

STUDIES ON HEMOLYTIC STREPTOCOCCI

VI. THE EPIDEMICUS GROUP

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In an earlier paper (1935) it was shown that, in hemolytic streptococci of Lancefield's group A which ferment lactose and salicin but not mannitol, there is some correlation between resistance to phage C/594 and ability to produce epidemics of septic sore throat. The present communication reports further studies on this resistant group of strains, as compared with the group of strains differentiated from it only by sensitivity to phage C/594, designated as *Streptococcus pyogenes* in the second paper of this series.

The group of resistant strains will here be designated the epidemicus group, deferring the description of the species *Streptococcus epidemicus* until further studies have determined more clearly whether or not certain strains which differ more or less from the type strain should be included in the species. Definitely, however, the type strain of *S. epidemicus* must be the strain known in the literature as X40, isolated by Davis and Rosenow in 1912 from the spleen of a fatal human case during the Chicago epidemic of septic sore throat. Davis gave it the species name *epidemicus*. It is maintained in the American Type Culture Collection and designated as *S. epidemicus* No. 624 in the catalog. Our number is 890. Essential characters are as follows: It belongs to Lancefield's group A, ferments lactose and salicin but not mannitol, resists phage C/594, and belongs to Griffith's type 13.

Eighteen of Griffith's 30 serologic type strains were found to belong to Lancefield's group A and to produce the specified fer-

mentation reactions. Using the technique described in previous papers it was found that the 18 type strains may be divided into three groups according to sensitivity to phage C/594, as shown in table 1. One group includes strains sensitive to the filtrate, one includes strains resistant to the nascent phage, and an intermediary group includes two strains sensitive to the nascent phage, but resistant to the filtrate. (Sensitivity to the filtrate implies sensitivity to the nascent phage; resistance to the nascent phage implies resistance to the filtrate.)

Using the technique described by Pauli and Coburn, agglutinating serums were prepared with strains resistant to phage C/594 representing all of those Griffith types which include

TABLE 1

Sensitivity to phage C/594 of the 18 Griffith type strains which belong to group A and ferment lactose and salicin, but not mannitol

	GRIFFITH TYPE STRAINS	SENSITIVITY TO PHAGE C/594	
		Nascent	Filtrate
Sensitive	2, 3, 4, 5, 8, 9, 11, 12, 24, 25, 26, 28	+	+
Intermediary	1, 29	+	-
Resistant	13, 22, 27, 30	-	-

chiefly resistant strains; and with strains of type 1 of the intermediary group. A representative strain of type 29 of the intermediary group was not available when the serums were prepared.

As the study progressed it was found that certain sensitive strains of Griffith's types 9, 11 and 12 are serologically related to the resistant type strain 993 of type 27. The unabsorbed serum prepared with strain 993 agglutinated these three type strains to full titer. Therefore, serums were prepared with strains representing the three types, and cross reactions in the absorbed serums shown by some strains gave further evidence of the relationships.

The histories of the strains used for the preparation of serums are as follows:

Strains sensitive to phage C/594 filtrate.

- No. 769*, received from Dr. Griffith, labeled, "Symons, type 9."
No. 770, received from Dr. Griffith, labeled "Blackmore, type 11."
No. 771, received from Dr. Griffith labeled "S.F. 42, type 12."
No. 1324, received from Dr. Griffith, labeled "Matthews, type 25."
(The reason for preparing a serum with this strain is given further on.)

Intermediary strains, sensitive to nascent phage but resistant to the filtrate.

- No. 650*, received from Dr. Griffith, labeled "S.F. 130, type 1."
No. 905, from a throat culture from a fatal human case in the Kingston, N. Y. (1930) epidemic of septic sore throat. It was previously studied by Wheeler, who reported that strains from the Kingston epidemic produced moist colonies and showed capsules typical of *S. epidemicus*; and that they produced toxin not entirely neutralized by "N.Y. 5" antitoxin. Griffith (1937) classified strain 905 in type 1.

