THE SEROLOGICAL CLASSIFICATION OF HAEMOLYTIC STREPTOCOCCI OBTAINED FROM CASES OF SCARLET FEVER.

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The serological classification of haemolytic streptococci has become increasingly important in recent years, and it has become especially desirable to investigate completely the strains of streptococci obtainable from cases of scarlet fever. Studies of this nature might lead to a simple method of identifying this particular organism, and might help to solve certain epidemiological problems associated with this infection. On the other hand, many pathogenic bacteria are found on the mucous membranes of normal individuals and it has been demonstrated in the case of *C. diphtheriae* that, at least, two main factors must operate before a human being can develop diphtheria. In the first place the organism must be toxigenic and secondly the human being must be susceptible. The serological classification of strains of *C. diphtheriae* has not proved of value as a means of identifying pathogenic types of this organism.

Summary of Literature.

The serological classification of strains of haemolytic streptococci obtained from cases of scarlet fever was attempted first by Moser (1902) and later by Moser and von Pirquet (1903). They prepared agglutinating sera by inoculating horses with strains of *Streptococcus scarlatinae* and found that strains obtained from scarlet fever cases were agglutinated specifically, whereas haemolytic streptococci from other sources were not. On the other hand, Aronson (1903) and Neufeld (1903) were unable to find any definite relationship between the streptococci of scarlet fever and were of opinion that it was not possible to differentiate between types of haemolytic streptococci by means of the agglutination reaction.

Weaver (1904) from extensive studies of streptococci cultivated from cases of scarlet fever concluded that some were agglutinated by sera obtained from patients in dilutions of 1 in 160 to 1 in 4000, while other strains were agglutinated in a lower dilution. He found also that the agglutinative reaction between streptococci cultivated from cases of scarlatina and the sera obtained from these cases was in no way specific and could not be of any value as a means of diagnosis.

Rossiwall and Schick (1905) confirmed in general the work of Moser and von Pirquet.

https://doi.org/10.1017/S0022172400017307 Published online by Cambridge University Press

Ruediger (1906) reported agglutination tests with the serum of a sheep that he had immunised by intravenous injection of a streptococus of scarlet fever origin. He found that the serum agglutinated most of the streptococci isolated from scarlet fever cases.

Nakayma (1919) found that the sera of rabbits immunised with haemolytic streptococci agglutinated the homologous strains readily but the heterologous strains less readily. He found considerable similarity amongst the strains obtained by scarlet fever cases. Tunnicliffe (1920) and Dochez and Bliss (1920) demonstrated that the type of haemolytic streptococcus found in the throats of scarlet fever patients is in general a specific type readily distinguishable from the types of *S. haemolyticus* causing angina and septic conditions. Bliss (1920) found that of 25 strains from the throats of patients suffering from scarlet fever 20 were agglutinated at equal titres by a serum prepared by immunising a rabbit with an individual strain. No agglutination was obtained when sera prepared against strains from other sources were tested against the 20 strains.

Gordon (1921) found that 18 strains of haemolytic streptococci isolated from scarlatinal cases were identical in their agglutination and absorption reactions. None of these strains absorbed the agglutinins from immune sera prepared from certain other types of haemolytic streptococci. On the basis of this evidence Gordon concluded that the streptococci from the throat secretions in scarlet fever constituted a single serological group distinct from other varieties of *Streptococcus pyogenes*.

Tunnicliffe (1922) confirmed and extended her previous work. She found that 72 strains from 75 cases of scarlet fever behaved in a similar manner when tested by the agglutination and absorption reactions. Bliss (1922) studied a second series of strains obtained from scarlet fever cases and found that 20 out of 24 strains were agglutinated specifically by anti-scarlatinal sera. In spite of the technical difficulties involved in preparing absorption test with streptococci he found that the absorption test confirmed the agglutination reaction and concluded that haemolytic streptococci from scarlet fever constitute a specific type which can be separated by the agglutination and absorption tests from haemolytic streptococci from other sources.

