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THE ANATOMICAL AREA OF INVOLVEMENT IN STREPTOCOCCAL INFECTIONS AND THE CARRIER STATE†

Questions concerning the precise anatomical location, the numbers and bacteriologic characteristics of hemolytic streptococci in streptococcal infections and in the carrier state are in need of clarification. This paper is a report of a study of these questions.

The time-honored diagnostic aid for streptococcal infections is the practice of swabbing the posterior pharyngeal wall, the tonsils or nasal passages, culturing the mucous or exudate on the swab and identifying the bacteria that subsequently grow. The presence of characteristic symptoms and signs of pharyngitis or tonsillitis with a throat culture positive for group A hemolytic streptococci plus a significant rise in streptococcal antibodies is evidence that the patient has had a streptococcal infection. In the absence of symptoms and signs, a carrier state is assumed to exist, although carriers may have a significant rise in streptococcal antibodies.[1,2] The cultures do not furnish any information regarding the exact anatomical site or extent of bacterial growth, whether it be the tonsils, the posterior oropharynx, the nasopharynx, or the posterior nares. In addition there is incomplete information concerning which histologic structures are involved, lymphoid tissue, mucous membrane or other tissues, or the microscopic anatomical location of streptococci during the carrier state or infection. Bacteria originating in the turbinates, the nasal mucosa, the sinuses, or the nasopharynx, if they are not taken up by leucocytes, may be swept backward in the mucous coat into the oropharynx by cilia of the lining (pseudostratified, ciliated epithelium) so that this locus becomes a disposal area for the whole upper respiratory tract. Hemolytic streptococci recovered here may have originated in locations extending from the nasal mucosa to the oropharynx.

METHODS

Population

Two groups of children were studied: (1) a group of 241 fourth and fifth grade school children who were participating in a long term study of streptococcal infections and the carrier state. They were from four schools randomly selected serving high-

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middle, middle, low Negro and low white socioeconomic areas; and, (2) 56 children with acute streptococcal infections seen in the Family Clinic of Vanderbilt Hospital and private physicians' offices. Most of the children were from ages 9-12, about equally divided between white and Negro and from socioeconomic areas similar to those of the 241 school children.

Swabbing techniques

Multiple swabs were obtained as soon as a child's throat culture, taken weekly in school, was known to be positive for hemolytic streptococci. Multiple cultures were taken before treatment in all but six of the 56 children with acute streptococcal infections. Seven swabs were taken from six different sites, one each from the left and right posterior nasal mucosa taken through the nares with a thin, soft, cotton-tipped wire; from the nasopharynx with a cotton-tipped wire bent so it could be guided up behind the uvula; two simultaneously from the posterior oropharynx and one from each tonsil or tonsillar fossa. Care was taken not to contaminate the cotton swab with material from any other site.

Bacteriologic methods

Within 30 minutes of swabbing, each swab was immersed and agitated in 1 ml. of Todd-Hewitt broth (Difco) and the broth minus swab incubated for two hours at 37°C. A pour plate was made from the 1 ml. of broth from each cultured site by inoculating 10 ml. of 10% defibrinated sheep's blood in agar with one loopful of the Todd-Hewitt broth. The broth cultures were returned to the incubator along with the pour plates. After incubation for a total of approximately 22 hours a new set of seven pour plates was inoculated with a loopful of the Todd-Hewitt broth which by now had incubated 24 hours. All plates were examined after 24 hours for hemolytic colonies. The positive cultures were removed, the rest incubated for another 24 hours (48 hours total) and examined again for hemolytic colonies. The plates were read by two observers independently and the number of hemolytic colonies classified as less than 10 colonies, 1+; 10-49 colonies, 2+; 50 or more colonies, but mixed with nonstreptococcal colonies, 3+; 50 or more colonies and practically a pure culture, 4+.

