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A SEROLOGICAL STUDY OF THE HAEMOLYTIC STREPTOCOCCI ASSOCIATED WITH SCARLATINA.

BY D. G. S. McLACHLAN AND T. J. MACKIE.

(*From the Bacteriology Department, Edinburgh University.*)

INTRODUCTION.

THOUGH the relationship of haemolytic streptococci to scarlatina has been widely accepted, the biological definition of the "*Streptococcus scarlatinae*" group remains a problem of considerable practical importance. Thus the question has arisen whether the streptococci associated with this disease can be clearly demarcated from haemolytic streptococci occurring in other pathological conditions. General biological characters, biochemical reactions and animal inoculation experiments reveal no essential difference. It has been shown that the scarlatina streptococci possess in a specially uniform and marked degree the property of elaborating a filterable toxic principle which, when injected intradermally in non-immune subjects, evokes an erythematous reaction, the so-called Dick Reaction; but this character is not restricted to these organisms and pertains in a greater or less degree to strains of haemolytic streptococci from other conditions (see McLachlan, 1927). The failure to delimit clearly the scarlatina streptococci by this means is of particular significance and it might seem doubtful whether they can be exactly defined by biological methods.

Attempts have been made to differentiate scarlatina streptococci by serological methods and it has been claimed by various workers that certain serological types of haemolytic streptococci are specifically associated with the disease. The question is of such importance in regard to the whole aetiological problem as to merit the most careful inquiry.

The serological investigation of the relationship of streptococci to scarlet fever dates back to 1902, when Moser and v. Pirquet found that the sera of scarlatina patients agglutinated streptococci isolated from the throats of early cases to a higher titre than streptococci obtained from other sources. They also stated that they could differentiate by agglutination tests scarlatina strains from streptococci occurring in other pathological conditions, employing for this purpose the serum of a horse immunised with a scarlatina streptococcus. They believed, therefore, that scarlatina strains differed specifically from other streptococci. Their results were confirmed by Rossiwall and Schick (1905), Meyer (1906), and Ruediger (1906). The investigations, however, of Neufeld (1903) and Aronson (1903) failed to confirm the serological unity of the scarlatina streptococci. Further, Detot (1904) concluded that there was no specific agglutination of scarlatina streptococci by the serum of convalescent

patients. In recent years agglutination and agglutinin-absorption tests have been again applied in the search for a satisfactory means of distinguishing these organisms and many observers have reported that the majority of strains from scarlatina possess common serological characters.

Bliss (1920) found that twenty out of twenty-five strains of haemolytic streptococci isolated from the throats of scarlet fever patients were agglutinated uniformly by sera prepared from four of these strains and by the immune serum for one of the type-strains of haemolytic streptococci identified by Dochez, Avery and Lancefield (1919), this strain being also of scarlatinal origin. None of the scarlatina streptococci which he investigated were agglutinated specifically by sera prepared from five strains of haemolytic streptococci derived from sources other than scarlatina.

Tunnicliff (1920) investigated the question of the serological unity of haemolytic streptococci from scarlet fever cases, and found that they formed a distinct group as judged by agglutination and agglutinin-absorption tests; 92 per cent. of scarlatina strains conformed to this group. This was confirmed in 1922 by the same worker.

Gordon (1921) found that eighteen scarlatina strains investigated by him were identical in their agglutination reactions, and inferred that scarlatina streptococci formed a serologically homogeneous group.

Stevens and Dochez (1924, 1926) concluded that about 74 per cent. of scarlatina strains could be classified in one serological group, and could be distinguished from other haemolytic streptococci by agglutination and agglutinin-absorption tests. They found that immune sera for scarlatina streptococci agglutinated the majority of strains isolated from cases of scarlet fever, the end-titre for these strains being generally much lower than the titre for the organisms from which the respective sera were prepared. Further, absorption tests demonstrated the existence of a common antigenic constituent among scarlatina streptococci; thus, treatment of a serum with a heterologous strain agglutinated by that serum removed the agglutinin for other heterologous strains, while the agglutinin for the homologous organism was only occasionally removed completely by a heterologous strain. Further tests, however, showed that while there was an antigenic similarity between streptococci belonging to the same group, *e.g.* the scarlatina group, strains within the group were seldom identical, and in general strain-specificity was more pronounced than group-specificity. They elicited analogous results with erysipelas streptococci and concluded that "erysipelas strains form a closely related group of haemolytic streptococci. Scarlatinal strains form an equally compact group. The two groups are related antigenically but less closely than strains within the groups. These groups are related to pyogenic strains but less closely than they are to each other."

Eagles (1924, 1926), as a result of agglutination tests, found that scarlatina streptococci fall into one serological group, all the strains which he employed being agglutinated by scarlatina sera to titres varying from 1 : 160 to 1 : 2560,

the end-titre of the sera in all cases being 1 : 2560. Frequently, however, the only strain agglutinated in this dilution was that for which the serum had been prepared. He obtained distinct evidence of cross-agglutination between erysipelas and scarlatina strains.

Birkhaug (1925) agreed with Stevens and Dochez that scarlatina and erysipelas strains form definite groups which can be distinguished from pyogenic streptococci by agglutination and agglutinin-absorption tests.

The term "group," as used by these authors, appears to be a loosely related assembly of strains, not a well-defined serological type, like, for example, strains of Type I pneumococcus. Other investigators have failed to confirm the serological homogeneity of scarlatina streptococci.

Williams and Hussey (1924) found that only 35 per cent. of strains fell into one serological group, and Dick and Dick (1924) have stated that there are at least two serological varieties.

In this country, Smith (1926) recognised two serological types containing together more than 80 per cent. of the strains examined by him at that time. "Type I" included about 57 per cent. of all strains tested.

Griffith (1926) added a third type to the two reported by Smith but the three types comprised only 46 per cent. of the strains which he investigated. He also noted that streptococci from sources other than scarlatina may belong to any of these serological groups. Thus, strains referable to Types I and III were isolated from cases of puerperal fever, and it was found that organisms belonging to any of these types might occur in the throat of persons not suffering from scarlatina. A Type I strain was obtained from the cerebrospinal fluid of a case of meningitis not known to be associated with scarlet fever.

Since the completion of the work which forms the subject of this paper two further contributions to the serology of scarlatina streptococci have been published by Griffith and Smith. Griffith (1927) examined a series of 222 scarlatina strains and added a fourth serological type to the three which he had reported previously. One hundred and fifty-six of his strains were included in these four groups. Organisms belonging to these types could generally be identified by direct agglutination tests.

Smith (1927) has made a further investigation of the 210 strains referred to in his previous report and has also examined a considerable number of non-scarlatina strains. He classified the majority of these organisms in ten serological types, 205 of the scarlatina strains falling into seven of these, while 62 out of 72 non-scarlatina streptococci were included in nine types, the remaining strains in each aetiological group being unclassified. It is noteworthy that many non-scarlatina strains were apparently identical serologically with scarlatina streptococci. The majority, however, of the scarlatina streptococci belonged to Types I and II, only 34 strains failing to react with the antisera to these types. Sixteen and eight scarlatina strains respectively were placed in Types IV and X; several of the other serological types had only

one representative. The Type X strains were remarkable in that antisera prepared for representatives of these strains reacted with the majority of the streptococci belonging to other groups in both direct agglutination and agglutinin-absorption tests, though the strains themselves did not react with antisera to organisms of different serological type. (See Addenda (1), p. 247.)

The serological investigation of scarlatina streptococci has revealed discrepancies among the results of certain observers particularly in regard to the important practical questions of the differentiation of these organisms from other haemolytic streptococci, and their antigenic constitution.

We have made a further study of this question in a serological analysis of a considerable number of strains of haemolytic streptococci from scarlatina and other sources, which had been carefully investigated as regards their toxigenic properties (McLachlan, 1927). While certain "groups" can be recognised among the scarlatina streptococci corresponding to those originally described by Smith and by Griffith, in which can be placed also various non-scarlatina strains, we have not found it possible to classify these organisms into well defined serological types. No clear demarcation has been elicited between haemolytic streptococci from scarlatina and from other conditions, though strains presenting particular serological characteristics are more prevalent in scarlatina. This study has also illustrated the complex antigenic constitution of these organisms.

