

Serological Groups and Types of Hemolytic Streptococci Isolated in Puerto Rico.*

A. POMALES-LEBRÓN, P. MORALES-OTERO, AND J. BARALT.

From the Department of Bacteriology of the School of Tropical Medicine, San Juan, P.R.

One hundred and sixteen pairs of tonsils were studied bacteriologically. The purulent exudate from the crypts and the inside of each pair of tonsils were cultured separately utilizing methods previously described¹ and the hemolytic streptococci obtained from these tonsils were studied serologically. The distribution of these cultures among Lancefield's groups is given in Table I. The results of a similar study of hemolytic streptococci from normal throats,² carried out in 1943-1944, is also included in Table I for purposes of comparison.

The tonsils yielded 62.8% cultures of β -hemolytic streptococci as compared with 17.4% positives among the normal throats. This difference was due mainly to a much higher percentage of Group A organisms obtained from tonsils (30.2%) than from normal throats (1.2%). The proportion of tonsil strains belonging to groups other than A was only twice as large (32.6%) as that obtaining among the throat cultures (16%).

Group B streptococci were obtained in 6% of the tonsils examined. No Group B strains were isolated from the normal throats, but there exists the possibility of these being overlooked if present in sparse numbers.

The distribution of the different Lancefield's groups among the aerobic tonsillar flora is given in some detail in Table II.

When the distribution of strains of different Lancefield's groups cultured from tonsils was compared with the distribution of strains

previously obtained from normal throats,² it was observed that Group A organisms predominated abundantly in 21 (60%) of the tonsils from which Group A strains were cultured. Contrasting markedly with this, no Group A strain were found in large number among the throats from which A organisms were cultured.

Group C organisms were abundant in 8 (57.1%) of tonsils from which C strains were obtained and in 26 (36.1%) of the normal throats from which C streptococci were isolated.

Group G organisms were obtained in large numbers in 4 (28.4%) and in 5 (16%) respectively of the tonsils and normal throats from which G organisms were cultured.

These figures may suggest that hemolytic streptococci of Groups G and C are of doubtful significance in the production of tonsillitis, however, streptococci belonging to these serological groups are occasionally obtained in abundant numbers and in pure or practically pure culture from diseased tonsils in the absence of any other common pathogen.

No conclusions can be drawn concerning Group B and F due to the small number of strains. It is of interest, however, to observe that these streptococci can be occasionally obtained in pure culture from the pus in the crypts and the inside of tonsils.

Our results agree closely with those reported by Keogh *et al.*³ in a similar study carried out in Melbourne, Australia.

The serological typing of 95 strains of Group A streptococci obtained from excised

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¹ Pomales-Lebrón, A., *P. R. J. Pub. Health and Trop. Med.*, 1929, **2**, 196.

² Pomales-Lebrón, A., Damin, G. J., Pons, C., and Morales-Otero, P., *P. R. J. Pub. Health and Trop. Med.*, 1946, 287.

³ Keogh, E. V., MacDonald, I., Battle, J., Simmons, R. R., and Williams, S., *British Med. J.*, 1939, **2**, 1036.

⁴ Swift, H. F., Wilson, A. T., and Lancefield, R. C., *J. Exp. Med.*, 1943, **78**, 127.

TABLE I.
Hemolytic Streptococci from 116 Pairs of Excised Tonsils and from 705 Normal Throats.

No. and %	Groups					Total positive	Negative
	A	B	C	G	F		
No. positive	35	7	14	14	3	73	43
% "	30.2	6	12	12	2.6	62.8	(37.2%)
No. "	9	0	72	42	?	123	582
% "	1.2	0	10.2	6.0	?	17.4	(82.6%)

TABLE II.
Distribution of Streptococci of Groups A, B, C, G, and F (Lancefield) Among the Aerobic Flora of Excised Tonsils.

Distribution	Cases in which this distribution occurred	
	No.	%
Group A		
Abundantly predominating or in pure culture	21	60.0
Numerous colonies, abundant growth <i>Staph. aureus</i>	3	8.6
Few colonies, <i>Staph. aureus</i> few colonies	2	5.7
Few colonies; abundant growth <i>Staph. aureus</i>	3	8.6
Few colonies; abundant growth of alpha strep.	3	8.6
Moderate growth; moderate growth of alpha strep.	2	5.7
Numerous; few colonies group G	1	2.8
Group B		
Abundant in pure culture	2	28.6
Predominating abundantly in crypts, few colonies of group B inside	1	14.3
Few colonies, abundant growth of <i>Staph. aureus</i>	2	28.6
Numerous colonies, few colonies of <i>Staph. aureus</i>	1	14.3
Moderate growth, sparse growth of <i>Staph. aureus</i>	1	14.3
Group C		
Abundantly predominating or in pure culture	8	57.1
Abundantly predominating in crypts, few group B colonies inside	1	7.1
Few colonies, <i>Staph. aureus</i> abundantly predominating	3	21.4
Few colonies and Gram negative cocci predominating	2	14.2
Group G		
Abundantly predominating or in pure culture	4	28.4
Moderate growth, <i>Staph. aureus</i> predominating	5	35.7
Predominating, few colonies of <i>Staph. aureus</i>	2	14.2
Moderate growth with moderate growth of <i>Staph. aureus</i>	2	14.2
Few colonies, abundant growth of group A	1	7.1
Group F		
Abundantly predominating	1	33
Numerous colonies mixed with abundant growth of <i>Strep. viridans</i>	1	33
Moderate growth and Gram negative cocci in sparse numbers	1	33

