcal pyoderma reflects repeated deposition of organisms dispersed from open lesions. Crowded living conditions and poor hygiene account for transmission of streptococci from one family member to another or to other close contacts during epidemics. Taplin's recent findings (personal communication, 1977) are highly instructive in this regard. He recovered Group A streptococci from inanimate environmental surfaces such as bed sheets and clothing in areas where the infection rate is very high.

A breach in the integrity of the skin appears to be an absolute requirement for streptococcal infection. Even the most superficial damage, provided that the stratum corneum is perforated, provides a suitable niche for streptococcal growth. A possible explanation is that serum provides the required nutrient support. It is also quite clear that the inoculum must be solidly entrenched in the wound before infection occurs. Little happens when a dense suspension is simply spread over the scarified surface. The inoculum must be rubbed into the damaged site. The bite of the Hippelates fly satisfies this requirement. Insect bites no doubt play a major role in transmitting the infection.

An inoculum of at least 10,000 organisms was required to establish an experimental infection. This most likely reflects the nonspecific loss of viability when transferring an organism from a culture medium to human skin. We observed a similar "transplantation shock" in experimental *S.aureus* infections [11]. Great decreases occurred upon inoculation of normal skin, followed a few hours later by a steady increase to high densities. Even in epidemic situations it is unlikely that as many as 10^4 organisms will arrive at a site where the horny layer has been perforated. It seems a likelihood that far fewer numbers can incite an infection if implanted into the skin through deeper wounds than we utilized, especially in the presence of other foreign substances which add insult to the original injury. It is important to emphasize that experimentally induced infections are erratic unless the organism is subcultured at least twice on sheep's blood agar. Evidently these organisms become more like lesional organisms when freshly grown. The inability of previous workers to induce experimental infections may have been due to the use of stock cultures. Also, the possibility exists that streptococci in infected lesions may be more virulent than laboratory strains and that spontaneous infections may occur with far fewer organisms than we found necessary.

S.aureus was recovered from 20.6% of lesions. This is somewhat lower than the prevalence of *S.aureus* in spontaneous streptococcal pyodermas. Dajani et al found 62% of 243 lesions contained *S.aureus* in addition to Group A streptococci [14]. While our observations are preliminary, the volunteers whose lesions yielded *S.aureus* were also found to harbor this organism in their anterior nares. *S.aureus* was rarely recovered from the lesions of those who did not harbor the organism elsewhere.

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