

The role of normal skin in the spread of streptococcal pyoderma

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SUMMARY

The primary body site of acquisition of group A streptococci was examined prospectively in a population with endemic streptococcal pyoderma. Weekly cultures were obtained during the skin infection season from apparently normal upper respiratory and cutaneous sites (and from skin lesions when present) in 44 children and adults living on the Red Lake Indian Reservation.

During the 9-week period of the study 705 of a total of 2305 cultures were positive for group A streptococci. The percentage of positive cultures from the various sites were: throat (20 %); nose (24 %); wrist (32 %); ankle (35 %); back (22 %); and skin lesions (81 %). Group A streptococci were also isolated from fingernail dirt, clothing and bedding as well as from a few household pets and insects.

Analysis of serial cultures obtained from the same individuals at weekly intervals suggested that the strains isolated from skin lesions first appeared on normal skin in the 2 weeks preceding the lesion. Spread to the nose and throat followed skin acquisition and/or skin lesions.

The high prevalence of group A streptococci on normal skin in the absence as well as the presence of pyoderma, and their appearance on normal skin before recovery from either skin lesions or the upper respiratory tract are consistent with the view that skin acquisition was a primary predisposing factor to pyoderma. Since the literature indicates that group A streptococci are rarely part of the normal skin flora, these findings raise the possibility of unique biological properties of these and perhaps other pyoderma strains, as distinct from other group A streptococci.

INTRODUCTION

In streptococcal infections of the upper respiratory tract, considerable epidemiological evidence points to the importance of human reservoirs, particularly nasal carriers, in the dissemination and transmission of infection (Hamburger, Green & Hamburger, 1945; Hamburger & Green, 1946; Wannamaker, 1954). In streptococcal pyoderma, the source and the primary site of infection are less certain and their determination is complicated by the observation that individuals with skin

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infection may also harbour the infecting strain in the upper respiratory tract (Anthony, Perlman & Wannamaker, 1967*a*; Fitcher, 1940). During a recent outbreak of post-streptococcal nephritis on the Red Lake Indian Reservation in northern Minnesota, U.S.A. (Anthony *et al.* 1967*b*), the epidemic strain first became prevalent in skin lesions and subsequently in the upper respiratory tracts of the population involved. Although this study suggested that the upper respiratory tract was an unlikely source of the streptococci causing pyoderma, it left unclear the origin of streptococci recovered initially in skin lesions. Despite the prevailing opinion in the literature that group A streptococci are rarely found on normal skin (Williams, 1965; Kligman, 1965) normal skin still seemed to be a possible site for acquisition of streptococci on the body before their appearance in skin lesions. In the summer of 1968 this hypothesis was tested during a prospective study of streptococcal pyoderma on the Red Lake Indian Reservation.

MATERIALS AND METHODS

Forty-four individuals from five families participated in the study. Beginning 1 July, weekly visits were made to each household for a 9-week period, and each participant had cultures taken of his anterior nares, throat, three normal skin sites (volar surface of one wrist, the medial aspect of one ankle and the middle of the back) and skin lesions if present. Cultures of fingernail dirt, personal clothing, bedding, throat cultures of household pets as well as cultures of a variety of insects trapped in or around each house were also obtained. Normal skin cultures and cultures of bedding and clothing were taken by moistening a cotton swab in Todd-Hewitt broth, rubbing a small, approximately 2-in. square, area of the skin or clothing surface firmly for several seconds and then immediately inoculating the swab on a sheep blood agar plate containing 0.1% crystal violet. Fingernail dirt from beneath one or two nails was collected in sterile screw cap vials and later suspended in 2 ml. of Todd-Hewitt broth. After overnight incubation at 37° C., a loopful of the broth culture was streaked on blood agar plates containing crystal violet. Insects were collected and processed intact in a similar manner. All other cultures were processed and beta-hemolytic streptococci classified serologically* as previously described (Anthony *et al.* 1967*b*). Sub-cultures of three individual colonies were made from each primary plate and were identified serologically.

RESULTS

During the 9 weeks of this study a total of 2305 cultures were obtained, and of these 705 (30.6%) were positive for group A streptococci. Between 230 and 275 cultures were obtained each week from 44 persons and their environment. The results of T-agglutination patterns and M-precipitation reactions indicated that 96.3% of all group A streptococci isolated belonged to two different strains.

