A Preliminary Report

Group A Beta Hemolytic Streptococci And Rheumatic Fever in Miami, Fla.

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RHEUMATIC FEVER seems to behave differently in warm climates than in northern areas. In the south, polyarthritis and chorea are relatively infrequent (*1*). Rheumatic fever is less severe, and the incidence of rheumatic heart disease is lower, as indicated by surveys of school children (*2*, *3*), necropsy reports (*4*, *5a*), and a low acute rheumatic fever hospitalization rate. A study of 4 large hospitals in Miami, Fla., over a 4-year period showed an average admission rate of 0.3 patient per month with acute rheumatic fever.

The explanation for this difference between the rheumatic fever picture in the north and that in the south may lie in some alteration in the pattern of the etiological relationship presumed to exist between group A beta hemolytic streptococci and rheumatic fever:

1. The organism may vary in incidence, frequency, type, or virulence.
2. The immunological response of the host to the organism may differ either in degree or pattern.
3. The host-organism relationship may be otherwise altered.

This is a preliminary report on the number and types of group A beta hemolytic streptococci isolated from the throats of normal, healthy Miami school children, with a discussion of the problems raised by our findings.

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Material and Methods

During the 4-month period from February through May 1953, a total of 1,154 pairs of throat swabs were taken at monthly intervals from 343 children residing in and attending grade 1 in three schools in Miami. Each child's throat was swabbed an average of 3.4 times. Written permission of the parents had been obtained for each child.

The schools were carefully selected to represent different socioeconomic levels: a low-income white group; a middle-income, relatively stable white population; and a mixed-income Negro group. A high-income group was not included because such a group in Miami does not maintain year-round residence, and therefore a long-term study would be impossible. The sample selected, though not truly random, represents the stable school population. It includes 83 percent of the 413 children attending grade 1 of the three schools in the study and is 3.8 percent of the 9,003 first graders attending all the public schools of Dade County.

Duplicate throat cultures were taken with sterile cotton swabs from all children present in school during the mornings set aside for this phase of the work. Nasopharyngeal swabs were not taken. All the swabbings were made by the same technician and required about 2 hours per morning.

The pair of swabs from each child was put into a sterile test tube immediately after being taken. When all the cultures had been collected, they were promptly taken to the laboratory. One swab was then streaked directly on Difco blood agar base (DBAB) enriched with 4 percent defibrinated sheep's blood, which was found to be preferable to a 5-percent concentration in revealing beta hemolysis (*6*). The second swab was initially cultured on a different medium each month for two reasons: first, to pick up additional isolates likely to appear on duplication; and second, to try to find a culture medium which would yield the highest possible number of isolates. In February, the second swab was initially cultured on Pike's (*7*) enriched medium, modified by the use of defibrinated sheep's blood in place of rabbit's blood and Todd-Hewitt broth (Difco) instead of fresh heart infusion. The following month, trypticase soy broth (BBL) was substituted for Todd-Hewitt broth (*8*). In April, the second swab was first cultured on Brewers' thioglycollate broth (Difco) and incubated for 6 hours at 37° C. Following incubation, the broth cultures for each of these 3 months were streaked with a transfer loop on DBAB enriched with 4 percent defibrinated sheep's blood. In May, the second swab was streaked directly on neopeptone heart infusion agar (Difco) containing 4 percent defibrinated sheep's blood.

All plates were incubated at 37° C. for approximately 24 hours and read with the aid of a microscope for the presence of beta hemolysis. In some cases in which beta hemolysis was questionable, several hours' additional incubation were required for a definitive reading.

Whenever a plate demonstrated colonies suspicious of beta hemolysis, subcultures were made to the surface of plates containing the same type of medium used for the initial plate. So that hemolysis could be viewed in deep as well as surface growth, a variation of Krumwiede and Kuttner's streak plate technique (*9*) was used. This involved the pouring of additional medium over about one-fourth of the streaked plate.