Methods.

Isolation and Cultivation of the Strains investigated. The strains of "*Streptococcus scarlatinae*" employed in this investigation were obtained from early cases of scarlet fever, generally about the 4th day of the illness. Throat swabs were taken from the patients and blood-agar plates were inoculated by successive strokes. After 24 hours' incubation numerous colonies of haemolytic streptococci were generally visible and from one of these a second blood-agar plate was subinoculated, also by successive strokes. After incubation overnight a subculture on a slope of coagulated sheep's blood was made from a single colony. Streptococci obtained from sources other than scarlatina were isolated by plating on blood-agar the material in which they occurred, and re-plating was performed as above. The strains collected were maintained on coagulated sheep's blood medium (on which they remained viable for several weeks), and subcultured at intervals of a month.

For purposes of comparison, strains of Types I and II were obtained from Dr J. Smith and two Type III strains, "61_A" and "62_A," from Dr F. Griffith (*vide supra*).

Preparation of agglutinating antisera. Rabbits were immunised according to the method used by Stevens and Dochez (1924) and by Eagles (1924). The organism was grown for 18–24 hours in phosphate-broth (Dochez, Avery and Lancefield, 1919) containing 0·1 per cent. glucose and standardised to *p*H 7·6. The culture was centrifuged until the growth had sedimented; the organisms were then re-suspended in saline and the emulsion standardised to equal Brown's opacity standard No. 2. The animals received 0·25 c.c. of this emulsion intravenously on each of 2 or 3 successive days, and this course of injections was repeated at intervals of a week. In all, four or five series of injections were given, the dosage being increased in the later stages. In the first two courses, organisms killed by an exposure to 57° C. for half an hour were injected. Afterwards, live organisms were used. A week after the last injection the end-titre of the serum was determined and if satisfactory the animal was bled and its serum carbolised and stored in the refrigerator. The end-titre varied from 1 in 1600 to 1 in 6400.

As about two months were required for the preparation of an agglutinating serum, the method was tried of giving an intravenous injection of about 50,000 million organisms detoxicated by 1 per cent. gold chloride, followed a week later by a dose of about 15,000 untreated living organisms (see Osman, 1927). By this means a potent serum was obtained in certain cases after about a fortnight, but there was a considerable loss of animals until the dosage was diminished. The end-titre of the serum varied in individual rabbits but attained even 1 in 12,800.

Preparation of organisms for agglutination and absorption tests. The strains to be tested were grown in tubes or flasks of phosphate-broth for 18–24 hours but if the growth was scanty the cultures were incubated for 48 hours.

In a few instances a uniform emulsion was obtained on the first subculture in broth, but as a rule the growth was extremely granular. When a uniform emulsion for agglutination tests was required, transplants of the organism were made in phosphate-broth every day till a non-granular emulsion was obtained. A few subcultures were generally sufficient to effect this, but in the case of some strains 15–20 transplants were necessary. Certain strains, however, remained granular even after repeated subculturing in phosphate-broth. The organisms were deposited by centrifuging and re-suspended in saline to a density suitable for agglutination reactions.

Direct Agglutination Tests. The test was performed by making a geometric series of serum dilutions, generally from 1 in 25 up to a dilution half that of the end-titre of the serum. To each of the dilutions an equal volume of bacterial emulsion was then added and the mixtures of serum and emulsions were transferred to narrow agglutination tubes. At the commencement of the investigation incubation was carried out at 55° C., but it was afterwards decided to conduct all agglutination tests at 37° C. since at this temperature the emulsions showed less tendency to spontaneous agglutination. After one and a half hours' incubation the tubes were withdrawn from the incubator and left on the bench for a few minutes before the results were read. The end-titre of the serum was taken as the highest dilution in which definite agglutination occurred.

Absorption Tests. In carrying out absorption tests with organisms which readily yield abundant growths in artificial culture, it is possible to saturate undiluted serum, but in the case of streptococci and particularly when large numbers of strains are studied, this is almost impracticable and sera have therefore been diluted before treatment with bacterial emulsion (*vide infra*). The final titration of the absorbed serum is thus restricted to a particular range of dilutions (*vide* p. 230) and results indicating absorption of agglutinin may have a relative value only, though sufficiently significant in indicating antigenic relationships. A considerable excess of bacterial emulsion has been employed for saturation, *i.e.* as compared with the minimum quantity of a particular organism required to remove agglutinin from its own antiserum. This diminishes any irregularities due to quantitative variations in technique but as pointed out later (*vide* p. 239) may tend to accentuate partial antigenic similarities.

The technique adopted was as follows: The organisms were grown for 18 to 24 hours in phosphate-broth and separated from the medium by centrifuging. Emulsions of the organisms in saline were then standardised to equal a special opacity standard four times as dense as Brown's opacity standard No. 10, and to a quantity of this standardised emulsion was added an equal volume of diluted serum 64 times the concentration of the end-titre of the serum for the strain from which the serum was prepared. Thus, if the end-titre of the serum was 1 in 3200, the dilution of the serum used in absorption tests was 1 in 50. The mixtures of serum and organisms were incubated at 37° C. for 4 hours, being shaken at intervals. The treated serum was then separated in the centrifuge and pipetted off. After storage overnight in a refrigerator, a series of dilutions were prepared and agglutination of the strain used for the preparation of the serum was tested. Some difficulty was experienced with one of the type strains on account of its tendency to agglutinate spontaneously when

used to test the absorbed serum. Diminution of the salt content of the diluent had little effect in stabilising the emulsion, but it was found that a suspension of the organism in phosphate-broth was more stable. In this case, the serum dilutions also were made with phosphate-broth instead of saline.

The residua of the emulsions not employed in the absorption tests were used for direct agglutination if they appeared to be relatively stable after standing at room temperature for 2 hours. The use of phosphate-broth as diluent instead of saline enabled many results to be obtained which would not otherwise have been possible until the organism had been repeatedly subcultured in broth.

Agglutinin-Absorption.

Scarlatina strains.

Agglutinin-absorption tests have been relied on almost entirely for eliciting the serological relationships and differences of the strains studied. The results of direct agglutination reactions will be recorded and discussed later.

After absorption, the agglutinating power of the serum was tested in dilutions ranging from a concentration 16 times the end-titre up to the actual end-titre. Complete absence of agglutination or only a trace of agglutination in the "titre $\times$ 16" dilution was taken to indicate a close serological relationship between the strain tested for its agglutinin-absorptive properties and the strain from which the serum had been prepared. This result has been expressed by the + sign in the tabulated results (Tables I and II). If no change in the agglutinating power of the serum was noticeable or only a slight weakening, this was regarded as signifying absence of antigenic relationship, and has been shown in the tables by the − sign. Intermediate effects, *i.e.* where a definite lowering of the end-point of the serum resulted (*e.g.* to the titre $\times$ 4 dilution), have been taken as indicative of partial antigenic similarity between the two strains. This type of result has been designated in the tables "partial absorption." Variable absorption effects on repeated testing have been noted with certain sera and strains. These are classified in the tables as "variable" and are discussed in detail later.

In the investigation of a large number of strains the preparation of agglutinating antisera for each strain and the carrying out of cross-absorption tests are almost impracticable, and though complete cross-absorption of the respective agglutinins has been considered necessary before two strains can be considered identical, even this does not prove their exact identity, as is well illustrated by certain of the results to be commented on. Agglutinating sera have been prepared for a number of selected strains and absorption of agglutinin from these sera by other strains has been taken as indicating a resemblance in part at least of the antigenic composition of the strain tested with that of the strain used for the preparation of the serum.