* Grouping sera as well as anti-T and anti-M sera were obtained from the Communicable Disease Center, Atlanta, Georgia. Additional anti-T sera were generously supplied by Dr M. T. Parker and Mr W. R. Maxted, Central Public Health Laboratory, Colindale, London, England.

Although these strains are not completely characterized they do possess sufficiently distinct markers to permit epidemiologic analysis (Table 1). The first of these two pyoderma strains, designated 'satellite strain', is readily distinguished by its failure to grow aerobically on routine sheep blood agar* except when the organisms appear as satellites around a variety of other bacterial species. Studies of the growth characteristics and nutritional requirements of this strain will be the subject of a separate report (S. S. Chapman, unpublished observations). Shortly

Table 1. *Characteristics of the two pyoderma strains*

	Percentage of total group A streptococci recovered	T-agglutination pattern	M-precipitation reaction
'Satellite strain'	62.7	3/B 3264	43
'Other pyoderma strain'	33.6	14/3/B 3264	41, 43

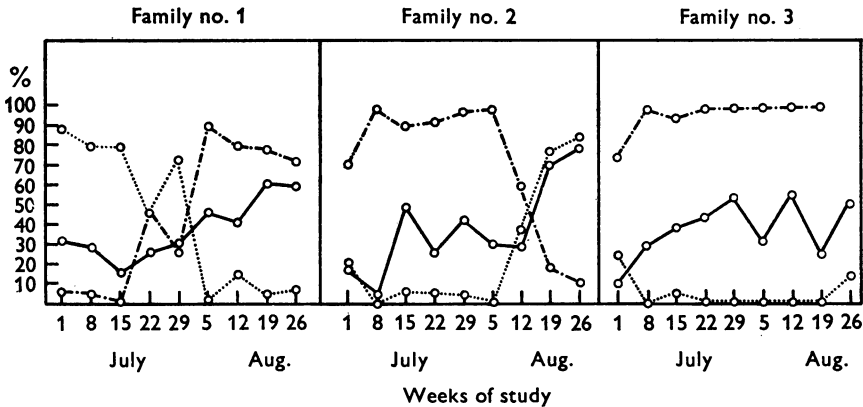


Fig. 1. Weekly recovery of group A streptococci in three families showing the percentage of total cultures positive for group A streptococci and the percentage of group A strains belonging to the 'satellite' and 'other pyoderma' strains. Weeks of study are indicated by the first day of the work week (7/1 = July 1, etc.). —, Percentage positive for group A streptococci; —.—, percentage positive for group A, satellite strain;, percentage of group A, other pyoderma strain.

after this strain was encountered during the first week of the study, trypticase soy sheep blood agar was modified by the addition of 1% Todd-Hewitt broth which permitted good aerobic growth. The 'satellite strain' gave a T-agglutination pattern of 3/B3264 and an M-precipitation reaction with type 43 M antisera. Further tests, including indirect bactericidal tests, will be required to determine whether the 'satellite strain' is an M type 43. The second strain, referred to as the 'other pyoderma strain', grew well on blood agar, showed a T-agglutination

* Routine sheep blood agar at the time of initiation of these studies was prepared from commercial trypticase soy blood agar base (Baltimore Biological Laboratories) plus 6% sheep blood.

pattern of 14/3/B3264 and gave precipitation reactions with both types 41 and 43 M antisera.

The isolation patterns of these two strains from three of the families studied are shown in Fig. 1. For each family the patterns are distinctly different. In Family No. 1 the 'other pyoderma strain' predominated at the outset but was replaced by the 'satellite strain' during the latter half of the summer. In Family No. 2 the converse was true. In Family No. 3 the 'satellite strain' predominated throughout the summer, accounting for 75–100% of all group A streptococci isolated each week.

Table 2. *Percentage of cultures positive for group A streptococci at various sites*

Culture site	All subjects (368 culture visits)	Without streptococcal pyoderma (297 culture visits)	With streptococcal pyoderma (71 culture visits)
Throat	20.8	20.7	19.7
Nose	24.4	22.3	33.8
Wrist	32.8	27.6	54.9
Ankle	35.5	30.9	54.9
Back	21.8	19.5	33.8

One or both pyoderma strains were isolated at some time from 43 of the 44 persons who participated in the study. Of these, 34 were children under 15 years of age. Thirty-three percent of all cultures taken from this age group were positive. Ten teenagers and adults participated in the study and considerably fewer of their total cultures were positive (17.5%).