Subcultures of typical colonies were made to slants of stock culture agar (Difco), to each 10.0 gm. of which were added 11.0 gm. neopeptone heart infusion agar (Difco) and 1.75 gm. agar. This medium was diluted to 200 cc. with distilled water, autoclaved at 120° C., 15-pound pressure, for 15 minutes, and then cooled to 45° C. After tubing in 5-cc. amounts in screw-capped tubes, approximately 0.3 cc. defibrinated sheep's blood was added to each tube, and the tubes were slanted. After overnight incubation of the subcultures at 37° C., the condensation fluid present at the bottom of the slants was gram-stained to check morphology. These slants were used as stock cultures for grouping and typing. Agglutination tests with serums for groups A through H and K through O were performed at the Streptococcus Laboratory, Communicable Disease Center, Chamblee, Ga. Type specificity was determined by the Lancefield precipitin method against types 1–6, 8, 11–15, 17–19, 22–26, 28–33, 36, 37, 39–44, 46, and 47.

Results

The number of groups and types of beta hemolytic streptococci isolated in our study are listed in tables 1 and 2. Fifty-eight children were found to have these organisms in their throats; in 14 instances children were found to be positive a second, third, or fourth time, giving a total of 72 isolates. Fifty-nine strains of group A beta hemolytic streptococci were isolated from the throats of 47 children. All of the 27 typable strains were type 12, with a single exception (type 28); in one child, type 12 was recovered in 2 successive months.

Group A beta hemolytic streptococci were not limited to the children of one school but were isolated from those attending each of the three schools studied.

Nine children had positive cultures more than 1 month: 2 months in 6 cases, 3 months in 1, and all 4 months in 2. Table 3 gives a breakdown of the findings in these 9 children.

Comparison of the number of positive cultures isolated with each of the various media used during the 4 months of the study yielded

Table 1. Grouping of beta hemolytic streptococci isolated from 1,154 throat cultures in 343 children over a 4-month period in Miami, Fla., 1953

Month	Number of children			Positive 1st time						Positive 2d, 3d, or 4th times			Total positive cultures	
	Total	Absent	Present and cultured	Total	Group A	Other groups			Ng[1]	Total	Group A	Ng[1]	Number	Percent
						B	C	G						
February	343	64	279	8	7		1			0			8	2.9
March	343	35	308	9	8	1				4	3	1	13	4.2
April	343	62	281	20	11	5	3	1		5	5		25	8.9
May	343	57	286	21	20				1	5	5		26	9.1
Total			1,154	58	46	6	4	1	1	14	13	1	72	6.2

[1] Nongroupable.

the following data: In February, the modified enrichment medium failed to reveal any positive cultures, while on enriched DBAB 8 strains of beta hemolytic streptococci were recovered. The trypticase soy enrichment medium used in March picked up only 1 strain (nongroupable), in contrast to 12 strains isolated on enriched DBAB. However, the thioglycollate broth used in April was effective in the isolation of 8 strains which were not isolated on enriched DBAB. Neopeptone medium in May was responsible for the recovery of 7 strains not found on enriched DBAB. Had a single swab been taken and planted only on enriched DBAB, we would have missed 1 strain in March, 8 in April, and 7 in May—a total of 16 strains, or 22.2 percent of the 72 strains recovered. Double cultures on enriched DBAB might have yielded the same results as those obtained by the use of other media. In our experience, it would appear that either thioglycollate broth or neopeptone heart infusion agar containing 4 percent defibrinated sheep's blood should be used as an initial culture medium, in addition to enriched DBAB. In the number of positive cultures or numbers of colonies demonstrated on original plates, none of the culture media used was found to be superior to DBAB containing 4 percent defibrinated sheep's blood.