In the first place an agglutinating serum was prepared for a standard strain (designated No. *1*), which had been isolated in 1924 from an early case of scarlet fever in Edinburgh and extensively used for the preparation of "Dick toxin." Direct agglutination tests were carried out with this serum

and other strains and one of these (No. *5*), which failed to agglutinate, was selected for the preparation of a second antiserum. As a result of agglutination and agglutinin-absorption tests with these two sera a third strain (No. *23*), apparently different in its serological characters, was also utilised for the production of a specific antiserum. Other strains were selected in a similar way at a later date and agglutinating sera prepared from them (*vide* Tables I–IV).

In Table I are shown the results of a preliminary sorting-out of the scarlatina strains by agglutinin-absorption tests. The organisms have been arranged in the table in five groups. The strains in the first four groups bear a close relationship to strains *1*, *5* (and *35*), *90*, and *23* respectively. The remainder, for convenience, have been designated Group V. Representative strains of Smith's Types I and II and Griffith's Type III can be classified with Groups I, II and III respectively.

The results obtained with the first four groups show that strains placed in any individual group may differ in their detailed antigenic composition from other organisms in the same group. Thus, some strains placed in the first group are closely related to strain *90*, viz. Nos. *18*, *33*, *48*, *91*, *93* and Smith's Type I strain. Others, including No. *1*, show no such affinity. This relation between certain strains placed in the Type I group and Type III strains has been noted by Griffith (1926). Strain *93* differs from other members of the first group, showing affinities with Nos. *1*, *5*, *23* and *90*. Thus, within the group there is no definite homogeneity when the antigenic relationships of strains are analysed in this way.

The strains in the second group show more antigenic homogeneity but are not entirely identical. Strain No. *5* was the original member of the group utilised for the preparation of an agglutinating serum, but it was found to possess certain peculiarities, discussed later, and an antiserum was prepared from No. *35*, a second member of the group, in order to check the results obtained with serum *5*. While strains *5* and *35* are closely related and would be considered identical as the result of cross-absorption tests, there is obviously some difference between them as may be seen from the results in Table I; thus, many strains among the other groups completely absorbed the specific agglutinin from serum *5* though not from serum *35* under the conditions of the test, *e.g. 30*, *11*, *62*, *51*, etc. On the other hand, one of the non-scarlatina strains, *Y* (Table II), absorbed the specific agglutinin from serum *35*, but not from serum *5*. The majority of the scarlatina and non-scarlatina strains studied showed some degree of relationship to No. *5*, but it was found that only the strains which completely and uniformly absorbed the agglutinin from serum *5* and were agglutinated directly to end-titre by this serum absorbed the agglutinin from serum *35*. Strain *35* has not therefore the generalised antigenic component or components possessed by strain *5*. Further, many strains, *e.g. 9*, *12*, *13*, *28*, *42*, *44*, *57*, etc., which partially absorbed the agglutinin from serum *5*, as judged by tests with strain *5*, failed to reduce the agglutinating titre of that serum for strain *35*.

Table I. *Agglutination Reactions of 101 Strains of Haemolytic Streptococci isolated from Cases of Scarlatina.*

Strains tested	Antisera to strains: *1* End-titre 1 : 3200		*5* End-titre 1 : 6400		*35* End-titre 1 : 6400		*23* End-titre 1 : 6400		*90* End-titre 1 : 6400		*22* End-titre 1 : 800		*73* End-titre 1 : 12,800		*4* End-titre 1 : 6400	
	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption	Direct agglutination	Agglutinin absorption
Group I																
1 (Type strain)	+	+	1 in 200	p. and v.	.	−	.	−	1 in 400	−	1 in 200	+	+	+	.	−
2, 3, 7, 26, 38, 47, 53, 63	+	+	.	p. and v.	.	−	−	−	.	−	.	+	+	+	.	.
14, 49, 87	.	+	.	p. and v.	.	−	−	−	.	−	.	+	.	.	.	.
18	+	+	.	p. and v.	.	−	−	−	.	+	.	+	+	+	.	−
27	1 in 800	+	.	p. and v.	.	−	.	−	.	−	.	+	.	+	.	.
33, 48	+	+	.	p. and v.	.	−	−	−	.	+	.	+	.	.	.	−
40	+	+	−	p. and v.	.	−	−	−	.	−	1 in 200	+	.	.	.	.
54	1 in 200	+	.	p. and v.	.	−	.	−	.	−	.	+	.	.	.	.
93	.	+	−	p. and v.	−	−	+	+	.	+	.	+	.	+	+	+
9, 28, 73, 99	+	+	.	p.*	.	−	−	−	.	−	.	+	+	+	.	.
13	.	+	.	p.*	.	−	.	−	.	−	−	+	.	.	.	.
60	−	+	.	p.	.	−	.	−	.	−	.	+	.	.	.	.
37	.	+	.	p.	.	−	−	−	.	−	.	+	.	.	.	.
30	1 in 400	+	.	+*	.	−	−	−	.	−	.	+	.	.	.	.
57	+	+	.	+	.	−	.	−	.	−	.	+	.	.	.	.
59	+	+	.	+*	.	−	.	−	.	−	.	+	.	.	.	.
91	.	+	.	+*	.	−	.	−	.	+	.	+	.	+	.	.
46	+	+	1 in 200	−*	.	−	−	−	.	p.	.	+	.	.	.	.
"*S. scarlatinae*," Type I (Smith)	+	+	.	−*	.	−	.	−	.	+	.	.	.	.	.	.
Group II																
5 (Type strain)	−	−	+	+	+	+	−	−	1 in 400	−	1 in 200	+	.	.	.	.
6, 10, 16, 20, 29, 32, 39, 50, 75, 79, 96	−	−	+	+	+	+	−	−	.	−	.	+	.	.	.	.
11, 71, 95	−	−	+	+	.	p.	.	−	.	−	−	+	.	.	.	.
21, 25, 36, 70, 94	−	−	+	+	.	+	−	−	−	−	.	+	.	.	.	.
35	−	−	+	+	+	+	−	−	+	−	−	+	.	−	.	.
58	1 in 1600	−	+	+	+	+	−	−	.	−	.	+	.	.	.	.
"*S. scarlatinae*," Type II (Smith)	−	−	+	+	+	+	−	−	.	−	.	.	.	.	.	.

