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APPENDIX 3

The Bacitracin Test for Recognition of Group A Streptococci

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The bacitracin test introduced by Maxted (1) is a useful procedure for the recognition of group A streptococci in routine diagnostic work; however, the author of the test himself pointed out a certain limitation in its specificity for Streptococcus pyogenes. In the course of time, further evidence has been brought of the existence of group A strains displaying relatively low sensitivity in the bacitracin test. At the same time, if the concentration of bacitracin is increased to the point of detecting almost all the group A strains, the test becomes positive also for some strains of certain other groups, in particular C and G (2, 3, 4, 5, 6).

For the bacitracin test, as performed at the Streptococcus Reference Laboratory in Prague, discs 6 mm in diameter containing 0.7 units bacitracin are employed. This concentration has been selected to make the test clearly positive in all group A strains, i.e. to give an inhibition ring of at least 5 mm. The test was used under standard conditions in samples of group A, B, C, G and L strains currently in circulation among people and animals in Czechoslovakia. Figure 1 shows that among the group C and G strains there were some whose sensitivity was comparable to that of the group A strains of low sensitivity. The strains of these groups were examined in quantitative sensitivity tests by means of the serial tube dilution method. Table 1 confirms the finding in Figure 1. The minimal concentration of bacitracin inhibiting the growth of the less sensitive group A strains equalled 0.075 units per ml; for some group C and G strains, this concentration was either identical or even lower.

Figure 1. Sensitivity of group A, C and G strains in bacitracin test.

Figure 2. Sensitivity of group A and L strains in bacitracin test.

rhannose	glucos -	galacto -	carbohydrate	
	amine	samine	group L	group A

Figure 3

The above should especially be kept in mind in using the bacitracin test in strains recovered from carriers, among whom the frequency of strains of groups C and G is substantially higher than in patients. This is illustrated in Table 2: whereas among strains isolated at random in 1960 to 1964 from patients, 6.9 percent were group C and 4.7 percent were group G strains, the respective values in carriers were 20.4 and 12.5 percent. Relatively high sensitivity in the bacitracin test was also observed among group L strains. Four standard strains and 20 strains isolated from animals were tested. As Figure 2 indicates, only part of the group L strains could be distinguished from group A strains in the bacitracin test. It is interesting that a certain biological relation between group A and L strains, as observed in respect to sensitivity to bacitracin, was also

Table 1. Minimal concentration of bacitracin inhibiting the growth of various group strains.

Minimal inhibiting concentration (units/ml)	Number of strains (in percentage) of group				
	A	C	G	B	L
2,5		5		20	
1,25		20	14	28	
0,63		20	24	20	6
0,32		13	14	4	
0,16		7	5	4	6
0,08	4*	13	14	16	42
0,04	12	17	24	8	42
0,02	22	5	5		6
0,01	26				
0,005	20				
0,0025	12				
0,0013	4				
Total number of strains	50	40	21	25	17

The strains were tested by the serial tube dilution method.

Table 2. Serological grouping of haemolytic streptococcal strains isolated from patients with streptococcal disease and from normal subjects in 1960 to 1964.

Disease	1960				1961				1962				1963				1964				Total				
	Patients		Healthy carriers	No. of strains	Patients		Healthy carriers	No. of strains	Patients		Healthy carriers	No. of strains	Patients		Healthy carriers	No. of strains	Patients		Healthy carriers	No. of strains	X				
	X	No. of strains	X		No. of strains	X	No. of strains		X	No. of strains	X		No. of strains	X	No. of strains		X	No. of strains	X			No. of strains			
A	236	81.4	94	63.5	263	68.0	111	63.0	224	90.4	85	57.0	196	64.1	89	61.0	210	67.1	103	64.0	1129	86.2	462	62.3	
B	6	2.1	5	3.4	1	0.3	6	3.5	3	1.3	6	4.1	3	1.1	1	0.7	4	1.7	4	2.5	17	1.3	22	2.9	
C	32	11.1	20	18.9	22	7.4	37	21.2	6	3.3	33	22.5	15	6.4	24	16.7	14	5.8	36	22.4	91	6.9	158	20.4	
D	1	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.6	1	0.1	1	0.1
F	-	-	-	-	2	0.6	1	0.6	4	1.7	4	2.7	2	0.9	7	4.8	2	0.8	1	0.6	10	0.8	13	1.7	
G	15	5.1	20	13.5	11	3.7	19	10.9	6	3.3	19	12.9	17	7.3	23	16.0	11	4.6	16	9.9	62	4.7	97	12.5	
K	-	-	1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.1	
Total	290	100.0	348	100.0	299	100.0	174	100.0	247	100.0	147	100.0	233	100.0	344	100.0	241	100.0	161	100.0	330	100.0	774	100.0	

Table 3. Serological relationship between group A and L strains.

Antigen	Antisera			
	Group A		Group L	
	unabsorbed	absorbed with group L cells	unabsorbed	absorbed with group A cells
Group A	++	++	-	-
Group L	+	-	++	++

The antigens were prepared by Fuller method. The ring test was used for precipitating reactions.

suggested by an immunological relationship between strains of these two groups as regards their group specificity. Our preliminary experiments suggested, as Table 3 shows, that group A serum cross reacts with group L strains; but group L serum did not react with group A strains. Absorption of anti-A serum by a group L strain did not change its reactivity with A strains; and vice versa, absorption of anti-L serum by a group A strain did not deprive the serum of its reactivity with L strains. A certain immunological relationship between groups A and L is also suggested by the considerable amount of glucosamine present in the carbohydrate of group L (Figure 3). The bacitracin test is beyond doubt a valuable tool in screening group A strains. However, the reliability of the test in routine work will depend on the source of the material to be tested. Its reliability will be high in analyzing material from human streptococcal disease. In examining carriers, among whom group C and G streptococci are substantially more frequent, its reliability will be less; it is low especially in strains from animal sources. Hence, it is clear that final differentiation of group A streptococci from other groups, as has already been shown by other authors, rests with the serological method.

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APPENDIX 4

Sensitivity of Group A Streptococcus Cultures
to Temperate Phages

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Studies on sensitivity of hemolytic streptococci to a phage were undertaken by Evans (1942, 1947), Kjems (1955), Krause (1957), Totolyan (1959), Wilson and Maxted (1962). The aforesaid investigators established a group-specific character of lysis of Streptococcus cultures. In addition, they revealed the absence of strict correlation between a serologic type of group A streptococci and sensitivity to a phage.

Different forms of streptococcal diseases (excluding glomerulonephritis) are not connected with a certain serologic type. Proceeding from this fact, there are good reasons for the search for additional marking of group A streptococcus cultures employing phages. Analogous assumption was suggested by Kjems (1955) and Maxted (1962). With this point of view, sensitivity of hemolytic group A streptococci to temperate phages was studied. In the present investigation, there were employed 25 temperate streptococcal phages isolated from lysogenic cultures of hemolytic group A streptococci by the method of ultraviolet induction. Their activity according to Cratia ranges between 1×10^6 and 1×10^9 cells per ml. While studying a lytic action, phages were used at the RTD dilution to exclude the nonspecific lysis without reproduction. Cultures of group A streptococci were isolated from patients with such Strepto-