Discussion

The finding of group A beta hemolytic streptococci in the throats of 16.3 percent of 343 children in Miami over a 4-month period is consistent with the 5 to 10 percent streptococcal rate reported by Denny (*10*) for the normal child population. It strongly suggests that

Table 2. Types of group A beta hemolytic strepto cocci isolated from 1,154 throat cultures in 343 children over a 4-month period in Miami, Fla., 1953

Month	Number of children cultured	Positive 1st time				Positive 2d, 3d, or 4th times			Total positive cultures	
		Total	Nontypable	Type 28	Type 12	Total	Nontypable	Type 12	Number	Percent
February	279	7	7						7	2.5
March	308	8	6	1	1	3	3		11	3.6
April	281	11	7		4	5	4	1	16	5.7
May	286	20	2		18	5	3	2	25	8.7
Total	1,154	46	22	1	23	13	10	3	59	5.1

Table 3. Nine children with repeatedly positive throat cultures, February–May, 1953

Month	Case number								
	2	54	102	139	353	380	604	667	686
February	A_0	A_0	A_0	Negative	Negative	Negative	A_0	C	A_0
March	A_0	Ng	A_0	A_0	A_0	A_0	Negative	Negative	A_0
April	Absent	A_0	Absent	A_0	A_0	A_0	A_{12}	do	A_0
May	Negative	A_0	do	Negative	Negative	Negative	A_{12}	A_{12}	A_0

A_0—Group A beta hemolytic streptococcus, nontypable.
Ng—Nongroupable beta hemolytic streptococcus.
A_{12}—Group A, type 12 beta hemolytic streptococcus.
C—Group C beta hemolytic streptococcus.

factors other than the mere presence of this group of organisms in a geographic location play a role in their etiological relationship to rheumatic fever. Investigation over a longer period is essential for a more comprehensive picture of the behavior of streptococci in this subtropical climate. Such prolonged study would enable determination of the following points.

More accurate isolation data. If the study had run throughout the year, if we could have taken cultures at more frequent intervals than once a month, and if we had been able to culture the children absent because of illness, it is reasonable to presume that the number of isolates of group A beta hemolytic streptococci would have been greater. The peak recovery rate observed in April and May may have continued into June, as has been reported in a long-range survey in New York by Coburn and Pauli (*11*).

The finding of only one type (type 12) among the typable organisms (with a single exception, type 28) could be interpreted as indicative of either an isolated epidemic or the predominance of this type during the season studied. The streptococcal rate found in our study could represent either an epidemic or the incidence prevailing in Miami at the time and paralleling the seasonal variations noted elsewhere (*11*).

"*Infection rate.*" The present study was directed only toward finding the extent to which group A beta hemolytic streptococci are present in Miami, Fla. As no serologic tests were performed and there was no complete followup of all illnesses during the study period, it cannot be stated with certainty that the organisms recovered were harmless parasites. There are certain indications, however, that they were pathogenic:

1. There was a shift from nontypable group A beta hemolytic streptococci in February and March to typable strains in April and May. That pathogenicity is related to typability has been reported by Rothbard and Watson (*12*).

2. The number of positive cultures increased in each succeeding month, reaching a peak in April and May. This finding parallels the seasonal streptococcal infection rates reported by Coburn and Pauli (*11*).

3. A preliminary followup study of 19 of the 25 children from whom type 12 was isolated indicated that for 13 either they or members of their families had upper respiratory infections. Of the remaining 6, 1 suffered from allergic symptoms and 3 from mumps. Only 2 were apparently free from any illness. In view of the possible relationship reported between viral diseases and streptococcal pathogenicity (*11*, *13*), it is of interest that at the time of our study there were numerous cases of mumps and chickenpox in the schools.

Frequency of reinfection. The number and frequency of group A beta hemolytic streptococcal infections in Miami may be factors in the incidence of rheumatic fever there. The interval between infections also may have a direct bearing on whether or not rheumatic fever follows streptococcal infection.

"*Rheumatogenicity.*" Strains and types of group A beta hemolytic streptococci may vary in their ability to precipitate attacks of rheumatic fever (*14*), a quality we have termed "rheumatogenicity." Our future studies will

attempt to answer the question as to whether such variations in rheumatogenicity occur. To date, none of the 343 children in our study, not even those from whom streptococci were isolated, have developed overt symptoms of rheumatic fever. Moreover, during the 3-month period, June–August 1953, following the study only one child (not in our study) in the area was hospitalized for acute rheumatic fever, as shown in the records of two of the largest hospitals in Miami.