Group III																		
90 (Type strain)	...	...	...	.	–	–	p.	–	–	–	p. and v.	+	+	+	+	.	–	1 in 400	–
8	...	...	...	–	–	1 in 400	+*	1 in 50	p.	–	–	.	+	–	+	.	.	.	.
47	...	...	...	–	–	1 in 3200	p.	1 in 200	–	–	–	+	+	–	+	.	.	1 in 100	–
62	...	...	...	–	–	–	+*	–	–	–	–	.	+	–	+	.	.	.	–
67	...	...	...	.	–	–	p.	–	–	–	–	–	+	.	+	.	.	.	–
22	...	...	...	–	–	.	p.	–	–	–	–	.	+	.	+	.	.	.	–
"*S. scarlatinae*," Type III (Griffith)																			
(*62 A*)	...	...	...	.	–	.	–*	–	–	.	–	.	+	.	.	.	–	.	–
Group IV																			
23 (Type strain)	...	...	...	1 in 100	–	–	p. and v.	.	–	+	+	–	p. and v.	1 in 200	+	.	.	+	+
4	...	...	...	–	–	–	–	.	–	+	+	.	+	.	+	.	.	++	+
24	...	...	...	–	–	–	–*	.	–	+	+	.	+	.	+	.	.	+	+
41, 69, 74, 86		...	...	.	–	.	p. and v.	.	–	.	+	.	+	.	+	.	.	.	+
61, 84		...	...	.	–	.	p. and v.	.	–	+	+	.	–	.	+	.	.	.	+
38	...	...	...	.	–	.	p.	.	–	.	+	.	+	.	+	.	.	.	+
34	...	...	...	–	–	.	p.	–	–	.	+	–	+	.	+	.	.	.	+
35	...	...	...	–	–	–	–*	–	–	.	+	+	+	.	p.	.	.	.	.
64	...	...	...	–	–	.	+*	.	–	.	+	–	+	.	+	.	.	.	+
29	...	...	...	–	–	+	+	.	–	.	+	.	+	–	+	.	.	1 in 200	–
76	...	...	...	.	–	.	p.*	.	–	+	+	.	–	.	+	.	.	.	+
Group V																			
82	...	...	...	.	–	–	p.	–	–	–	–	1 in 1600	–	+	+	1 in 200	–	.	.
42	...	...	...	–	–	1 in 400	p.	1 in 200	–	–	.	–	–	–	+	.	.	.	.
12, 56, 82, 85, 89, 92		...	...	.	–	.	p. and v.	.	–	–	–	–	–	.	+	.	.	.	.
28	...	...	...	.	–	.	p.*	.	–	–	–	–	–	.	+	.	.	.	.
43, 52		...	...	.	–	1 in 200	p.	–	–	–	–	–	–	.	+	.	.	.	.
44, 55		...	...	.	–	1 in 200	p.	–	–	–	–	–	–	–	+	.	.	.	.
45	...	...	...	–	–	1 in 400	p. and v.	.	–	–	–	–	–	–	+	.	.	.	.
65	...	...	...	–	–	1 in 400	p.*	.	–	–	–	1 in 200	–	–	+	.	.	.	.
51	...	...	...	–	–	1 in 800	+	–	–	–	–	–	–	.	+	.	.	.	.
66	...	...	...	–	–	–	p.	–	–	1 in 800	–	.	–	+	+	.	–	.	.
68	...	...	...	1 in 100	–	.	p.	.	–	.	–	.	–	.	+	.	.	.	.
77, 81		...	...	.	–	.	p.*	.	–	.	–	.	–	.	+	.	.	.	.
80	...	...	...	.	–	.	+*	.	–	.	–	.	–	.	+	.	.	.	.
83, 97		...	...	.	–	.	p.	.	–	.	–	.	–	.	p.	.	.	.	.
88	...	...	...	–	–	–	p.	–	–	–	–	.	–	.	p.	.	.	.	.

\+ signifies complete, or almost complete, absorption of the specific agglutinin from a serum.
p. signifies a lesser degree of absorption of agglutinin for the homologous strain.
p. and v. signifies that absorption of agglutinin has been partial and variable.
– signifies absence or traces only of absorption of agglutinin for the homologous strain.
* signifies that the test has been performed on one occasion only. This has been shown only in the case of tests with serum *5*.

The figures in the "Direct-agglutination" columns denote the highest dilution in which agglutination was observed.
\+ signifies agglutination to end-titre.

Table II. *Agglutination Reactions of Strains from Sources other than Scarlatina.*

Strain	Source	Dilution of toxin and results obtained	Antisera to strains: *1* Direct agglutination	*1* Agglutinin absorption	*5* Direct agglutination	*5* Agglutinin absorption	*35* Direct agglutination	*35* Agglutinin absorption	*23* Direct agglutination	*23* Agglutinin absorption	*90* Direct agglutination	*90* Agglutinin absorption	*22* Direct agglutination	*22* Agglutinin absorption	*A* Direct agglutination	*A* Agglutinin absorption
Group I																
V	Empyema	1 : 1000 β	+	+	.	−*	.	+	.	+	−	−	.	+	.	.
Group II																
M	Septicaemia	1 : 1000 β	−	−	+	+	.	+	.	−	.	−	.	+	−	−
Q	Purulent conjunctivitis	1 : 100 γ	.	−	.	+	.	+	.	−	.	−	.	+	.	−
Z	Tonsillitis	1 : 1000 α	.	−	1 in 3200	+	.	+	.	−	−	−	.	+	.	−
Group III																
B	Abscess of finger	1 : 100 γ	.	−	.	partial	.	−	.	−	.	+	.	+	.	−
D	Peritonitis	1 : 100 α	−	−	−	partial	−	−	−	−	.	+	−	+	1 in 100	−
E	Infection of knee joint	1 : 100 γ	.	−	.	partial	.	−	.	−	.	+	.	+	.	−
T	Throat of healthy person	1 : 250 α	.	−	.	−	−	−	.	partial	.	+	.	+	.	.
Group IV																
K	Mastoiditis	1 : 1000 α	.	−	−	−*	.	−	+	+	.	partial	.	+	−	−
P	Mastoiditis	1 : 250 α	.	−	.	−	.	−	+	+	−	−	.	+	.	−
R	Abscess of finger	1 : 1000 α	.	−	−	−	.	−	+	+	−	−	.	+	−	−
U	Throat of healthy person	1 : 1000 α	.	−	−	partial	−	partial	+	+	.	partial	.	+	.	−
T_2	Throat of healthy person	1 : 250 α	1 in 800	−	1 in 400	partial	.	partial	−	+	−	−	.	−	.	−
T_5	Throat of healthy person	1 : 100 β	.	−	.	partial	.	partial	.	+	.	partial	.	−	.	−
Group V																
C	Erysipelas	1 : 100 β	.	−	.	partial	−	−	−	−	.	−	.	+	.	−
H	Nose	1 : 1000 β	.	−	−	partial		−	.	−	.	−	−	+	−	−
L	Tonsillitis	1 : 100 γ	−	−	+	partial	.	−	.	−	.	−	.	+	−	−
N	Otitis media	1 : 1000 α	−	−	−	−	.	−	.	−	−	−	−	+	.	−
O	Simple abscess	1 : 250 α	.	−	.	−	.	−	.	−	.	−	.	+	.	−
S	Cervical abscess: case of diphtheria	1 : 100 β	.	−	.	−*	.	−	.	−	.	−	.	+	.	−
W	Tonsillitis	1 : 250 β	.	−	.	.	.	−	.	−	.	−	.	+	.	−
X	Subcutaneous abscess of foot	1 : 250 α	.	−	.	.	.	−	.	−	.	.	−	+	.	−
M_A	Tonsil of case from which "*M*" was obtained	1 : 100 γ	.	−	−	−*	.	−	.	−	−	−	−	+	−	−
T_1	Throat of healthy person	1 : 100 γ	.	−	.	−*	.	−	.	−	.	−	.	+	.	.
T_3	Throat of healthy person	1 : 100 β	.	−	−	*partial*	−	−	.	−	−	−	.	+	−	.
T_6	Throat of healthy person	1 : 100 γ	.	−	.	.	.	−	.	−	.	−	.	−†	−	−
A	Cervical abscess: case of diphtheria	1 : 100 γ	−	−	−	−	−	−	−	−	−	−	−	−	+	+
F	Simple abscess	1 : 100 γ	−	.	−	−*	−	−	−	−	.	−	−	−	+	+
G	Simple abscess in leg	1 : 100 γ	−	.	−	−*	−	−	−	−	.	−	−	−	+	+
I	Nasal catarrh	1 : 100 γ	−	−	−	−	.	−	−	−	.	−	−	−	−	−

α Toxin gave results like scarlatina toxin
β Toxin gave irregular results
γ No toxin demonstrated in given dilution
} (*vide* McLachlan, *J. Hyg.* 1927, **26**, 84).

† Gave a partial absorption reaction with serum *66*; this serum behaved like serum *22* when tested with various scarlatina and non-scarlatina strains.

* Signifies that the test has been performed on one occasion only. This has been shown only in the case of tests with serum *5*.

Other signs—explanation as in Table I.

It will be seen from Table I that all the scarlatina strains—and the same is true for the majority of the non-scarlatina strains (*vide infra*)—are related to strain *22*, whose characteristic antigenic constituent is generalised and non-specific. Serum *22* showed such generalised reactions in an even more marked degree than serum *5*.