Stevens and Dochez (1924, *a*, *b*, *c*), examined the throats of 40 acute cases of scarlet fever and obtained haemolytic streptococci in 87 per cent. of cases. Of these strains 74 per cent. were agglutinated by immune sera prepared from a scarlatinal strain. They repeated the examination of the cases 30 days after the onset of illness and found *S. haemolyticus* in 47 per cent. This time, however, only 55 per cent. of the strains could be agglutinated. Absorption tests were carried out with fresh immune sera and strongly agglutinable strains with the result that they concluded that the strains of haemolytic streptococci associated with scarlet fever are closely related biologically and probably constitute a single unit group. They examined further seven strains *of streptococci obtained from suppurative processes in the course of the*

disease and only one strain could be agglutinated by the scarlatinal serum. As a result of these observations they expressed the opinion that these non-agglutinating strains occurring in cases from which scarlatinal strains can be isolated represented secondarily invading streptococci, and that the complications in scarlet fever may be caused either by the original scarlatinal strain or by pyogenic strains.

Eagles (1924) examined 19 strains of *S. scarlatinae* and found that they could be all placed in one group by the agglutination test.

Dick and Dick (1924) found no cross agglutination between the two strains of haemolytic streptococci with which they obtained scarlet fever in human beings. While these two strains differed culturally in their effect on mannite and were not agglutinated by the same immune serum, they had in common the property of producing the specific toxin of scarlet fever. They concluded that the agglutination test could not be relied on for the identification of scarlet fever streptococci.

Williams (1925) found that out of 70 strains tested, only about 30 per cent. fell into one group; 10 per cent. consisted of two small groups, one of three strains, and one of two strains; 63 per cent. were not classified serologically. From sources other than scarlet fever she found that 8 per cent. of the strains were agglutinated by the Type I scarlatinal serum, and she concluded that the agglutination test is not of much practical worth in the diagnosis of scarlet fever or in the identification of carriers.

RECENT WORK ON TECHNICAL PROCEDURES ASSOCIATED WITH THE STUDY OF STREPTOCOCCI BY MEANS OF THE AGGLUTINATION TEST.

There is little doubt but that the serological classification has been hampered by the want of a suitable technique. The very frequent occurrence of spontaneous agglutination and the fact that emulsions of cultures of these organisms are often extremely granular has made it difficult to distinguish between false and specific agglutination. It is therefore necessary to summarise briefly the results obtained by certain American workers who have devised methods for avoiding these difficulties.

Northrop (1921, 1922), Northrop and De Kruif (1921, *a*, *b*; 1922, *a*, *b*; 1923) and De Kruif (1921, *a*, *b*), in a series of papers on the stability of bacterial suspensions have added much to the knowledge concerning the factors which govern bacterial agglutination.

These workers consider that two forces play their parts in bacterial agglutination, a repelling force (the electrical charge on the bacteria) which keeps bacteria apart, and an attracting or cohesive force which is a function of surface tension. In experiments with auto-agglutinable bacteria suspended in glycine sodium acetate buffer solution they have shown that agglutination occurs whenever the difference in electric potential between the surface of the organism and the surrounding fluid falls below + or − 15 millivolts. All

factors affecting the cohesive force must be excluded however. They confirmed the work of others that the electric charge on organisms is negative when they are grown in an alkaline medium and positive when grown in acid medium. Between the two extremes the charge on the bacteria is low and spontaneous agglutination is liable to occur. Further experiments showed that electrolytes in a concentration of less than 0·01 *N* to 0·1 *N* affect primarily the potential while in concentration greater than 0·1 *N* the effect is to reduce the cohesive force. When the cohesive force is decreased the critical potential is also decreased and when the cohesive force is made very small no agglutination occurs even although the potential is reduced to zero.

The presence of protein or serum alters the results already described. The addition of protein to a bacterial suspension widens the acid agglutination zone and shifts the isoelectric point to that of the added substance. When immune serum was added to a suspension of organisms which did not agglutinate spontaneously it was shown by indirect experiment that a film was formed on the surface of the bacteria. Salt solution in concentration above 0·1 *N* did not then affect the cohesive force and agglutination was produced by the film of immune serum reducing the potential to 15 millivolts. They found that only 12 agglutinating doses of the immune serum could combine with the suspension.