*Grouping and typing procedures**

A single hemolytic colony was picked from each positive plate and incubated overnight in 10 ml of Todd-Hewitt broth. The streptococci were grouped and typed* serologically according to the precipitin method.[3]

Serologic methods for streptococcal antibodies

A blood serum specimen was obtained from each child as soon as group A streptococci were recovered from any of the culture sites and again one and two months later. Antistreptolysin O titres (ASO) were determined using a standard method,[4] and antihyaluronidase (AH) levels by the mucin-clot prevention test.[5] An increase or decrease in titre of two or more dilutions was interpreted as a significant serological change of AH or ASO.

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* Typing sera were available from the National Communicable Disease Center (NCDC) for types 1-7, 8, 11, 12, 14, 15, 17-19, 22-26, 28-33, 36-44, 46, 47, 51. The term non-typable (NT) refers to strains of group A streptococci that did not react with the available typing sera.

Diagnostic criteria

On the basis of symptoms and signs, bacteriologic and serologic results, the children were placed in two groups (after completion of the laboratory studies): those with streptococcal infections, and those considered to be carriers. There was no way to know for certain to which diagnostic group each child should be assigned until all studies had been completed.

Streptococcal infections (56 subjects)

Patients with a streptococcal infection had characteristic symptoms and signs among which were a sore throat, fever, nausea and vomiting, dysphagia, a scarlatiniform rash in a few, leucocytosis, and pharyngeal mucosal and tonsillar edema and erythema, usually without exudate. These patients all had a throat culture positive for beta hemolytic streptococci, 89.1% of which were group A, and all but 12 had a significant increase in titre for AH or ASO, or both. The 12 children who did not have a significant increase were treated with penicillin in therapeutic doses. Had antibiotics not been administered, a significant rise in streptococcal antibodies probably would have occurred. Since the clinical manifestations of these 12 children resembled closely those of the children with streptococcal infections, and they all had group A streptococci in their cultures, they were assigned to the streptococcal infection group.

Carriers (22 subjects)

Ten of these children were without evidence of an upper respiratory infection (URI); 12 had symptoms and signs of mild corrhyza. Among these 12 there was no fever, nausea, vomiting, dysphagia, or rash. A few had a mild sore throat and slightly injected pharyngeal mucosa but no exudate. All of these 22 children had throat cultures positive for beta hemolytic streptococci, 53.1% group A. No significant rise in streptococcal antibodies occurred in any of the carriers. None were treated with penicillin.

RESULTS

Percentage distribution of children with streptococcal infections and children who were carriers, according to number of sites positive for hemolytic streptococci (Fig. 1)

Over two thirds of the children with streptococcal infections had five or more sites positive for hemolytic streptococci. This finding is in marked contrast to those for carriers in whom over 85% had fewer than five sites positive. A word of explanation is necessary here to clarify a possible source of confusion. Figure 1 shows some children with no sites positive, even among those with streptococcal infections. Thirty-five percent (84) of the carriers whose multiple cultures were negative had fewer than ten colonies of hemolytic streptococci on the initial culture. It has been our experience that children whose cultures are not consistently positive for hemolytic streptococci are almost always those whose cultures yield ten or fewer colonies. Those whose cultures yield 50 or more colonies are consistently positive. This is the most logical explanation of why their multiple