The strains in the third and fourth divisions of Table I have been grouped round Nos. *90* and *23* respectively. Strain *90* corresponds to Griffith's Type III. No. *23* and certain other strains in this group are related antigenically to those grouped with No. *90*, but have apparently an antigenic constituent not possessed by members of that group. A few strains, *61*, *76* and *84*, which have been placed in the "*23*" group do not possess the antigenic constituent characteristic of the organisms ascribed to the "*90*" group.

The strains in the last division of Table I have affinities with *5* and *22* only. Antisera were prepared for certain other strains in this group, viz. *66*, *65* and *42*. The serum for No. *66* in absorption tests with various strains behaved like No. *22*; the other antisera reacted, in agglutination and absorption tests, only with the strain used for immunisation. Thus, while strain *66* showed non-specific antigenic characters, the other representatives of Group V examined appeared to possess a highly restricted antigenic specificity.

While it has been possible to distinguish four serological groups among the scarlatina strains, the first three corresponding generally to Smith's and Griffith's Types I, II and III, it is doubtful whether such a classification gives a true impression of the differentiation and relationship of the scarlatina streptococci. A classification into groups or types implies a similarity between members of the same group and a degree of separation between strains belonging to different groups that are not found here.

Tables III and IV illustrate the antigenic relationships of the strains investigated in so far as these can be analysed by means of the sera employed. The system of indicating the antigenic relationships between strains (Table III) has been adopted from Torrey and Buckell (1922, *vide infra*).

In Table IV it will be seen that the scarlatina strains form twenty-one different serological categories; forty-one per cent. of the strains, however, are included in two of these groups. It is probable that further categories might have been noted if additional sera had been employed. These tables illustrate the complexity and overlap in the antigenic constitution of the strains examined and represent their relationships more truly than an attempted classification into serological types. Even with the classification adopted in Table IV it has been impossible to represent minor differences between certain strains, *e.g. 5* and *35*.

Certain of the strains studied, *e.g. 5*, *22* and *66*, possess as more or less predominant antigenic constituents, non-specific components common to other scarlatina strains as judged by the fact that practically all other strains absorb agglutinin from their antisera. This peculiarity has also been noted by Torrey and Buckell (1922) in a serological study of the gonococcus. Thus

Table III. *Strains of Haemolytic Streptococci from Scarlatina and other sources.*

Scarlatina strains numbered *1–99*.
Non-scarlatina strains designated *A–Z*.

Absorption reactions	Antisera to strains		
	1	*5*	*35*
Complete	*1–3, 7, 9, 13, 14, 18, 26–28, 30, 33, 37, 38, 40, 46–49, 53, 54, 57, 59, 60, 63, 73, 87, 91, 93, 99* Type I (Smith), *Y*	*5, 6, 8, 10, 11, 16, 19–21, 25, 29, 30, 32, 35, 36, 39, 50, 51, 57–59, 62, 64, 70, 71, 75, 79, 80, 91, 94–96* Type II (Smith), *M, Q, Z*	*5, 6, 10, 16, 20, 21, 25, 29, 32, 35, 36, 39, 50, 58, 70, 75, 79, 94, 96* Type II (Smith), *M, Q, Y, Z*
Partial or variable	—	*1–3, 7, 9, 12–14, 17, 18, 22, 23, 26–28, 33, 34, 37, 38, 40–45, 47–49, 52–56, 60, 61, 63, 65–69, 72–74, 76–78, 81–90, 92, 93, 97–99* *B, C, D, E, H, L, U,* T_2, T_3, T_5	*8, 11, 71, 95* *U,* T_2, T_5
Negative	All other strains	*4, 15, 24, 46* Type I (Smith) Type III (Griffith) *A, F, G, I, K, N, O, P, R, S, T, Y,* M_A, T_1, T_6	All other strains
Not tested	—	*W, X*	—

Absorption reactions	Antisera to strains		
	90	*23*	*22*
Complete	*4, 8, 15, 17, 18, 19, 24, 33, 34, 41, 48, 62, 64, 67, 69, 72, 74, 86, 90, 91, 93, 98* Type I (Smith) Type III (Griffith) *B, D, E, T*	*4, 15, 19, 23, 24, 34, 41, 61, 64, 69, 74, 76, 84, 86, 93, 98* *K, P, R, Y, U,* T_2, T_5	*1–14, 16–82, 84–87, 89–96, 98, 99* *B, C, D, E, H, K, L, M, N, O, P, Q, R, S, T, U, W, X, Y, Z,* T_1, T_3, M_A
Partial or variable	*23, 46* *K, U,* T_5	*90, T*	*15, 83, 88, 97*
Negative	All other strains	All other strains	*A, F, G, I,* T_2, T_5, T_6 Type I (Smith) Type II (Smith) Type III (Griffith)
Not tested	—	—	

it would appear that in serum *5* there are fractions or combinations of fractions, which react with a corresponding antigenic moiety present in many scarlatina strains. On the other hand, haemolytic streptococci isolated from other conditions have also possessed the same power of absorbing agglutinins from serum *5* so that this serum in no way serves to distinguish scarlatina streptococci from those occurring in other pathological conditions.

Antisera were prepared from strains *73* and *4*, members of Groups I and IV respectively, to ascertain whether their reactions corresponded with those of sera *1* and *23*. Strains *1* and *73* were found to be very closely related, and likewise *4* and *23*, complete absorption of agglutinin from their sera occurring in cross-absorption tests (Table I). A limited number of strains representative of each group were investigated with these sera. Absorption tests with serum *73* gave results parallel to those obtained with serum *1*, and the results obtained with serum *4* corresponded in the majority of instances with those

Table IV. *Classification of Scarlatina and Non-Scarlatina Strains by Analysis of their Serological Relationships to Certain Selected Strains.*

Serologically related to the following strains*	Scarlatina strains: Serial numbers	Scarlatina strains: Total number	Non-scarlatina strains: Strains	Non-scarlatina strains: Total number
1. 5. 22	*1, 2, 3, 7, 9, 13, 14, 26, 27, 28, 37, 38, 40, 47, 49, 53, 54, 60, 63, 73, 87, 99*	22	—	—
1. 5. 22	*30, 57, 59*	3	—	—
1. 5. 90. 22	*18, 33, 48*	3	—	—
1. 5. 90. 22	*91*	1	—	—
1. 5. 90. 23. 22	*93*	1	—	—
1. 90. 22	*46*	1	—	—
1. 35. 23. 22	—	—	*Y*	1
5. 35. 22	*5, 6, 10, 16, 20, 21, 25, 29, 32, 35, 36, 39, 50, 58, 70, 75, 79, 94, 96*	19	*M, Q, Z*	3
5. 35. 22	*11, 71, 95*	3	—	—
90. 5. 22	*62*	1	—	—
90. 5. 22	*17, 67, 72*	3	*B, D, E*	3
90. 5. 35. 22	*8*	1	—	—
90. 23. 5. 22	*19, 64*	2	—	—
90. 23. 5. 22	*34, 41, 69, 74, 86, 98*	6	—	—
90. 23. 22	*4, 24*	2	—	—
90. 23. 22	*15*	1	—	—
90. 23. 22	—	—	*T*	1
90. 23. 5. 22	*90*	1	—	—
90. 23. 5. 22	*23*	1	—	—
23. 5. 22	*61, 76, 84*	3	—	—
23. 90. 22	—	—	*K*	1
23. 22	—	—	*P, R*	2
23. 5. 35	—	—	T_2	1
23. 5. 35. 90	—	—	T_3	1
23. 5. 35. 90. 22	—	—	*U*	1
5. 22	*12, 22, 42, 43, 44, 45, 52, 55, 56, 65, 66, 68, 77, 78, 81, 82, 85, 89, 92*	19	*C, H, L,* T_3	4
5. 22	*83, 88, 97*	3	—	—
5. 22	*51, 80*	2	—	—
22	—	—	*N, O, S,* M_A, T_1	5
A	—	—	*A, F, G*	3
I	—	—	*I*	1
	Total number of strains tested	98		27

* Numbers in heavy type signify close relationship to the particular strain; small type, a lesser degree of relationship.

given previously by serum *23*. Certain differences, however, were noted. For example, strain *19* failed to react with serum *4* though it absorbed the agglutinin from serum *23*.