Further they found that electrolytes could be divided into two classes by their action on sensitised bacteria. The salts of the heavy metals and hydrogen ion behaved as though they combined chemically with the bacteria suspension and the amount present in solution had to be increased with any increase in the density of the suspension in order to allow agglutination to occur. The second class of electrolytes consisted of the alkalis and alkali earths which do not form complex compounds with proteins. With those the concentration required to produce agglutination in the presence of immune serum is independent of the concentration of the suspension.

From the foregoing findings they concluded that a bacterial suspension could be made more stable by (1) the addition of alkali or acid in such a way as to bring the suspension to a *p*H far from the isoelectric point, (2) by the addition of substances which affect the critical potential. The stability could be decreased by (1) the addition of alkali or acid so as to approach the isoelectric point, and (2) the addition of any ion of the opposite charge to that of the organism. In order to prepare a stable suspension of a strain of streptococcus the organisms were grown in 10 per cent. rabbit serum broth at 37° C. for 16 hours. The culture was centrifuged and the deposit was repeatedly washed in distilled water. The organisms were then suspended in sufficient 0·001 NaOH (*p*H 7·0) to give the required density. The immune serum was diluted with *M*/320 NaCl in water and an equal quantity of various dilutions added to the bacterial suspension. It was found that specific agglutination could then be obtained without any sign of spontaneous agglutination in the control tube containing the salt solution and the bacterial suspension.

Mellon, Hastings, and Anastasia (1924, *a*, *b*, *c*) carried out further experiments on the spontaneous agglutination of bacteria with special reference to interfacial surface tension. They suggested that suspensions of auto-agglutinable diphtheria bacilli could be made stable by first preparing the suspension in a 2·5 per cent. solution of sodium chloride and then diluting with two volumes of distilled water. They found that there was considerable evidence that the high cohesive power of certain bacteria, which is responsible for much of their spontaneous agglutinability, could be diminished by such a substance as sodium oleate. This substance acted by reducing the interfacial surface tension between the organism and its medium.

Shibley (1924, *a*, *b*) also studied the problem of spontaneous agglutination in streptococci and in general confirmed the work of Northrop and De Kruif. He concluded that factors which reduced the cohesive force or made the repelling force relatively greater than the cohesive force made for stable suspensions. He introduced the method of reducing the cohesive force by growing the streptococci in a large amount of bouillon at 23° C. for 18 hours. The density of the suspension thus obtained was increased by centrifuging and resuspending in fresh culture fluid.

METHODS.

As each acute case of scarlet fever was admitted to hospital a swab was obtained from the tonsillar and nasal secretions, while, if complications arose, various purulent materials were also obtained for culture. Scarlet fever, however, is a relatively mild disease at the present time and septic complications are infrequent.

In order to conserve the blood agar, the bottom of each petri dish was first covered with a layer of nutrient agar and after this had solidified a layer of agar to which 10 per cent. of defibrinated sheep's blood had been added was superimposed. As each swab was obtained it was emulsified in broth and a drop of the emulsion spread over a blood agar plate. If after incubation haemolytic colonies were obtained, three such colonies were selected and replated. From this second plating colonies were then picked into broth tubes containing 5 per cent. sheep's blood. These were incubated overnight, the morphological characteristics of the strains were studied and two strains (from each specimen) were then retained for further cultural and serological tests. The blood broth cultures were stored in the refrigerator at − 2° C. so as to obviate the necessity for repeated subculturing.

If the haemolytic activity of a strain was doubtful the haemolysin production was estimated by the titration method advocated by De Kruif and Ireland (1920). That is, a subculture of the strain under examination was made into broth and incubated overnight; one cubic centimetre of this broth culture was added to a tube containing 25 c.c. of 20 per cent. horse serum in broth. This was incubated for eight hours and the culture was then centrifuged at high speed. Half a cubic centimetre of various dilutions of the

supernatant fluid was placed in small test-tubes and an equal volume of 2 per cent. washed sheep's red blood cells added. The tubes were then incubated in the water bath at 37° C. for one hour and the haemolytic titre of a strain was thus determined. A control tube containing inoculated medium, salt solution, and red blood cells was included in each series.