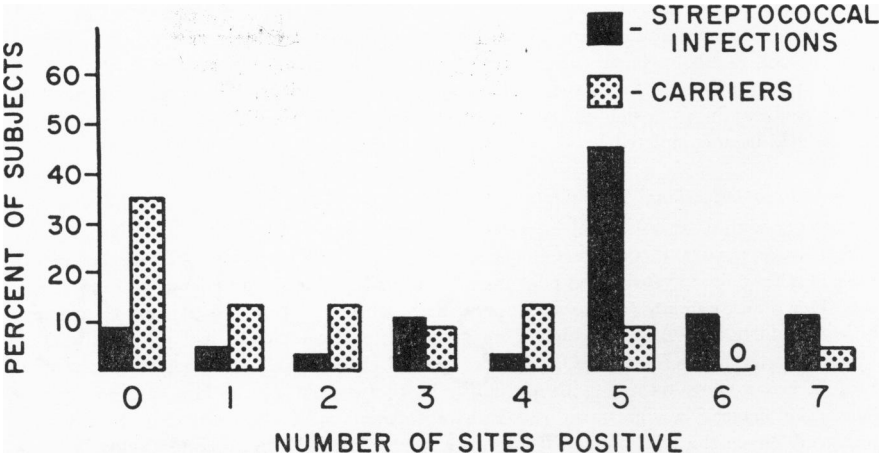

FIG. 1. Percentage distribution of children with streptococcal infections and carriers, according to number of sites positive for hemolytic streptococci. (For an explanation of carriers and streptococcal infections with 0 sites positive, see text.)

cultures were negative on the next day. Cultures from the six sites were not taken from six children with infection until it was known for certain, usually the next day, that the original throat culture was positive for hemolytic streptococci. In the meantime these six of the children with streptococcal infections had been treated with penicillin and their throat cultures had become negative.

Degree of positivity for all cultures for all serological groups (Table 1)

The degree of positivity of the initial multiple cultures was significantly greater for children with streptococcal infections (Table 1). Three fourths had 50 or more colonies (3+ or greater) on all culture plates. Over half

TABLE 1. DEGREE OF POSITIVITY OF CULTURES FOR HEMOLYTIC STREPTOCOCCI

Degree of positivity	*56 patients with infections*		*22 Carriers*	
	No.	*%*	*No.*	*%*
1+ − <10 colonies	38	14	20	26
2+ − 10-50 colonies	27	9.9	21	27.3
3+ − >50 colonies	190	69.9	36	46.7
4+ − >50 colonies almost pure culture	17	6.2	0	0

of the carriers (53.3%) had fewer than 50 colonies per plate (2+ or less). None of the carriers had cultures designated 4+.

Percent of individuals with positive cultures for hemolytic streptococci from seven different sites

The percentage of individuals with cultures positive for hemolytic streptococci was consistently higher when swabs were taken from the nasopharyngeal, the posterior oropharyngeal and both tonsillar sites (Fig. 2). This finding is true for both groups, those with infections and carriers. Swabs taken from the nares yielded hemolytic streptococci much less frequently than the other sites. This is clear evidence that the preferred sites for growth and for taking swabs for isolation of hemolytic streptococci are