Table 2 shows the percentage of cultures positive for group A streptococci at individual culture sites. The term 'culture visit' refers to one complete set of cultures obtained from one person during one weekly visit. When all subjects were considered, the highest percentage of positive cultures came from the wrist, 32.8%, and ankle, 35.5%. The data were also analysed to permit separate consideration of those culture visits associated with pyoderma and those not associated with streptococcal pyoderma in the individual. The percentages of positive cultures from the nose and from all three normal skin sites are definitely higher when streptococcal pyoderma is present. This suggests that endogenous shedding (Williams, 1965) may be one factor resulting in positive normal skin cultures. However, in the absence of pyoderma, there is still a significantly higher prevalence of group A streptococci on normal skin (wrist and ankle) than in the upper respiratory tract based on observed and expected frequencies of positive cultures at all sites ($P = < 0.01$).

Of 368 culture visits, 233 (62.2%) were associated with recovery of group A streptococci from one or more apparently normal body sites. When consideration is limited to these 233 positive culture visits (Table 3), the greatest percentage (77.3%) was associated with recovery of group A streptococci from normal skin at one or more sites whereas fewer of such visits were associated with positive

cultures from the upper respiratory tract and skin lesions. Furthermore, over one-third of the positive normal skin cultures occurred at a time when streptococci were *not* recovered from any other site (Table 3, footnote). The two pyoderma strains accounted for virtually all of the group A isolations from normal skin sites and skin lesions.

Table 3. *Frequency of recovery of organisms at various sites when present at some site*

Site of recovery	Group A streptococci (233 'culture visits')	Two pyoderma strains (216 'culture visits')
Upper respiratory tract (nose and/or throat)	125 (53.6%)	104 (48.1%)
Normal skin (one or more of three sites)	180* (77.3%)	176 (81.5%)
Skin lesions	71 (30.5%)	70 (32.4%)

* 66/180 associated with recovery of organisms from normal skin only.

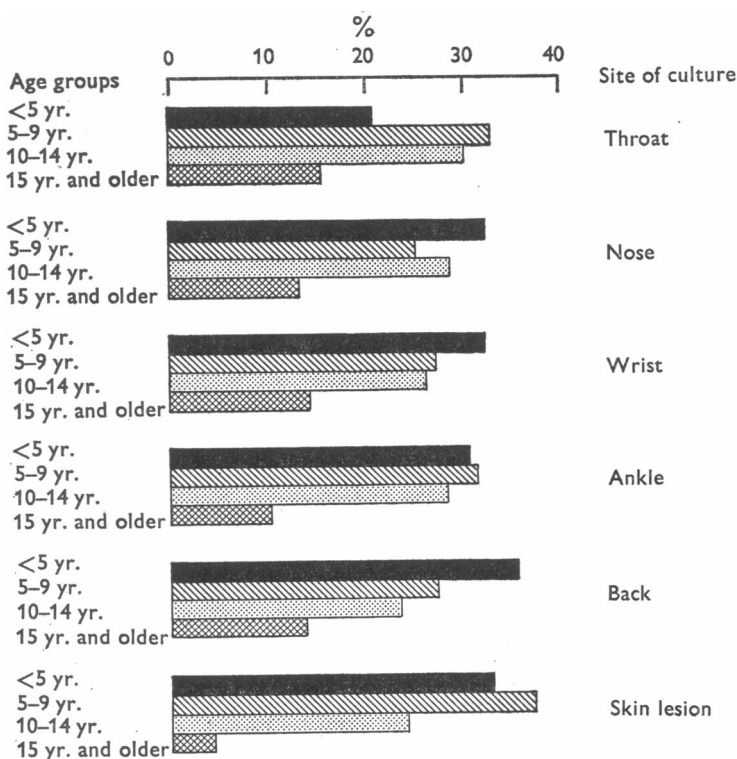


Fig. 2. Percentage distribution of positive cultures for group A streptococci among various age groups by culture sites.