The biochemical tests included the determination of bile solubility and the fermentation reactions of the various strains on lactose, salicin, mannite, inulin, raffinose, melezitose and sorbite. One per cent. of the various sugars was incorporated in sugar-free broth along with brom cresol purple as an indicator. The fermentation reactions were read at the end of 24 hours and again after seven days.

To prepare agglutinating sera rabbits were immunised by giving intravenous injections of a washed suspension of streptococci which had been grown on 10 per cent. serum broth. Their sera were repeatedly tested and immunisation was stopped when a titre of 1 in 1600 to 1 in 3200 was obtained against the homologous strain.

For the agglutination test the method of De Kruif (1922) with slight modification was employed. Cultures were grown overnight in flasks containing 50 c.c. of 10 per cent. horse serum beef broth. No salt was added to the medium and the final hydrogen ion concentration was *p*H 7·6. The cultures thus obtained were centrifuged and the resulting deposit was washed twice with distilled water. From the washed deposit a suspension of the cocci was made in 0·001 *N* sodium hydroxide so that the density corresponded to a standard tube containing 500 millions cocci per cubic centimetre. The immune serum was diluted with *M*/25 sodium chloride solution and 0·4 c.c. of various dilutions was placed in the agglutination tubes and an equal volume of the coccal emulsion was added. A control tube containing a volume of *M*/25 sodium chloride solution and a volume of the coccal emulsion was included in each test. Each series of agglutination tests was also controlled by testing the agglutinating serum against the homologous strain. The tubes were incubated in the water bath for two hours at 55° C.

The absorption tests were carried out by suspending the washed deposit from a 50 c.c. serum broth culture in 1·2 c.c. *M*/25 sodium chloride solution, and 0·98 c.c. of this suspension was then added to a tube containing 0·02 c.c. of immune serum. As a control, the immune serum was absorbed by the homologous strain each time a series of these tests was carried out. The absorption tests were incubated in the water bath at 55° C. for two hours, and the tubes were then centrifuged. One volume (0·4 c.c.) of various dilutions ranging from 1 in 50 to 1 in 800 of the absorbed serum was then placed in the agglutination tubes and 0·4 c.c. of a suspension of the homologous strain in 0·001 *N* NaOH was added. This second series of agglutination tubes was also incubated in the water bath at 53° C. By using this technique spontaneous agglutination is practically eliminated and only two strains have been encountered which gave persistent non-specific results. Repeat absorp-

tion tests had to be carried out not infrequently, and it was on account of the supposed variable antigenic character of the haemolytic streptococci that in practically every instance two strains from each source were employed.

RESULTS.

A complete serological classification of all strains from cases of scarlet fever and other sources has not yet been accomplished but the results at present available are of considerable interest.

From 247 cases of scarlet fever 209 strains of haemolytic streptococci were obtained on culturing the tonsillar secretions, while another strain was obtained from the pus in a case of surgical scarlatina. Table I shows the number of cases admitted on various days of the disease in relation to the presence of haemolytic streptococci in the throat cultures.

Table I.

Number of cases showing Streptococcus haemolyticus *in throat cultures.*

Day of illness when admitted	1	2	3	4	5 and over
Total number	69	82	47	24	34
Number positive	63	77	36	15	18
Number negative	6	5	11	9	16
Per cent. positive	91	93	76	62	52

The titration method of estimating the amount of haemolysin produced gave remarkably constant results for all strains investigated. Provided the conditions governing the experiments were kept uniform, it was usual to find that after an eight-hour incubation period, 0·001 c.c. of the supernatant fluid from a serum broth culture would completely haemolyse the red blood cells and that 0·0005 c.c. would produce partial haemolysis. In general if the supernatant fluid was tested after a three-hour incubation period 0·5 c.c. would be required to cause haemolysis. From the three to the eight hour period of growth there occurred a rapid outpouring of haemolysin; the maximum having been reached, the haemotoxin quickly disappeared until at the 12 hour incubation period 0·1 c.c. was required. After incubating for 24 hours no haemolysin could be detected in many cultures. The estimation of haemolysin production does not distinguish between streptococci isolated from various sources.