Apparent variations in agglutinin-absorption reactions have been observed: these have been specially pronounced with serum *5*. All the strains we have classified in Group II completely and consistently absorbed the agglutinins from this serum and similarly absorbed the agglutinins from serum *35*. As noted above, the great majority of the strains investigated among the various other groups also exhibited some degree of absorption reaction with serum *5* but not with serum *35*. The reaction with serum *5* showed considerable variability, however, on repeated testing. For example, when strain *41* was first tested with serum *5*, the absorption of agglutinin was marked though not

complete, but on the next occasion only a trace of agglutinin was removed. On testing a third time marked absorption was again noted. On a fourth occasion the absorption of agglutinin was almost complete. On testing a fifth time absorption was marked. This type of result has been tabulated as "partial and variable," and has been specially characteristic of the reactions with this particular serum. Such results might be interpreted as due to alterations in the antigenic structure of the strain whose absorptive power was tested, or to variability in the agglutinogenic composition of strain *5* when used to test the removal of the specific agglutinin from the absorbed serum. On one occasion it was observed that the absorption reaction between strain *5* and its own serum was practically negative. In this case the growths used for the absorption test and the subsequent agglutination reaction were not identical. This suggested some variation in the agglutinogenic characters of strain *5*, and it seems unlikely that the variable reactions referred to above were dependent on lability of all the strains with which such results were obtained on repeated testing with serum *5*. These variations also seemed greater than those attributable to any slight variations in technique, and form a marked contrast to the clear-cut results obtained with serum *35* and other sera.

Similar variations have been noted with strains *23* and *90*. Thus, on first testing, strain *23* apparently absorbed but little agglutinin from serum *90*. On re-testing after an interval of a few weeks strain *23* almost completely absorbed the specific agglutinin from serum *90*. A few days later the strain again failed to remove agglutinin from the serum. This variation in the absorption reaction between strain *23* and serum *90* has been noted over a large series of repeated tests. While such variability has been encountered in certain instances, the majority of the strains have apparently undergone little alteration in their antigenic constitution and tests after an interval of a year or more have given the same result as that obtained on first testing. It seems reasonable to assume that some strains may be variable in a part, at least, of their antigenic structure.

Though only a few of the strains were agglutinated by serum *22* nearly every strain in our series completely absorbed the agglutinin from that serum. It was thought possible that the culture might consist of a mixture of "specific" and "non-specific" colonies such as has been described by Andrewes (1922) in the Salmonella group. The culture was therefore plated out and subcultures made from three different colonies and labelled 22_A, 22_B and 22_C. Antisera were prepared from each of these and cross-absorption and agglutination tests performed with them and with serum *22*. It was found that cultures 22_A, 22_B and 22_C were serologically identical and also absorbed agglutinin from the original serum *22*. Tests with various other strains, however, showed that their sera were "specific" as contrasted with the "non-specific" generalised reactions of the original serum *22*. If "non-specific" colonies were present in the culture our selection failed to demonstrate them. A considerable interval

had elapsed from the time of preparing the original *22* serum and antigenic variation of the strain, *e.g.* loss of its non-specific constituents, may have accounted for these results.

Serum *66* also exhibited generalised reactions, though not to the same extent as serum *22*.

It is of special interest that strains *5* and *22*, from whose sera agglutinin was absorbed by the majority of the strains examined, did not react appreciably with the other group sera. On the other hand, certain strains, *e.g. 93*, possessed the property of absorbing agglutinin from the majority of the sera available. Such results might be interpreted according to the predominance or otherwise of particular antigenic constituents in the strains. It must be noted that the technique of the absorption experiments was arranged so that the serum could be treated with a considerable excess of bacterial antigen; an antigenic constituent not necessarily predominant in the organism might, under the conditions of the test, be present in sufficient amount to absorb completely the corresponding agglutinin from the serum. For example, strain *1*, containing even as a minor component the antigenic constituent characteristic of strain *22*, might thus absorb the agglutinin from serum *22* while strain *22* would only absorb from serum *1* a minor agglutinin and not exhibit a demonstrable absorption effect.

Strains from Sources other than Scarlatina: their relationship to Scarlatina Streptococci with reference to Serological Characters and Toxigenic Properties.

The agglutinating sera prepared from scarlatina strains have also been used to investigate a number of haemolytic streptococci obtained from sources other than scarlatina. Table II shows that the majority of these, like the scarlatina strains, cannot be classified into well-defined serological types. The strains have been grouped, however, on a similar basis to that adopted in the case of the scarlatina streptococci and among them representatives of the scarlatina groups may be recognised. The majority resembled the scarlatina strains in absorbing agglutinin from sera *5* and *22*. Strains *A*, *F* and *G* form a very well delimited serological group, and strain *I* likewise showed marked serological individuality.

Many streptococci from non-scarlatinal sources apparently possess an antigenic structure indistinguishable from that of scarlatina strains. Thus, strains *M*, *Q* and *Z* are closely related to *35* (and *5*) and can be classified in Group II. It may be noted, however, that these strains differ from scarlatina strains in their toxigenic properties.

The results of intradermal tests with culture filtrates of all the strains we have examined, scarlatinal and non-scarlatinal, have already been reported (McLachlan, 1927), but for convenience the results obtained may be briefly restated. All the scarlatina strains tested, with three exceptions, yielded a toxin, which, in a dilution of 1 in 1000 gave a positive skin reaction in known positive subjects and a negative result in known negative reactors. The

three exceptional strains (Nos. *68*, *71* and *89*) yielded toxic preparations which were quantitatively less active. The results obtained with preparations from the non-scarlatina strains are included in Table II, and it will be seen that while some strains formed "toxins" resembling those obtained from scarlatina streptococci, others failed to produce a "toxin" demonstrable in the highest concentration tested (1 in 100); others, again, yielded preparations which gave irregular results, evoking reactions in both "Dick-positive" and "Dick-negative" individuals. Scarlatina streptococci, therefore, differed from the other strains in their marked and uniform toxigenic properties.

Of the three non-scarlatina strains placed in Group II, only one, *Z*, produced a toxin similar and quantitatively comparable to that formed by scarlatina streptococci, and it is of interest to note that this organism was obtained from the throat. Streptococcus *M*, isolated from a case of septicaemia, produced a very active toxin which gave rise to marked cutaneous reactions in both "Dick-positive" and "Dick-negative" subjects, while *Q*, from a case of purulent conjunctivitis, failed to produce a toxin demonstrable even in a dilution of 1 in 100. Thus, though these three organisms are related serologically to a group of scarlatina strains with a well-marked serological stamp, they differ widely in their toxigenic properties from each other and from the scarlatina strains.

Strain *Y*, from a case of empyema, is related to strain *1*, but also to *23* and *35*. It differs to some extent from the scarlatina strains classified in Group I. Toxin preparations from this strain induced positive skin reactions in known "Dick-positive" and "Dick-negative" persons.

Four strains, *B*, *D*, *E* and *T*, are related in their antigenic structure to No. *90* and may be classified in Group III. Of these four strains *D* and *T* formed toxins which evoked a cutaneous reaction when tested in dilutions of 1 in 100 and 1 in 250 respectively. In these dilutions the toxins behaved like preparations from scarlatina strains. Filtrates from cultures of *B* and *E* did not produce a cutaneous reaction when tested in a dilution of 1 in 100.

Strains *K*, *P*, *R*, *U*, T_2 and T_5 are related serologically to No. *23* and may be classified in Group IV, but other constituents are present in their antigenic structure. *K*, *R* and *U* yielded toxic preparations similar to those formed by scarlatina strains, while toxic filtrates, qualitatively similar but quantitatively weaker, were obtained from *P* and T_2. The toxin produced by T_5 acted only in low dilutions and sometimes gave a positive reaction in known negative subjects.