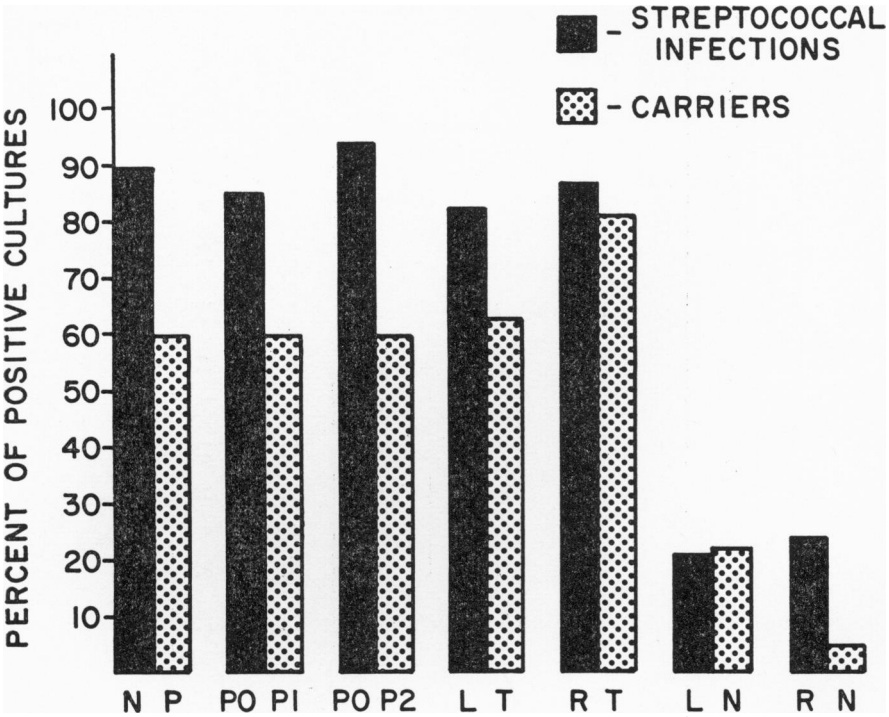

FIG. 2. Percent of individuals with positive cultures for hemolytic streptococci from seven different sites.

NP = Nasopharynx
PoP1 = Posterior Oropharynx } two cultures
PoP2 = Posterior Oropharynx }
LT = Left Tonsil
RT = Right Tonsil
LN = Left Nares
RN = Right Nares

TABLE 2. BACTERIOLOGIC RESULTS OF INITIAL MULTIPLE CULTURES

	Number of subjects	*Number of cultures positive for typable strains of group A streptococci* — *Types*						*Total group A typable*	*Percent group A typable*	*Number cultures positive for group A non-typable (A-NT)*	*Percent positive A-NT*	*Total number of group A*	*Percent positive group A*	*Number of cultures positive for non-group A* — *Group*				*Total non-group A*	*Percent non-group A*
		1	*3*	*5*	*6*	*12*	*18*							*B*	*C*	*F*	*G*		
Patients with infections	56	22	9	10	13	27	14	95	37.5	158	62.5	253	89.1	6	14	1	10	31	10.9
Carriers	22	0	0	0	0	0	0	0	0	13	53.1	43	53.1	5	15	0	18	38	46.9
Totals	78	22	9	10	13	27	14	95	32.1	201	67.9	296	81.1	11	29	1	28	69	21.1

the nasopharynx, the posterior oropharynx, and the tonsils, rather than the posterior nasal mucosa, with little to choose among the first three sites.

Serologic results

Forty-four of the children with streptococcal infections had a significant rise in either ASO or AH and 16 had a significant rise in both ASO and AH. Thirty-nine of the 56 children with infection are known to have received penicillin early in the course of their infection (within 1-3 days of onset) in therapeutic doses. Twenty-seven of these children exhibited a significant rise of one or the other streptococcal antibodies indicating that penicillin does not always prevent a significant increase in streptococcal antibodies. Twelve of the children assigned to the streptococcal infection group did not have a significant antibody increase. Presumably, penicillin prevented the antibody response in these 12 since they all were treated with this antibiotic.

Bacteriologic results (Table 2)

Of all the initial multiple cultures that were positive for beta hemolytic streptococci, the percentage positive for group A streptococci was significantly higher for patients with infection (89.1%) than it was for carriers (53.1%). The remainder of the positive cultures of multiple sites were non group A, which were identified in significantly higher numbers and percentages among carriers (46.9%) than the infection group (10.9%). The non-group A strains recovered most frequently were groups C and G. The percent of the initial multiple cultures positive for typable strains of group A was 37.5 for the infection group. Typable strains of group A were not cultured from carriers. Of all the group A strains, the majority (67.9%) were non-typable with the available typing sera. Non-typable strains of group A comprised 62.5% of all group A strains in patients with infections. Typable strains of group A identified most frequently were 12, 1, 18, 6, 5, and 3.

All multiple cultures

It was possible to obtain weekly cultures from all sites from most of the carriers but from only a few of the group with streptococcal infections. The results of grouping and typing these strains were similar to those for the initial multiple cultures.