The fermentation reactions of the haemolytic streptococci have shown very great similarity. According to the Holman classification (1916) the vast majority of the strains belong to the type *S. pyogenes* fermenting lactose and salicin. Eight strains from four sources produced acid in mannite (*S. infrequens*) in addition to acid in lactose and salicin. Eight strains from four sources gave acid in salicin only (*S. equi*), and two strains produced acid in raffinose as well as acid in salicin and lactose. None of the strains fermented inulin, sorbite and melezitose.

The serological classification by the agglutination and absorption tests showed that the strains obtained from the throat cultures of 209 cases of

scarlet fever could be divided into various groups. Type I strains were obtained from 118 cases, Type II strains from 57 cases and the strains from 34 cases have remained meantime unidentified. In addition the pus from one case of surgical scarlet fever, from which no haemolytic streptococci could be obtained from the throat culture, showed a Type I strain. Different types have not been encountered in the throat cultures from cases at the commencement of the illness even although two strains were tested from each source.

From nasal swabs from 210 cases, haemolytic streptococci were obtained 16 times. Ten cases showed Type I streptococcus, five cases Type II and in one case the strain was not classified. In every instance these strains corresponded to the type which had been found in the throat.

In addition to the case of surgical scarlet fever pus was obtained from ten cases during the course of disease and the relationship of these strains to those obtained from the throat and nose of the same case is shown in Table II.

Table II.

The serological relationship of strains obtained from septic complications to those obtained from the throat and nose cultures.

		Serological type isolated from		
Case No.	Septic complication	Throat	Nose	Pus
1	Mastoiditis	I	0	II
2	,,	I	0	I
3	,,	II	0	II
4	Otitis	I	0	I
5	,,	II	II	I
6	,,	II	0	II
7	Adenitis	II	0	II
8	Otitis	II	0	II
9	Adenitis	+	+	+
		All strains unidentified		
10	,,	I	0	I

It will thus be seen that the septic complications of scarlet fever can be caused by a type different from that found in the throat culture on admission to hospital. This is probably due to the fact that cross infection with various types of haemolytic streptococci must occur in a scarlet fever ward.

The strains found in the throat cultures from members of the same family suffering from scarlet fever have with one exception shown agreement in their serological classification, as seen in Table III. In family No. 3 the second case

Table III.

Serological classification of strains obtained from members of the same family.

Family No.	No. of cases in family	Serological type	Family No.	No. of cases in family	Serological type
1	2	II	6	2	Unidentified
2	3	II	7	3	I
3	2	I and II	8	2	I
4	3	I	9	2	I
5	2	I	10	2	I

https://doi.org/10.1017/S0022172400017307 Published online by Cambridge University Press

occurred after the first case had been discharged from hospital and it is probable that, although the first case on admission was found to harbour a Type I strain, the patient became a carrier of Type II streptococcus in hospital.

Further an outbreak of scarlet fever occurred in a ward in a children's hospital involving five patients and two nurses. All these cases showed a Type I streptococcus. In another ward three children developed scarlet fever and all showed Type II *S. scarlatinae* in cultures obtained from their throats. Throat and nose swabs from 20 nurses and patients from the latter outbreak showed that one nurse harboured a Type I strain, another a Type II strain and in a third the serological type was not identified; further three patients were found to give a Type II streptococcus, two strains being obtained from the throat and one from the nasal secretions.

Strains of haemolytic streptococci have also been obtained from 43 individuals not suffering from scarlet fever. Four strains obtained from cases of otitis, cervical abscess following tonsilitis, purulent rhinitis and from a normal throat belong to Type I; while Type II strains were obtained from two cases of tonsilitis, and from the respiratory passages of four individuals in a scarlet fever infected ward.