Counting of beta-haemolytic streptococcal colonies present on each culture plate was difficult in some instances because of the nature of the growth of the predominant strain in a satellite pattern. In those instances where it was possible to make some estimation of the number of colonies, approximately two-thirds of the

wrist and ankle cultures contained 1–10 colonies whereas over 80% of the back cultures contained this number. Both wrist and ankle sites yielded similar percentages of cultures containing 10–50 colonies (17 and 18% respectively) and 50–500 colonies (14 and 17% respectively).

Fig. 2 shows the percentage distribution of positive cultures for group A streptococci by age for each culture site. The 44 persons were divided into four age groups of approximately equal numbers: less than 5 years (11 children); 5–9 years (10 children); 10–14 years (13 children); and 15 years and older (10 adolescents and adults). The oldest age group accounted for the lowest percentage of positive cultures at all sites but especially with respect to skin lesions, where only 4.4% of positive cultures occurred in this age group. The youngest age group had the greatest percentage of positive cultures from both the back and the wrist as well as the nose.

Table 4. *Environmental cultures*

Site cultured	Number of cultures	Percent of cultures positive	
		Group A streptococci	Two pyoderma strains
Fingernail dirt	196	29.5	29.5
Personal clothing	43	80.4	76.8
Bedding	33	60.6	60.6
Insects	54	5.5	5.5
Household pets	38	5.5	5.5

The results of environmental cultures obtained during the study are shown in Table 4. The percentage of fingernail dirt cultures positive for group A streptococci was similar to that obtained for both the wrist and ankle. At the time of the initial recovery of either of the two pyoderma strains from nail dirt, 80% of the normal skin cultures were positive for these strains at one or more sites and 40% of the individuals had pyoderma. High percentages of clothing (80.4%) and bedding (60.6%) cultures were positive for group A streptococci. In each instance the majority of individuals wearing the clothing or sleeping in the bed had the same strain recovered from normal skin or skin lesions at the same time. Only an occasional household pet or insect culture was found to have group A streptococci. The number of environmental cultures taken varied considerably from week to week. Because of this variation, no attempt was made to correlate in detail the results of these cultures with those obtained from the upper respiratory tract, normal skin and skin lesions.

The finding that normal skin yielded such a high percentage of positive cultures for the two group A pyoderma strains recovered during this study suggested that the presence of these streptococci on normal skin could well be a significant factor in the spread of streptococcal pyoderma. In order to examine this possibility the sequence of appearance of specific pyoderma strains at various body sites was determined. The results of culture visits in the 2 weeks preceding the first appearance of a strain at a specified body site were considered. Each site of appearance and each individual were analysed separately and the combined results are shown

in Table 5. While the majority of the preceding weeks' cultures were negative before the appearance of a strain at any of the four sites studied, significantly more of the culture visits were associated with negative cultures before the initial appearance of a strain on normal skin (86.6%) compared with other sites ($P = < 0.001$). This suggests that normal skin was the primary site of acquisition of a strain on the body and this is further supported by the results of culture visits associated with positive cultures. Relatively small percentages of cultures were positive at other sites during the 2 weeks preceding the appearance of either pyoderma strain on normal skin. In particular the finding that only 6.1% of preceding culture visits were associated with pyoderma is perhaps the most compelling evidence suggesting that shedding of organisms from skin lesions of the

Table 5. Results of cultures preceding appearance at a specific site

Specific sites of appearance studied	Culture results at other sites 1-2 weeks previously				
	Percent negative at all sites	Percent positive for group A streptococci			
		Normal skin	Lesions	Nose	Throat
Normal skin	86.6	—	6.1	3.6	6.1
Skin lesions	56.8	36.4	—	15.9	6.8
Nose	59.4	30.4	18.8	—	4.3
Throat	51.3	30.7	23.7	20.5	—

same individual was not the only factor resulting in the high prevalence of positive cultures from normal skin. During the 2 weeks before initial recovery of pyoderma strains in skin lesions, 36.4% of normal skin cultures were positive for the same strain whereas significantly small percentages of nose, 15.9% ($P = > 0.02 < 0.05$) and throat 6.8% ($P = < 0.001$) cultures were positive for the same strain during the preceding 2 weeks. Before appearance in the nose the highest percentage of preceding positive cultures was again from the normal skin (30.4%) but 18.8% of the culture visits were associated with pyoderma. This suggests that both of these sites contributed to acquisition of strains by the nose ($P = > 0.10, < 0.20$). Finally previous to the appearance of strains in the throat, the percentages of positive cultures from normal skin, skin lesions and the nose did not differ significantly. This is consistent with the view that all of these sites contributed to throat acquisition ($P = > 0.2, < 0.3$).

