FURTHER STUDIES ON THE SEROLOGICAL CLASSIFICATION OF HAEMOLYTIC STREPTOCOCCI.

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IN a preliminary paper (1926) I have shown that most strains of haemolytic streptococci obtained from scarlet fever may be placed in two very definite serological groups. The strains from 119 cases were found to belong to Type I, from 57 cases to Type II, while from 34 cases the strains were unidentified. That the scarlatinal streptococci do not form one serological group has been confirmed by Griffith (1926) and by James (1926). Further, the sera and type cultures used in this investigation have been interchanged with those of Dr Griffith, and his Types I and II strains have been found to conform with the main serological types found in Aberdeen.

Since the results of the preliminary investigation were published the work has been extended. Further serological types have been found amongst the scarlatinal streptococci and the immunological relationship of the scarlatinal streptococci to haemolytic streptococci obtained from 72 non-scarlatinal sources have been fully studied.

METHODS.

Cultural methods. All strains used in this investigation were twice plated on blood agar, and from the second plating colonies were picked into tubes containing blood broth and incubated overnight. These were stored at -2°C . until required. A comparative test for haemolysin production was made with 72 strains. Thus 0.5 c.c. of a 24-hour broth culture of a strain was added to a tube containing 5 c.c. of 20 per cent. inactivated horse serum broth, and after an incubation period of 8 hours and of 24 hours 0.1 c.c. of the culture fluid was added to 1 c.c. of 2 per cent. washed sheep's red blood cells and the mixture was incubated for 1 hour in the water bath at 37°C . These tests showed that haemolysin was present in all tubes at the 8-hour period of growth, but only 41 cultures showed active haemolysin at the 24-hour period. It would appear that the 24-hour culture is of little value as a test for haemolysin production, and as a routine this test was therefore made at the 8-hour growth period. The strains were further tested for their action in media containing lactose, salicin, mannite, inulin, and raffinose, and for their resistance to bile.

Immunisation of rabbits. For the preparation of agglutinating sera some 70 rabbits were immunised with various strains. The strains of streptococci were grown for 24 hours at 37°C . in 10 per cent. horse serum broth medium. The cultures were then centrifuged, the deposits suspended in distilled water

and the resulting emulsions again centrifuged. The deposits thus obtained were resuspended in 10 c.c. of normal saline solution containing 0.4 per cent. of carbolic acid. The emulsions were then heated at 55° C. for half an hour, and rabbits were given increasing doses of streptococci twice weekly by the intravenous route. After immunisation had been carried out for six weeks, the blood serum was tested for specific agglutinins. Usually it was found to have the necessary agglutinin content by the sixth to eighth week, and only sera with a titre of 1 in 1600 to 1 in 3200 were used in this investigation.

Agglutination test. The method of obtaining stable suspensions was a modified form of that described by De Kruif and Northrop (1923). The strains of streptococci were grown in a broth medium consisting of beef extract to which 2 per cent. proteose peptone was added; no sodium chloride was used and the hydrogen ion concentration was adjusted to pH 7.6. Fifty c.c. of the medium were distributed into flasks having a total capacity of 100 c.c. and sterilised in the autoclave. After cooling, 5 c.c. of sterile inactivated horse serum were added, and the flasks were afterwards placed at 37° C. for 48 hours to test their sterility. For the agglutination test a flask of this medium was inoculated with a strain of streptococci and incubated at 37° C. overnight. The culture was then centrifuged, the organismal deposit was washed once with distilled water, again centrifuged, the supernatant fluid poured off and the deposit resuspended in sufficient 0.001 *N* NaOH to give a density corresponding to No. 1 tube of the Brown and Kirwan (1915) scale (302 millions of streptococci per c.c.). The agglutinating serum was diluted with salt solution to give a concentration of 1 in 50. One volume (0.4 c.c.) of various dilutions of the serum (1 in 50, 1 in 100, 1 in 200, 1 in 400, and 1 in 800) was placed in five agglutination tubes and an equal volume of streptococcal suspension was then added. A control tube containing a volume of sodium chloride solution and a volume of coccal emulsion was included in each test. The agglutination tests were incubated for 4 hours at 55° C.