DISCUSSION.

The serological classification indicates that there are probably many serological types of haemolytic streptococci capable of causing scarlet fever. In certain cases it is possible, however, that on culture of the throat secretions the colonies of the causative organisms may be completely outnumbered by saprophytic or non-scarlatina producing types. Some workers have noted that a large number of inagglutinable strains appeared when throat cultures were obtained during convalescence, the inagglutinability being ascribed to a change in the antigenic character of the streptococcus. It would appear more reasonable to infer that the hospitalisation of cases of scarlet fever offers every opportunity for cross infection, with various serological types. It seems likely, moreover, that special types predominate in certain areas in epidemic times.

The fermentation reactions of the Type I and Type II strains have shown that all strains with one exception belonged to the *S. pyogenes* group of Holman. One Type I strain produced acid in mannite, as well as acid in lactose and salicin.

Agglutinating sera were prepared from three Type I strains and from three Type II strains and all sera showed that these two types were very well defined, there being only occasional cross agglutination in low dilution of the sera. Further, there was only slight cross agglutination with the various unclassified strains. In so far as Type I and Type II strains are concerned the agglutination test would indicate the group to which the strain belonged without an absorption test. Marked variation in the antigenic properties of the type strains has not been observed, but, on certain occasions, cultures were

encountered which gave relatively poor agglutination and incomplete absorption tests. A repeat test on the following day would then show that the strains had acquired greater antigenic properties.

SUMMARY.

Haemolytic streptococci have been obtained from throat cultures of 92 per cent. of cases of scarlet fever in the first two days of the disease.

Strains from 210 cases were found to belong to two main serological types, Type I strains being obtained from 119 cases, and Type II strains from 57 cases.

Strains obtained from cases occurring in members of the same family were found to be mainly of the same serological type.

Strains obtained from cases in small isolated outbreaks were found to be of the same serological type.

NOTE. These Type I and Type II strains have been examined by Dr Griffith, Ministry of Health, Pathological Laboratory, London, and have been found to conform to his main types.

REFERENCES.

ARONSON, H. (1903). Weitere Untersuchungen über Streptococcen. *Deutsch. med. Wchnschr.* XXIX. 439.

BLISS, W. P. (1920). A biologic study of haemolytic streptococci from the throats of patients suffering from scarlet fever. *Bull. Johns Hopkins Hosp.* XXI. 173.

—— (1922). Antigenic relationships between strains of *Streptococcus haemolyticus* isolated from scarlet fever. *J. Exp. Med.* XXXVI. 575.

DE KRUIF, P. H. (1921 *a*). Change of acid agglutination optimum as index of bacterial mutation. *J. Gen. Physiol.* IV. 387.

—— (1921 *b*). The mechanism of granular growth of the rabbit septicaemia bacillus type G. *Ibid.* IV. 395.

DE KRUIF, P. H. and IRELAND, P. M. (1920). Streptolysin. *J. Inf. Dis.* XXVI. 285.

DICK, G. F. and DICK, G. H. (1924). The prevention of scarlet fever. *J. Amer. Med. Assoc.* LXXXIII. 84.

DOCHEZ, A. R. and BLISS, W. P. (1920). Biologic study of haemolytic streptococci from throats of patients suffering from scarlet fever. *Ibid.* LXXIV. 1601.

EAGLES, G. H. (1924). The significance of serological groups of haemolytic streptococci with special reference to *Streptococcus scarlatinae*. *J. Exp. Path.* V. 199.

GORDON, M. H. (1921). A serological study of haemolytic streptococci. *Brit. Med. J.* I. 632.

HOLMAN, W. L. (1916). The classification of streptococci. *J. Med. Res.* XXXIV. 377.

MELLON, R. R., HASTINGS, W. S. and ANASTASIA, C. (1924 *a*). Rôle of surface tension and potential difference in the stability of a diphtheria-like bacillus. *Proc. Soc. Exp. Biol. and Med.* XXI. 279.