DISCUSSION

The finding that only two easily differentiated strains accounted for 96% of all group A streptococci was fortunate. The epidemiologic findings with respect to the appearance of either strain in families and more specifically at various body sites were probably clearer than would have been the case had multiple less well-defined strains been encountered. Both the 'satellite strain' and the 'other pyoderma strain' belong to the T-antigen complex 3/13/B3264, a pattern known to be associated with streptococcal pyoderma (Parker & Williams, 1961; Dillon, 1967). The unique biologic property of the 'satellite strain' (i.e. the inability to grow aero-

bically on routine sheep blood agar) permitted separate analysis of these two strains. The finding that the 'other pyoderma strain' showed precipitation reactions with both types 41 and 43 M antisera is of interest in view of the recent report by Wiley & Bruno (1968) which describes precipitin and bactericidal cross-reactions between types 33, 41, 43, 52 and Ross. This strain apparently has many of the characteristics of the Ross strain, but the exact nature of the precipitation reactions and possible interrelationships of these two strains will await more detailed studies.

Earlier studies have indicated the rarity with which beta-haemolytic streptococci may be recovered from cultures of normal skin (Colebrook & Maxted, 1933; Colebrook, Maxted & Johns, 1935; Williams & Miles, 1949). Indeed, the investigations by Colebrook & Maxted (1933) also showed that these organisms do not survive when artificially inoculated on the surface of human skin. The experimental work of Ricketts, Squire & Topley (1951) demonstrated that human skin lipids, in particular unsaturated fatty acids of sebum, are highly effective in killing beta-haemolytic streptococci both *in vitro* and *in vivo*. Their study provided a biologic basis for the earlier observations and since that time the possibility of finding beta-haemolytic streptococci on normal skin has seemingly been dismissed by most investigators. An exception is the study of Markham & Stenhouse (1959) in which 13% of normal skin cultures from healthy inhabitants of Rarotonga in the Cook Islands yielded group A streptococci. In this same population 81% of pyoderma lesions were positive for group A organisms.

The results of the present study indicate an even higher prevalence of group A beta-haemolytic streptococci on normal skin among the individuals studied and in fact suggest that skin was the best normal body site for isolation of the two pyoderma strains prevalent at the time of the study. It is of interest that, although the pyoderma strains were isolated from normal skin in both adults and children, those children less than 5 years old provided the best source for normal skin isolations. Whether this reflects biological differences in skin properties among different age groups or simply greater contamination is unknown.

Analysis of the sequence of initial appearance of these pyoderma strains suggests that in the individuals studied the body site where the organisms were most likely to appear initially was normal skin, and, further, that appearance of streptococci on normal skin was a primary factor in the development of streptococcal pyoderma. Following skin acquisition it is possible that some type of trauma such as an insect bite, a laceration or an excoriation permitted the organism to penetrate the skin and produce pyoderma. From pyoderma lesions, as well as from normal skin, the strains appeared to spread to the upper respiratory tract. Whether this pattern of spread is characteristic of all pyoderma strains or certain selected ones remains to be determined. Based on these observations it is not possible or necessary to postulate that pyoderma strains parasitize or multiply on normal skin. However, these findings do support the possibility of survival of these streptococci on normal skin without loss of infectivity, which may have been an important factor in the development of the streptococcal pyoderma reported in this study.

Although the ability to culture these pyoderma strains readily from clothing,

bedding and an occasional pet or insect may reflect nothing more than environmental contamination, the possible role of these sources as secondary reservoirs has yet to be explored under these circumstances. The viability of group A streptococci in blankets and dust has been well-documented (Loosli, Lemon, Wise & Robertson, 1948) but later studies of the transmission of these organisms in Army barracks suggested that there was no increased risk of streptococcal respiratory infections among men who were issued with blankets naturally contaminated with streptococci or who inhaled contaminated dust (Perry *et al.* 1957*a, b*). However, despite many basic similarities, strains of group A streptococci that predominantly cause pyoderma may differ in their serologic characteristics (Parker & Williams, 1961) and perhaps in other respects from those strains primarily associated with respiratory disease. Thus what applies epidemiologically in one situation may not in the other.

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