The agglutinin absorption test. The strain under examination was grown in 50 c.c. serum broth flasks at 37° C. for two days. The culture was centrifuged at high speed, the supernatant clear fluid poured off and the deposit resuspended in 5 c.c. of distilled water. The emulsion was transferred to a specially designed centrifuge tube graduated to 0.2 c.c. with subdivisions of 0.01 c.c. This was then centrifuged for ten minutes at a speed of 2000 revolutions per minute, and a packed volume of cocci was obtained. When the supernatant fluid was pipetted off the dense deposit was diluted 1 in 10 with *M*/25 salt solution and a series of emulsions was compared with the emulsions of the standard Brown and Kirwan (1915) opacity tubes. It was found that all emulsions corresponded to No. 5 tube of the scale so that the diluted suspensions contained 1522 millions of cocci per c.c.

The emulsion having been standardised, the absorbing dose for each serum was estimated. The measured deposit was diluted 1 in 10 with *M*/25 NaCl and quantities, 0.1 c.c., 0.2 c.c., 0.3 c.c., 0.4 c.c. were added to tubes containing

0.02 c.c. of the serum to be tested, $M/25$ salt solution being then added to bring the total volume up to 1 c.c. The tubes were incubated in the water bath at 37°C . for 4 hours and left at room temperature overnight. They were then centrifuged and volumes (0.4 c.c.) of dilutions of the absorbed serum (1 in 50 to 1 in 800) were placed in agglutination tubes together with one volume of the homologous strain suspended in 0.001 N NaOH, and incubated at 55°C . for 4 hours. A control series of tubes containing various dilutions of unabsorbed serum and the homologous strain was included.

It has been assumed by most workers that for purposes of serological classification of numerous strains of organisms two or three times the amount of the coccal suspension as estimated for the homologous strain and serum should be employed. Accordingly in this study 0.06 c.c. to 0.08 c.c. of the packed volume of cocci diluted 1 in 10 have been added to a constant quantity of serum, namely 0.02 c.c., and the total volume made up to 1 c.c. by adding the requisite amount of $M/25$ NaCl solution. As estimated by the opacity scale 800 to 1200 millions of cocci are required for the absorption of 0.02 c.c. of an agglutinating serum having a titre of 1 in 1600.

IMMUNOLOGICAL RELATIONSHIPS.

In the preliminary serological study of streptococci obtained from cases of scarlet fever, strains were examined by means of both the agglutination test and the agglutinin absorption test. On account of the labour involved in setting up these various tests, and on account of difficulties encountered, the primary agglutination test was not performed as a routine in the second part of this work. Further, it was frequently found that if a serum contained a small amount of group agglutinins, a strain might be so sensitive to them that it would agglutinate to full titre on one occasion and only to $\frac{1}{16}$ of the titre or less on another occasion. Again another phenomenon interfered with the preliminary agglutination test. Not infrequently no agglutination could be obtained even with the serum diluted 1 in 100, but when the same culture was used for the absorption test it would completely absorb the agglutinins from the serum. This phenomenon, of course, has been encountered in the classification of other organisms. In the application of the absorption test it was found that whereas a culture of streptococci could absorb the agglutinins from a serum completely on one occasion, a second culture of the same strain could only partially absorb the agglutinins for the homologous strain. This was especially manifest when certain sera were absorbed with certain type strains and will be referred to in more detail later.

The classification of haemolytic streptococci which has been accomplished is based on the results obtained by first testing the whole of the scarlatinal strains (453 strains obtained from 210 cases) by means of the agglutination and absorption tests with Types I and II sera. Later 10 scarlatinal Type I strains, 10 scarlatinal Type II, 34 scarlatinal strains unclassified by Types I and II sera, and 72 non-scarlatinal strains were tested for their power to absorb

the agglutinins from 15 agglutinating sera representing ten serological types (Table I). Although it has been found that the majority of the scarlatinal strains fall into one or other of two serological groups, it has been definitely established that the scarlatinal streptococci cannot be distinguished by means