"*Nephritogenicity.*" Rammelkamp and Weaver (*15*), in a review of the literature and on the basis of their own studies, found that attacks of acute glomerulonephritis generally followed type 12 streptococcal infection. None of the children in our study from whom strains of type 12 were isolated developed frank clinical manifestations of nephritis. However, since urine examinations and Addis counts were not performed, it is recognized that the existence of subclinical nephritic infections cannot be ruled out.

Lancefield's precipitin technique was used in the study for typing group A beta hemolytic streptococci. It is known (*16*) that type 12 organisms determined by this method include both types 10 and 12 when ascertained by Griffith's agglutination procedure, and it may be that only one of these types is nephritogenic.

Immunological response. The determination of serologic changes evoked by streptococcal infection, both clinical and subclinical, in relationship to rheumatic fever, is now under investigation in the laboratory of the National Children's Cardiac Hospital, Miami. Such data will aid in differentiating between pathogenic and possibly parasitic strains of group A beta hemolytic streptococci recovered. The pattern of immunological response to single and multiple streptococcal infections will be followed and compared with that observed in northern areas (*5b*).

The isolation of large numbers of group A beta hemolytic streptococci in Miami in our preliminary study has raised many provocative questions concerning the host-organism relationship in a subtropical climate. What is the significance of the presence of group A beta hemolytic streptococcus in the throat of a normal child? Does it evoke an increase in the antistreptolysin-O titer? What effect, if any, does it have on the subsequent development of rheumatic fever, nephritis, or other infections, clinical or subclinical? To what extent are streptococcal infections followed by rheumatic fever in the southern States? Is the 3 percent figure quoted by Rammelkamp (*15*) valid here? Does residence in a subtropical climate alter the host's clinical or serologic response, or both? How is the pattern of immunological response related to the lower incidence of rheumatic fever in a southern climate?

Summary

1. Duplicate throat swabs were taken once a month from those present among 343 healthy school children in grade 1, Miami, Fla., during the 4-month period of February through May, 1953. In all, 1,154 pairs of throat swabs were collected, giving an average of 3.4 cultures per child and 288.5 children per month.

2. Based on the monthly average of 288.5 children studied, 20.1 percent (58 children) were found to harbor beta hemolytic streptococci in their throats at least once; 16.3 percent (47 children) had group A at least once; and 8.7 percent (25 children) had Lancefield's type 12 at least once.

3. An increasing number of cultures positive for group A beta hemolytic streptococci were found in each succeeding month of the study period. There was also a shift from nontypable isolates in February and March to typable strains in April and May.

4. Although the total number of children from whom streptococci were recovered was too small to warrant elaborate statistical analysis, it is of interest that followup studies revealed no frank cases of rheumatic fever or glomerulonephritis in the study group. Data collected on 19 children from whom the type 12 strain was isolated showed that upper respiratory infections occurred either in themselves or in their immediate families in 13 instances.

5. The importance of continuing and extending this epidemiological study over a period of years is emphasized to obtain further information on the behavior of group A beta hemolytic streptococci, their incidence and infection rates, the effect of streptococcal reinfections on the

incidence of rheumatic fever, "rheumatogenicity" and "nephritogenicity" of different types and strains, and the pattern of immunological response to these organisms in a subtropical climate.

ACKNOWLEDGMENTS

The authors wish to thank Drs. T. E. Cato, S. D. Doff, Albert V. Hardy, Alexander Langmuir, and Elaine L. Updyke, and Lilly Harman and the Dade County nursing staff for their active advice and cooperation; and Alba Colon for technical assistance.

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Legal Note on Meat Inspection Fees

A recent finding of the Court of Appeals of Ohio bears on the power of health departments in that State to charge fees for meat inspection.

In *Brunner et al.* v. *Rhodes*, 119 N. E. 2d 105, decided March 23, 1953, the Court of Appeals of Ohio held that under Ohio State law a board of health does not have the power by regulation to exact inspection fees from operators of slaughterhouses.

The court based its decision on the grounds that Ohio does not give boards of health this authority by direct statute or by strong legislative implication.