A considerable proportion of the non-scarlatina strains corresponded to the scarlatina streptococci included in Group V, reacting only with *22* or with *22* and *5* sera.

Certain non-scarlatina strains, *e.g.* *A*, *F*, *G*, *I*, showed complete absence of relationship to the scarlatina strains for which antisera were available. Immune sera were prepared from *A* and *I*. It was found that *F* and *G* belonged to the same serological group as *A*, and these three organisms formed a group which was sharply demarcated from all the other streptococci studied. Culture filtrates did not evoke a cutaneous reaction even when tested in a dilution of

1 in 100. Strain *I* also proved to be distinct serologically from the other streptococci examined. Toxin preparations were inert in the dilutions employed for the intradermal tests. These four only among 30 strains from sources other than scarlatina could be clearly differentiated by serological methods from the scarlatina streptococci.

The results recorded above show that there is no correspondence between toxigenic properties and serological characteristics. Eagles (1926) has arrived at a similar conclusion.

In this connection attention may be drawn to the observation by Smith (1927) that a standard strain of *Streptococcus scarlatinae* obtained from Dochez and noted for its toxigenic properties was quite distinct serologically from all the other streptococci examined, both scarlatinal and non-scarlatinal.

A more complete investigation of the serological characteristics of the whole class of haemolytic streptococci has not been attempted and no general statement can be made regarding the serological classification of these organisms. Further antisera were not prepared from non-scarlatina strains as this study was more immediately concerned with the serology of the scarlatina streptococci. The results show that the scarlatina streptococci may not differ serologically from many—possibly the majority—of the haemolytic streptococci occurring in other pathological conditions. There may be, however, serological groups of haemolytic streptococci devoid of aetiological relationship to scarlatina and also sharply demarcated serologically from the scarlatina streptococci.

The distribution of the strains among the different serological groups is shown in Tables I and IV. In all, 101 scarlatina strains were examined, and 77 were included in the provisional Groups I–IV, 55 of these strains being comprised in Groups I and II. The remaining 24 strains have been classified in Group V, but are probably heterogeneous. Of 30 non-scarlatina strains investigated, only one (*Y*) could be classified with Group I but differed in certain respects from all other Group I strains; three others belonged to Group II. The non-scarlatina strains which could be assigned to Groups III and IV were more numerous, four and six strains respectively being placed in these groups. Group *A* comprised three strains only and did not include any scarlatina strains among its members and *I* was the only representative of its type.

It will be noted that strains with certain serological features are obtained more frequently from scarlatina than from other conditions. While haemolytic streptococci belonging to Groups I and II have been isolated much more frequently from scarlatina than from other sources, organisms belonging to these two serological groups are by no means restricted to scarlatina. Haemolytic streptococci belonging to Groups III and IV have been obtained from non-scarlatinal sources in as large a percentage of cases as from scarlatina. The number, however, of non-scarlatina strains investigated has been relatively small and several of the strains placed in these two groups have been obtained

from the throat or from infections traceable to the throat. In Table IV are shown the numbers of scarlatina and non-scarlatina strains respectively assigned to different serological categories. This table indicates the distribution of strains showing a particular serological stamp in the two aetiological classes. It is obvious that the recognition of serological groups among the scarlatina streptococci is of little aid in determining the source of origin or the pathogenic properties of an individual strain.

Direct Agglutination.

Owing to the difficulty of obtaining uniform and stable emulsions suitable for direct agglutination tests, it has not been practicable to carry out such tests according to any complete system, and the aim of the investigation was to study serological relationships in terms of agglutinin-absorption reactions. A considerable number of direct agglutination tests have been carried out, and considered along with absorption effects they provide interesting data. With certain sera, *e.g. 1* and *35*, parallelism between the reactions was fairly well marked; in other cases divergence was obvious. Even in the case of serum *1*, lack of correspondence was noticed. For example, strain No. *54* was agglutinated to a titre of only 1 in 200 by the immune serum to strain *1* (end-titre 1 in 3200) but completely absorbed the agglutinin from that serum. Strain *60* exhibited no direct agglutination reaction though it completely fixed the agglutinin. Strain *58* was agglutinated in a dilution of 1 in 1600 but did not react with serum *1* in the agglutinin-absorption test. Strain *67* was not agglutinated directly by the antiserum to strain *90* but completely absorbed agglutinin from it. The results of direct agglutination tests do not necessarily indicate the absorptive capacity of a strain, and in general, direct agglutination tests have yielded less information than absorption tests in the determination of the principal serological relationships of a strain.

No evidence of the group agglutination described by other workers (Stevens and Dochez, 1926; Eagles, 1924, 1926) have been elicited with the sera we have employed.

Discussion.

The existence of serological types among haemolytic streptococci had been described by Dochez, Avery and Lancefield (1919), and in their investigation strains from scarlatina were assigned to one serological group. The results obtained in recent years by other workers seemed at first to support this conclusion. In particular, the work of Stevens and Dochez (1924, 1926) indicated that most strains derived from cases of scarlatina resembled each other in part at least of their antigenic constitution and could be identified as a result of this similarity. A group agglutinogen could be demonstrated by either direct agglutination or absorption tests, but was not entirely restricted to scarlatina strains. The system of agglutination tests which they employed was rendered possible by the fact that the majority of their strains were

agglutinated (in varying degrees) by a serum prepared from any one scarlatina strain, and saturation of the serum with any strain removed the group agglutinin for other scarlatina streptococci, though, as a rule, without effect on the specific agglutinin. In 1924, they noted that 26 out of 35 strains, *i.e.* 74·3 per cent., were uniformly agglutinated by immune sera prepared from different scarlatina streptococci. In a study of scarlatina, erysipelas and pyogenic strains (1926) they found that 79 per cent. of scarlatina streptococci were agglutinated by a serum prepared from one scarlatina strain. Immune sera prepared from erysipelas strains agglutinated some scarlatina strains, but the strains agglutinated by an individual serum were not the same as those agglutinated by other sera.

Bliss (1920) found that 20 out of 25 scarlatina strains were agglutinated by sera prepared from four different scarlatina streptococci, the same strains being agglutinated in each case.

In our experience, a minority only of scarlatina streptococci have been agglutinated by antisera for individual strains, and no group agglutinin, as described by some workers, has been noted. The majority of our strains failed to agglutinate with any particular scarlatina serum even in dilutions as low as 1 in 50 when the titre of the serum was 1 in 6400 for the strain from which it was prepared. Evidence of the existence of common or generalised agglutinogenic constituents has been elicited, but they are in no way group characters of the scarlatina strains and can be recognised in our series among the majority of other haemolytic streptococci.

Smith (1926, 1927) and Griffith (1926, 1927) have described a number of serological "types" among strains obtained from scarlatina, and at an early stage of this investigation it seemed possible that the majority of scarlatina streptococci might be included within certain serological groups. As the work progressed, however, it became evident that there was considerable antigenic overlap between strains assigned to these different groups and such classification seemed somewhat artificial. Though two strains might appear to be entirely different or apparently identical as the result of agglutination and agglutinin-absorption tests with one serum, yet when another serum was used the strains would be found to present a similarity or difference, as the case might be, in part of their antigenic structure.

Strains in the first four groups of Table I might be held to constitute different serological types, but, save possibly in the case of those placed in the second group, there are so many inter-relationships that such an arrangement is hardly justifiable. This is illustrated in the tabulated results. Even strains that have apparently been proved identical as the result of cross-absorption tests may differ in their relationship to other strains. This has also been pointed out by Torrey and Buckell (1922) with regard to gonococcus strains.

In their serological characteristics the haemolytic streptococci obtained from cases of scarlatina seem analogous to gonococcal strains, among which,

as a whole, individual characteristics are more marked than group resemblances. From their study of gonococcus strains, Torrey and Buckell (1922) concluded that these might be classified into three groups. In the first group they placed strains which were closely related but not identical in antigenic constitution. Certain of the strains in this group were highly generalised or representative. In the second group were placed strains whose antigenic components were more specialised but certain strains in this group showed a close relationship to certain members of the first group. Strains included in the third group were more definitely separated from the first group and showed marked individual characteristics.