Table I. *Agglutination sera used in the investigation.*

Serum No.	Prepared for strain	Source of strain	Serological type
SF1	SF3TA	Scarlet fever	I
SF3	SF4TA	Scarlet fever	I
H/1	H/56	Tonsilitis	I
SF2	SF5TA	Scarlet fever	II
SF4	SF10TA	Scarlet fever	II
H/2	H/4	Tonsilitis	II
SF6	SF255TA	Scarlet fever	III
SF5	SF129TA	Scarlet fever	IV
SF7	SF167TA	Scarlet fever	IV
SF9	SF158TA	Scarlet fever	V
H/7	H/66	Erysipelas	VI
H/6	H/9	Erysipelas	VII
H/5	H/1	Rhinitis	VIII
H/8	H/2	Tonsilitis	IX
SF8	SF193TA	Scarlet fever	X
H/3	H/26	Osteomyelitis	X
H/4	H/28	Empyema	X
Dochez	Dochez	Scarlet fever	—

of serological tests from certain haemolytic streptococci causing puerperal fever, erysipelas, or suppurative processes. It therefore follows that the serological characteristics of the scarlatinal strains, and of the strains from non-scarlatinal sources must be considered together.

As it was considered necessary to determine the agglutinative action of the various sera on certain type strains, the direct agglutination results recorded in Table II have been obtained with cultures of the various strains which were selected after testing them, firstly, for sensitivity to spontaneous agglutination, and secondly, for agglutination with their homologous sera.

From Table II it will be seen that the three Type I sera and the three Type

Table II. *Results of agglutination tests.*

Strains used	Type	Agglutinating sera																
		SF1 I	SF3 I	H/1 I	SF2 II	SF4 II	H/2 II	SF6 III	SF5 IV	SF7 IV	SF9 V	H/7 VI	H/6 VII	H/5 VIII	H/8 IX	SF8 X	H/3 X	H/4 X
SF3TA	I	1600	1600	1600	0	100	0	100	100	100	0	100	100	0	0	200	200	400
SF4TA	I	1600	1600	1600	0	100	0	100	100	100	0	100	100	0	0	200	200	200
H/56	I	1600	1600	1600	100	100	0	100	100	100	0	100	100	0	0	200	200	200
SF5TA	II	0	0	0	1600	1600	1600	200	100	100	0	100	100	0	0	1600	800	1600
SF10TA	II	0	0	0	1600	1600	1600	200	200	200	0	100	100	0	0	1600	800	1600
H/4	II	0	0	0	1600	1600	1600	100	100	100	0	100	100	0	0	1600	1600	1600
SF255TA	III	100	0	0	0	0	0	1600	100	100	0	100	100	0	0	1600	1600	1600
SF129TA	IV	0	0	0	0	0	0	200	1600	1600	100	400	200	400	0	1600	1600	1600
SF167TA	IV	0	0	0	0	0	0	100	1600	1600	100	400	200	800	0	1600	1600	1600
SF158TA	V	100	0	100	0	0	0	200	0	0	1600	100	0	400	0	800	400	800
H/66	VI	0	0	0	0	0	0	100	100	0	0	1600	100	200	0	1600	1600	1600
H/9	VII	0	0	0	0	0	0	200	0	0	0	100	1600	0	0	1600	1600	1600
H/1	VIII	100	100	100	0	0	0	200	0	0	0	100	100	1600	0	1600	1600	1600
H/2	IX	0	100	100	0	0	0	100	100	200	0	100	0	100	1600	1600	1600	1600
SF193TA	X	100	100	100	0	0	0	100	100	100	200	400	400	100	0	1600	1600	1600
H/26	X	100	100	100	0	0	0	100	0	100	100	400	400	0	0	1600	1600	1600
H/28	X	100	100	100	0	0	0	100	0	100	100	400	400	0	0	1600	1600	1600

II sera gave comparatively little cross agglutination with the heterologous strains. Types III, IV and V sera agglutinated some of the heterologous strains to a dilution of 1 in 200. Type VI serum agglutinated Type IV and Type X strains to 1 in 400, and the other heterologous strains to 1 in 100; Type VII serum agglutinated Type IV strains to 1 in 200, and Type X strains to 1 in 400; Type VIII serum agglutinated Type IV strains to 1 in 400, and 1 in 800 respectively, Type V strains to 1 in 400, and Type VI strains to 1 in 200; while Type IX serum agglutinated the homologous strain only. On the other hand, the three Type X sera agglutinated all the heterologous strains to full titre with the exception of Types I and V. Likewise the results of the various agglutinin absorption tests are summarised in Table III from which it will