* These figures refer to the percentage distribution of strains of the different groups among the tonsils from which organisms of that particular group were isolated.

tonsils and other sources was attempted utilizing the method described by Swift *et al.*⁴ The results are given in Table III.

Representatives of the following types were found among the strains tested: 44, 41, 1, 14, 19, 33, 4, 36 and 39. Approximately

one-half of the cultures were negative with all the serums tested.[†] About the same proportion of untypable strains was isolated from the different sources. Types 44 and 41 were the ones more commonly encountered. The number of strains studied being too small no

TABLE III.
Typing of Hemolytic Streptococci from Different Sources.

Source	No. of strains tested	No. and %	Types										Cross reacted*	Negative with serums tested
			44	1	4	41	19	39	33	14	36			
Excised tonsils	33	No. 8 % 24.3	2	2	2	2	1	0	0	0	0	3	15	
Pathological throats, abscesses, ear and skin infections	22	No. 1 % 4.5	0	0	0	3	1	1	0	0	0	4	11	
From lesion or affected limb. Recurrent lymphangitis	9	No. 1 % 11.1	0	0	0	13.7	4.5	4.5	0	0	0	18.2	50	
Normal throats	31	No. 2 % 6.4	0	0	0	2	0	0	1	0	0	0	5	
		No. 2 % 12	3	0	1	22.2	0	0	11.1	0	0	0	55.5	
		No. 6.4 % 12.6	9.7	0	3.2	9.7	0	3.2	3.2	3.2	3.2	1	18	
Totals	95	No. 12 % 12.6	5	2	8	5	1	3	1	1	1	8	49	
		% 51.6	5.3	2.1	8.4	5.3	1	3.2	1	1	1	8.4	51.6	

* One strain gave a slight precipitate with types 3, 5, 22, 29, 31. Negative with the others. One culture gave a precipitate with No. 28 and No. 44 type serums. The other 6 cross reacting strains gave very slight precipitates with the majority of the type serums.

* One strain gave a slight precipitate with types 3, 5, 22, 29, 31. Negative with the others. One culture gave a precipitate with No. 28 and No. 44 type serums. The other 6 cross reacting strains gave very slight precipitates with the majority of the type serums.

conclusions can be drawn concerning the distribution of the different types among the various sources.

It must be noted that approximately one-half our strains were untypable. Swift *et al.*⁴ state: "negative reactions may also be due to loss of type-specific M substance after isolation of the strain. In some strains and types the M substance remains constant, but in others it may disappear rapidly during subculture on artificial media." The extracts of the strains from excised tonsils were made from stock cultures kept on blood agar slants for many months. The fact that approximately the same proportion of strains from different sources were untypable suggests that this failure was not mainly due to degradation *in vitro* of the organisms with loss of the M substance. There exists the possibility that these strains may be capable of losing their M substance *in vivo*, or may belong to types other than those for which they were tested.

Summary. A bacteriological study of 116 pairs of excised tonsils showed that 62.8% harbored β -hemolytic streptococci. In contrast 705 cultures of normal throats similarly studied showed an incidence of 17.4% of this organism. This difference is due mainly to a higher percentage of Group A streptococci (30.2%) found in excised tonsils as compared to 1.2% in normal throats.

The proportion of streptococci cultured from excised tonsils belonging to groups other than A was twice as large (32.6%) as that obtained from normal throat cultures (16%). In typing 95 strains of hemolytic streptococci by the precipitin method described by Swift *et al.*⁴ representatives of types 44, 41, 1, 14, 19, 4, 36 and 39 were found. Approximately half the strains were untypable with the serums tested. Types 44 and 41 were most commonly found.

† We are greatly indebted to Dr. R. C. Lancefield for supplying type serums and homologous extracts for this study. Extracts from each of our strains were tested with the following type serums, 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22, 23, 24, 26, 28, 29, 30, 31, 32, 33, 36, 37, 38, 39, 41, 43, 44, and 46.