DISCUSSION

The results strengthen the belief and confirm previous findings from studies in Nashville[1] and elsewhere[6] that children with streptococcal infec-

tions have larger numbers of hemolytic streptococci than carriers. Furthermore, group A streptococci were recovered much more frequently from children with streptococcal infections than from carriers. Not unexpectedly, based on the larger number of sites positive for hemolytic streptococci, the results show that the anatomical area involved in those with infections is greater than for carriers. The relatively smaller number of streptococci present in the carriers and the much higher frequency of negative cultures following the initial positive culture is a plausible explanation of why streptococcal antibodies did not increase.

It was unusual to recover beta hemolytic streptococci other than group A from children with streptococcal infections, but quite common in children who were carriers, indicating that in these children streptococci other than group A were common inhabitants of the pharynx and tonsils. However, there were three children with clinical and serologic evidence of a streptococcal infection from whom no group A streptococci could be recovered. These three children's cultures yielded groups C, G, and C respectively in large numbers. It doesn't seem likely that group A streptococci would have been missed had they been present. Treatment was not administered until after the swabs had been taken so there is no possibility that group A streptococci could have been eliminated by antibiotics. It is known that both hyaluronidase and streptolysin O are produced by some strains of group C and G, so it is very likely that these children did have infection and antibody response due to these non-group A strains. A throat culture positive for hemolytic streptococci from a child with clinical manifestations of a streptococcal infection contained the etiologic agent in nearly 90% of cases. In carriers, however, strains of hemolytic streptococci belonging to serologic groups other than A were found in almost half of the cultures. In a pediatric practice these results would suggest that serologic grouping of streptococci would not have been a necessary diagnostic procedure since most children with symptoms and signs of a streptococcal infection had group A streptococci. Breese[7] reached a similar conclusion and stated that "since 98.5% of cultures of beta hemolytic streptococci from sick children were group A, there is little advantage in determining the specific group of each organism in pediatric disease."

It would appear that culturing the nares would yield a very low percent of positive cultures even in children with streptococcal infections. This was true among the children from whom multiple cultures were taken at the onset of infection. This finding argues against the nasopharynx as the preferred site for multiplication of hemolytic streptococci in these children. Culturing from any other site in the upper respiratory tract is preferable but there is little to choose from any one of the other sites—nasopharynx,

oropharynx, or tonsils—so far as obtaining positive cultures is concerned. Hamburger, *et al.*[8] showed that the nasal carrier was the "dangerous" one in the spreading of hemolytic streptococci. His studies were done in young adult army personnel under epidemic conditions and it was suspected that as many as 60% of these "dangerous" carriers had sinusitis. There was no evidence of sinusitis in any of the children in the present study. Lemon's[9] studies also done on young adult U.S. Army patients, using a standard nose blow test in which the nose was blown three times into a sterile handkerchief, led him to the conclusion that in many cases marked proliferation of streptococci occurs in nasal mucosa alone. Earlier work[10] on nurses in which blood agar plates were held in front of patients with acute tonsillitis while the patient talked, sneezed, or coughed, showed that it was unusual for streptococci to be expelled by these respiratory maneuvers. These curious contradictions remain unexplained but regardless of the explanation it would appear that when direct culturing techniques were used, beta hemolytic streptococci were not common inhabitants of the posterior nares in carriers or children with streptococcal infections.

SUMMARY

Children with streptococcal infections harbored group A streptococci in greater numbers and in a larger anatomical area of the upper respiratory tract than carriers. Swabs taken from tonsils, the oropharynx, or the nasopharynx of children yielded a much higher percentage of positive cultures than those taken from the posterior nares. These results indicate that the posterior nares are not the most frequent sites of multiplication or the preferred sites for recovery of beta hemolytic streptococci from children. Children with symptoms and signs characteristic of a streptococcal infection yielded group A streptococci in approximately 90% of the cases. In carriers, a positive culture contained serologic groups other than A over four times more frequently than it did in children with streptococcal infections.

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