Table III. *A summarisation of the agglutinin absorption tests.*

Strains	Sera									
	Type I	Type II	Type III	Type IV	Type V	Type VI	Type VII	Type VIII	Type IX	Type X
Type I	0	1600	1600	1600	1600	800	1600	1600	1600	0-400
Type II	1600	0	1600	1600	1600	800	1600	1600	1600	0
Type III	1600	1600	0	1600	1600	800	1600	1600	1600	200-800
Type IV	1600	1600	1600	0	1600	400-800	1600	1600	1600	0
Type V	1600	1600	1600	1600	0	800	1600	1600	1600	1600
Type VI	1600	1600	1600	1600	1600	0	1600	1600	1600	0
Type VII	1600	1600	1600	1600	1600	800	0	1600	1600	200-400
Type VIII	1600	1600	1600	1600	1600	800	1600	0	1600	0
Type IX	1600	1600	1600	1600	1600	800	1600	1600	0	400-800
Type X	1600	1600	1600	1600	1600	400-800	1600	1600	1600	0

Figures indicate the agglutinin titre of a serum of the homologous strain after absorption.
0 = complete absorption: 1600 = agglutination to titre and no absorption.

be seen that none of the heterologous strains absorbed any of the agglutinins from Types I, II, III, IV, V, VII, VIII and IX sera. When Type VI serum was absorbed with the heterologous strains the agglutinin titre was reduced by Types I, II, III, VII, VIII and IX to 1 in 800, and by Types IV and X strains to between 1 in 400 and 1 in 800. When Type X sera were absorbed the heterologous strains belonging to Types II, IV, VI and VIII completely removed the agglutinins, and the titres were reduced by absorption with Types III and VII strains to 1 in 200 and 1 in 400, with Type IX strains to 1 in 400 and 1 in 800, while Type V strain did not affect the homologous agglutinins. The results obtained by absorbing Type X sera with Type I strains were very irregular. It was found that one culture of one strain would completely absorb the agglutinins from a serum, and a second culture of the same strain would only partially absorb them. Further, when this occurred double the usual absorbing dose did not reduce the titre, but rather tended to leave a greater amount of residual agglutinins. The absorption tests with the Type I strains and Type X sera were repeated on no less than four occasions, and on at least two occasions complete absorption was obtained.

Five scarlatinal strains and ten non-scarlatinal strains remained unclassified after the whole of the strains had been tested against the various sera. One

scarlatinal strain SF119TA completely absorbed the agglutinins from Type VI serum, but did not absorb any of the specific agglutinins from Type X sera and therefore could not be classified as a Type VI strain. Another scarlatinal strain SF256TA was capable of absorbing the agglutinins from Type IV, Type VI, and Type X sera and therefore could neither be placed as a Type IV strain nor a Type VI strain. Scarlatinal strain SF211TA reduced the titre of Type VI serum to 800 but did not affect Type X sera. The remaining two scarlatinal strains and the ten non-scarlatinal strains were capable of reducing the titre of Type VI serum to 800 and markedly absorbed the specific agglutinins from Type X sera. The absorption of three Type X sera was repeated with these latter strains, using double the amount of the estimated dose of cocci for absorption, the results being shown in Table IV, but complete absorption could not be obtained.

Table IV. *Absorption of Type X sera by the unclassified strains.*

Absorbing strain	Agglutination of the homologous strains after absorption of three Type X sera with various doses of cocci					
	Serum SF8 Dose of cocci		Serum H/3 Dose of cocci		Serum H/4 Dose of cocci	
	0.08 c.c.	0.16 c.c.	0.06 c.c.	0.12 c.c.	0.08 c.c.	0.16 c.c.
SF42TA	800	400	400	200	400	200
SF241TA	400	200	400	200	400	200
H/3	400	200	200	200	200	200
H/5	200	200	200	200	200	200
H/18	400	200	400	200	400	400
H/22	200	100	200	200	200	200
H/24	200	200	200	200	200	200
H/27	400	200	200	200	200	200
H/29	400	200	200	100	200	200
H/30	200	200	100	200	200	200
H/51	200	200	200	200	200	200
H/52	200	200	200	200	200	200

The Dochez strain was obtained from the National Collection of Type Cultures and a serum was prepared for it. This strain apparently is a typical example of the common scarlatinal type found in the United States of America. The whole of the scarlatinal and non-scarlatinal strains have been tested with this serum, and the Dochez strain has been tested against the various agglutinating sera. Not a single homologue has been found.