—— —— —— (1924 *b*). A paradoxical stability phenomenon with spontaneously agglutinating diphtheria bacilli and its practical import. *Ibid.* XXI. 421.

—— —— —— (1924 *c*). Spontaneous agglutination of bacteria. *J. Immunol.* IX. 365.

MOSER, P. (1902). Ueber die Behandlung des Scharlachs mit einem Scharlach-streptococcenserum. *Wien. klin. Wchnschr.* XV. 1053.

MOSER, P. and VON PIRQUET, C. (1903). Zur Agglutination der Streptococcen. *Centralbl. f. Bakteriol.* XXXIV. 506, 714.

NAKAYAMA, Y. (1919). Agglutination of streptococci. *J. Inf. Dis.* XXIV. 489.

NEUFELD, F. (1903). Ueber Immunität und Agglutination bei Streptococcen. *Zeitschr. f. Hygiene,* XLIV. 161.

NORTHROP, J. H. (1921). The stability of bacterial suspensions. I. A convenient cell for microscopic cataphoresis experiments. *J. Gen. Physiol.* IV. 629.

—— (1922). The stability of bacterial suspensions. VI. The influence of the concentration of the suspension on the concentration of salt required to cause complete agglutination. *Ibid.* V. 605.

NORTHROP, J. H. and DE KRUIF, P. H. (1921 *a*). The stability of bacterial suspensions. II. The agglutination of bacillus of rabbit septicaemia and of *B. typhosus* by electrolytes. *Ibid.* IV. 639.

—— —— (1921 *b*). The stability of bacterial suspensions. III. Agglutination in the presence of proteins, normal serum and immune sera. *Ibid.* IV. 655.

—— —— (1922 *a*). The stability of bacterial suspensions. IV. The combination of antigen and antibody at different hydrogen ion concentrations. *Ibid.* V. 127.

—— —— (1922 *b*). The stability of bacterial suspensions. V. The removal of antibody from sensitised organisms. *J. Gen. Physiol.* V. 139.

—— —— (1923). Stable suspensions of autoagglutinable bacteria. *J. Exp. Med.* XXXVII. 647.

ROSSIWALL, E. and SCHICK, B. (1905). Ueber spezifische Agglutination von Streptokokken aus Scharlachanginen und extrabukkalem Primäraffekt. *Wien. klin. Wchnschr.* XVIII. 3.

RUEDIGER, G. (1906). The streptococci from scarlatinal and normal throats and from other sources. *J. Inf. Dis.* III. 755.

SHIBLEY, G. S. (1924 *a*). Studies in agglutination. I. The agglutination of streptococci. *J. Exp. Med.* XXXIX. 245.

—— (1924 *b*). Studies in agglutination. II. The relationship of reduction of electrical charge to specific bacterial agglutination. *Ibid.* XL. 453.

STEVENS, F. A. and DOCHEZ, A. R. (1924 *a*). Agglutination and absorption of agglutinins with *Streptococcus scarlatinae. Ibid.* XL. 253.

—— —— (1924 *b*). Studies in the biology of streptococcus. IV. The occurrence of *S. scarlatinae* in convalescence and in the complications of scarlet fever. *Ibid.* XL. 493.

—— —— (1924 *c*). The study of haemolytic streptococci associated with scarlet fever. *Proc. Soc. Exp. Biol. and Med.* XXI. 37.

TUNNICLIFFE, R. (1920). The specific nature of the haemolytic streptococcus from scarlet fever. *J. Amer. Med. Assoc.* LXXIV. 1386.

—— (1922). Group specificness. *J. Inf. Dis.* XXXI. 372.

WEAVER, G. H. (1904). Agglutination of streptococci, especially those cultivated from cases of scarlatina, by human sera. *Ibid.* I. 91.

WILLIAMS, ANNA (1925). The relationship between antibodies. A report on scarlet fever. *Amer. J. Pub. Health,* XV. 129.

(*MS. received for publication* 20. II. 1926.—Ed.)