Strains resistant to nascent phage:

- Strain 890*, previously referred to as the type strain of *S. epidemicus*.
Strain 989, received from Dr. Griffith, labeled "63T type 22."
Strain 993, received from Dr. Griffith, labeled "G 112 type 27."
Strain 1403, received from Dr. Griffith, labeled "Quinn, Middleborough, Type 30."
Strain 951, from a case of erysipelas, identified as type 30.

Strain 1321 was of interest in this study because it is a resistant epidemic strain which has been studied by other investigators. The details of its history were reported by Coburn and Pauli, who designated it the "Westwater" strain. They isolated it from a case of pharyngitis in an outbreak at the Pelham Home in which rheumatic attacks followed throat infection. Hooker studied it and found that it produced strong B toxin and weak A toxin. Griffith placed it in his type 25.

Because attempts to produce an agglutinating serum with strain 1321 were unsuccessful, a serum was prepared with strain 1324 of type 25 (its history is given above) although this, Griffith's type strain, differs from strain 1321 in being sensitive to C/594 filtrate.

Using Griffith's technique for rapid agglutination in absorbed serum, 212 strains were classified as to serologic type. Among them 69 were sensitive to C/594 filtrate, 60 were sensitive to the nascent phage but resistant to the filtrate, and 83 were resistant to the nascent phage.¹ The correlation between serologic types and resistance to phage C/594 is shown in table 2.

Among the 83 strains resistant to nascent phage C/594, 63.9 per cent were classified in five of Griffith's types: 13, 22, 25, 27

TABLE 2
Correlation between sensitivity to phage C/594 and agglutination typing in 212 strains

GRIFFITH TYPE	SENSITIVE TO FILTRATE		SENSITIVE TO NASCENT PHAGE; RESISTANT TO FILTRATE		RESISTANT TO NASCENT PHAGE	
	Number	Per cent	Number	Per cent	Number	Per cent
13	0	7.2	3	25.0	9	63.9
22	0		0		2	
25	1		2		8	
27	1		9		16	
30	3		1		18	
1	7	21.7	11	18.3	8	19.3
11	9		0		8	
Not agglutinated in the above 7 types	48	71.0	34	56.7	14	16.8
Total.....	69		60		83	

and 30; 19.3 per cent fell in the two types 1 and 11, leaving only 16.8 per cent of strains not agglutinated by those seven type serums. On the other hand among the 69 sensitive strains only 7.2 per cent were agglutinated by those five serums which agglutinated the majority of the resistant strains; 21.7 per cent were agglutinated by serums of types 1 and 11, leaving the majority of sensitive strains (71 per cent) untyped by the seven serums.

On further examination, the data presented in table 2 show that

¹ The comparative numbers of strains selected for this study to represent the various groups are not indicative of their prevalence as the cause of human disease.

more than half of the resistant strains (51.8 per cent) fell into three of Griffith's types, 13, 27 and 30, the serums of those types having been prepared with strains selected on account of resistance to the phage. Griffith's type 22, with the type strain resistant to phage C/594, was represented in our collection by only one other strain.

Approximately 10 per cent of all resistant strains fell in type 25, although as already stated, Griffith's type strain, No. 1324, used for the preparation of the agglutinating serum, was sensitive to the filtrate. In our collection of 11 strains belonging to type 25, strain 1324 was the only one sensitive to C/594 filtrate; 8 were resistant to the nascent phage; two were sensitive to the nascent phage but resistant to the filtrate.

Almost 10 per cent of resistant strains fell in type 1, the agglutinating serums having been prepared with strains which were sensitive to the nascent phage but resistant to the filtrate, a character common to many type 1 strains.

Although the strain of type 11 used for the preparation of agglutinating serum was sensitive to the filtrate, almost 10 per cent of resistant strains fell in this type, which, as already stated, was shown to be serologically related to type 27. On the other hand, an insignificant number of resistant strains were agglutinated by the serums of types 9 and 12, prepared against sensitive strains serologically related to type 27.