From their studies on the serology of haemolytic streptococci Stevens and Dochez (1926) concluded that the antigenic composition of streptococci was probably extremely complex, and consisted of an "antigenic mosaic" built up of a considerable number of fractions, certain constituents possibly being common to streptococci from widely different sources, while other constituents occurred chiefly in strains associated with particular pathological conditions. From the results of agglutination and absorption tests devised to demonstrate the presence of group agglutinins, they inferred that the majority of scarlatina strains probably contained certain antigenic fractions characteristic of this group which would serve to distinguish scarlatina strains from streptococci occurring in other conditions. They indicated, however, that such serological stamp is still insufficient to identify scarlatina strains. As Stevens and Dochez's own protocols show, strains from other sources may contain the antigenic fractions which they have postulated as characteristic of, though not entirely restricted to, scarlatina strains. The tests which we have carried out support the conclusion that the antigenic structure of streptococcal strains is exceedingly complex, but we have found no evidence that scarlatina streptococci possess fractions in their antigenic constitution by which they may be identified. It is noteworthy, however, from our results that certain of the serological categories we have been able to recognise by the antisera used, are represented only among the scarlatina organisms (Table IV) and if strain *Y* were excluded from Group I in virtue of its exceptional serological characters, this group might be regarded as a specific scarlatina group unrepresented among haemolytic streptococci from other sources. It is of special interest that 31 per cent. of our scarlatina strains belong to this group. On the other hand, the characteristic antigenic constituent represented by strain *1* is recognisable also in a non-scarlatina strain (*Y*) from a case of empyema, and Griffith has isolated two organisms which he classified as "Type I" from puerperal fever and from meningitis respectively. Smith (1927) has also obtained strains belonging to this type from non-scarlatinal conditions, viz. erysipelas and puerperal fever; he has occasionally isolated similar strains from the throat of healthy persons and from infections traceable to the throat. Further, other serological categories which each comprise a definite proportion of scarlatina streptococci are represented also by multiple

non-scarlatina strains in our series. Just as Group I appears to be related specially to scarlatina, certain serological types (*e.g.* *A*) are unrepresented among the scarlatina streptococci.

In some respects our observations agree with those recently recorded by Smith (1927). Thus, among the ten serological types described by him, strains belonging to Types I and II were found to be associated much more frequently with scarlatina than with other conditions. Further, his Type X strains possessed, apparently as a major constituent, an antigenic fraction present to a varying extent in the majority of scarlatina and non-scarlatina strains. Type X antisera agglutinated to end-titre the majority of strains representative of the other types; strains belonging to Types I and V, however, reacted only in lower dilutions. In agglutinin-absorption tests the titre of Type X sera was considerably reduced, and in some cases the agglutinins were completely removed, by organisms of different serological type. A Type V strain, however, the only representative of its type, did not react with Type X sera in this test. The organisms which he has classified as Type X would thus present some analogy to Nos. *22* and *66* in our series, though they are evidently dissimilar since antisera for strains *22* and *66* agglutinated only a few of our organisms even in low dilutions while the majority of the streptococci tested completely absorbed agglutinin from sera *22* and *66*.

As the result of a full investigation of a limited number of strains representing Types I and II, and of all other strains not included in these two types Smith has described serological types of haemolytic streptococci which, with the exception of Type X are sharply demarcated from one another and can be readily recognised by agglutination or agglutinin-absorption tests. Certain of these types, however, are represented by only one strain. Our results also indicate that there may be a number of small, well-defined serological groups and serologically individualistic strains (exemplified by Nos. *A*, *42*, *65* and *I*). In a more detailed analysis of the serological constitution of individual strains within the main groups we have found that there is a considerable amount of antigenic overlap between strains in different groups and that there may even be differences between strains placed in the same group. A system of analysis of the antigenic composition of the strains which we have studied is illustrated in Table IV.

It is of interest to note that the distribution of scarlatina streptococci into serological types or groups as elicited by Smith, Griffith and ourselves varies in different parts of the country. Thus, 57 per cent. of strains isolated in Aberdeen were placed in Type I, while 27 per cent. belonged to Type II. Griffith, on the other hand, working with strains of which about two-thirds were obtained from Manchester and Liverpool, and one-third from London, found that 15 per cent. and 17 per cent. belonged to Types I and II respectively. When investigating organisms obtained from cases in London 4·5 per cent. of strains were attributable to Type I and 25·6 per cent. to Type II. We have found that 31 per cent. of our strains could be assigned to Type I and 22 per

cent. to Type II. A further difference in the geographical incidence of strains of a particular serological stamp is brought out by Smith, who found that a standard American strain of "*Streptococcus scarlatinae*" isolated by Dochez differed serologically from all the local strains which he examined. (See Addenda (2), p. 247.)

Signs of antigenic lability have been noted in certain strains during the course of the investigation and variations in agglutinogenic constitution appear to have occurred with remarkable rapidity, though the conditions of culture and testing have been kept constant as far as possible. Antigenic lability has been described as occurring in certain organisms, *e.g.* meningococcus, gonococcus. Torrey and Buckell (1922) noted that different type-strains of gonococcus after cultivation for 14 years had tended to revert to one regular group, and in general, where antigenic variations have been observed, they have developed gradually. The existence of antigenic lability in a group of organisms is an additional argument against the formulation of sharply defined serological types, and though serological groupings can be effected among the scarlatina strains, it is doubtful whether the acceptance of distinct serological types is warranted. Though strains of a particular serological stamp may be specially related to scarlatina, these may comprise only a minority of scarlatina streptococci and no definite serological demarcation exists between this group and other haemolytic streptococci.

Summary and Conclusions.

1. A minority only of scarlatina streptococci are agglutinated by antisera for individual strains and no group agglutination has been observed.

2. Absorption tests reveal the existence of common or generalised agglutinogenic constituents but these are not restricted to scarlatina strains and can be recognised among the majority of the haemolytic streptococci isolated from various sources.

3. Though certain serological groups among scarlatina streptococci may be recognised as corresponding generally to "types" described by Smith and Griffith, there is such considerable antigenic overlap among the different groups that the differentiation and relationship of strains cannot be expressed satisfactorily by formulating definite serological types.

4. A system of serological analysis of scarlatina and other haemolytic streptococci is suggested (see Table IV).

5. While strains presenting particular serological characters may be associated more frequently with scarlatina, haemolytic streptococci with common serological characters occur both in scarlatina and other conditions. No essential serological distinction can be drawn between scarlatinal and other haemolytic streptococci and a "*Streptococcus scarlatinae*" group cannot be defined by serological methods.

6. Among the haemolytic streptococci derived from conditions other than scarlatina no relationship exists between serological characteristics and

toxigenic properties (as evidenced by cutaneous reactions to culture filtrates).

We have to thank Drs J. Smith and F. Griffith for cultures of the type strains referred to in the text.

ADDENDA.

(1) In a further recent investigation of scarlatina streptococci Williams, Gurley and others (1927) have recognised four serological types among 65 strains isolated from cases in New York City. Three of these strains could be assigned to Type I, and 32 strains were placed in a sub-group of Type I. This sub-group is identical with Griffith's Type III, which is not regarded by Williams as a separate type. None of the strains examined could be classified in Type II (Type-strains obtained from Griffith were used for comparison). Williams has recognised two other types, 7 strains being placed in Type III and 14 strains in Type IV. 10 out of 15 erysipelas strains investigated could also be classified in Type IV. The 9 remaining scarlatina strains were unclassified. A certain relationship between serological characters and carbohydrate reactions was noted.

(2) The authors cited under (1) found that only 3 strains were attributable to Type I. No representatives of Type II were met with; 32 of the strains, however, could be assigned to Type III (Griffith). *Vide supra.*

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