The correlation between resistance to phage C/594 and association with septic sore throat is shown in table 3.

In our collection there are group A strains, with the given fermentation characters, from 27 epidemics of septic sore throat which occurred in various parts of this country and in England, the earliest having been the Chicago epidemic of the winter 1911-12. Only one strain from a given epidemic was included in the table if all were found to be identical. From eight epidemics the strains were found to belong to two of Griffith's types, and from those epidemics two strains, representing the two types, were included in the table.

It was shown in an earlier publication (1936) that among the scarlet fever strains of our collection the majority of those having

the given fermentation characteristics are sensitive to nascent phage C/594. On the other hand, only 5 of the 35 septic sore throat strains (14.3 per cent) were found to be sensitive to the lytic filtrate; nine strains (25.7 per cent) were sensitive to the nascent phage but resistant to the filtrate, leaving the majority (60 per cent) completely resistant to the phage.

TABLE 3
*Sensitivity to phage C/594, and agglutination typing of 35 septic sore throat strains from 27 epidemics**

SENSITIVITY TO PHAGE		NUMBER OF STRAINS	TYPE	NUMBER OF STRAINS OF THE GIVEN TYPE
Nascent	Filtrate			
+	+	5	9	1
			11	1
			27	2
			28†	1
+	-	9	Untyped	3
			1	2
			13	1
			27	2
-	-	21	29†	1
			Untyped	6
			1	2
			11	4
			13	4
			25	2
			30	3

* Nine duplicate strains, with all reactions identical with the type strain of the given epidemic, were omitted from this table.

† Griffith's type strains, not studied serologically by the writer.

Further observations made on the data presented in table 3 show that more than half of the 35 strains from septic sore throat epidemics (51.4 per cent) belonged to four of Griffith's types, 1, 11, 13 and 27, each of those types being represented by four or five strains.

DISCUSSION

In the previous paper of this series it was shown that distinctions made on the basis of sensitivity to phage B/563 were of an

order similar to the group distinctions made on the basis of precipitin reactions according to Lancefield's technique. In this paper it is shown that distinctions made on the basis of sensitivity to phage C/594 are of another order, some strains of group A being sensitive, and other strains being resistant. Studies reported in this paper were undertaken to determine whether relationships indicated by sensitivity to phage C/594 might be correlated with other characters which are of epidemiologic or immunologic significance.

The data show evidence of some correlation between distinctions made on the basis of sensitivity to phage C/594, and distinctions made on the basis of agglutinin absorption reactions. The data suggest, however, that antigenic structure and resistance to bacteriophage are linked together genetically, rather than that they depend on the same substance in the bacterial cell, because marked discrepancies in the relationship between the two properties may occur, as in the case of the sensitive strain 1324, belonging to type 25, a type which includes chiefly resistant strains.

The data confirm the observation previously reported, that there is some correlation between resistance to phage C/594 and ability to produce epidemics of septic sore throat. It is unknown to what extent such a reaction may be linked with those properties which determine protective responses in the host. Studies are in progress to show whether cross protection may be demonstrated between those Griffith types which are grouped together by the common character of resistance to phage C/594.

SUMMARY

Eighteen of Griffith's 30 type strains belong to Lancefield's group A and ferment lactose and salicin but not mannitol. Twelve are sensitive to phage C/594 filtrate; two are resistant to the filtrate but are sensitive to the nascent phage; four are resistant to the nascent phage.

Among 212 strains of Lancefield's group A which ferment lactose and salicin but not mannitol, the majority of strains resistant to phage C/594 (63.9 per cent) fell in Griffith's serologic types 13, 22, 25, 27 and 30. Only a few (7.2 per cent) strains sensitive to C/594 filtrate fell in those five serologic types.

Among 35 strains with the given characters from epidemics of septic sore throat, the majority (60 per cent) were resistant to nascent C/594 phage. Only 5 (14.3 per cent) were sensitive to the filtrate.

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