From the foregoing it is therefore evident that the haemolytic streptococci can be divided into a number of closely related serological groups. Certain types such as Types I and II can be identified readily by means of the agglutination test alone provided they do not exhibit the phenomenon of inagglutinability. When, however, Type X sera are employed, most of the heterologous strains are not only agglutinated to full titre, but are capable of completely absorbing the specific agglutinins from these sera. On the other hand, Type X strains neither agglutinate to any extent with the heterologous sera, nor do they absorb the agglutinins from them. These findings would seem to indicate that

the antigenic structure of the Type X strains is the least complex of all, and it is believed, therefore, that this latter group contains members which could be separated off from it if further agglutinating sera were prepared for certain strains.

Griffith (1926) and James (1926) have previously called attention to the fact that cultures of certain strains (even the homologous strain) may on one occasion completely absorb the agglutinins from a serum, and yet on another occasion can only partially remove the specific agglutinins. They have suggested that this phenomenon may be due to the occurrence of a specific and non-specific phase in cultures of streptococci. So far as the present investigation goes, this question remains unanswered. It seems, however, certain that the phenomenon is not dependent on any modification of the absorbing dose, and further, that double the usual absorbing dose of cocci can be used without effecting complete absorption of the agglutinins.

The distribution of the various serological types of streptococci in the scarlatinal and non-scarlatinal groups of cases is given in Table V, from which

Table V. *The distribution of the serological types.*

Type	Scarlatinal group No. of cases showing the various types in throat cultures	Non-scarlatinal group No. of cases showing the various types
I	119	8
II	57	7
III	3	2
IV	16	2
V	1	0
VI	1	2
VII	0	1
VIII	0	1
IX	0	1
X	8	38
Unclassified	5	10
Total cases	210	72

it will be seen that the Types I and II strains predominated in the scarlatinal group and the Type X strains in the non-scarlatinal. The homologous strains belonging to Types V, VII, VIII and IX were the only examples of their respective types.

SEROLOGICAL TYPES IN RELATION TO CASES OF SCARLET FEVER.

For the investigation of the scarlatinal streptococci two strains were isolated from cultures made from the throat and nose secretions, and if septic complications arose later, strains of streptococci were obtained from the pus. Thus actually 259 strains of Type I streptococci were obtained from 119 cases of scarlet fever. Of these cases, 118 showed two Type I strains in the cultures made from the throat secretions, while one case from which no haemolytic streptococci were obtained from the throat showed a Type I streptococcus as a secondary invader of an empyema cavity originally containing a Type I

pneumococcus. Again, 125 strains of Type II streptococci were obtained from 57 cases of scarlet fever, and 69 strains from 34 cases were found to belong to neither of the two main groups. It was obvious that the two main serological types of streptococci were undoubtedly capable of causing scarlet fever, but after these strains had been eliminated, there still remained strains from 34 cases to be classified. These were obtained from cases admitted to hospital on the day on which the rash appeared, or on the day following, and it is unlikely that if non-scarlatinal haemolytic streptococci were present in the throat that they would have had time to outgrow the scarlatinal strains. Further, since two strains were obtained from the throat culture in each case and neither belonged to the main types, the only conclusion that could be made was that other serological types of streptococci were also capable of causing scarlet fever. When the typing of these strains had been accomplished, 3 were found to belong to Type III which conforms to the Type III described by Dr F. Griffith (1926), 16 were found to belong to Type IV, 1 to Type V, and 8 to Type X, and the strains from 5 cases remained unclassified.

Strains obtained from nasal secretions. From nasal swabs from 210 cases of scarlet fever, haemolytic streptococci were obtained 16 times and in every instance corresponded to the type which had been found in the throat culture. Thus ten cases showed a Type I streptococcus in the throat and nose cultures, five cases a Type II streptococcus, and in one case a Type X streptococcus.

Strains from septic complications. In addition to the case of surgical scarlet fever following upon a secondary infection of an empyema cavity, strains of haemolytic streptococci were obtained from the pus in ten cases in which septic complications occurred and at necropsy from two cases of scarlet fever who died from streptococcal septicaemia. The serological classification of these strains showed that the septic complications of scarlet fever were in the main caused by the same type of streptococcus which was found in the throat cultures at the beginning of the disease. In one case (SF55), however, a Type I strain was obtained from the throat, while later a Type II strain was obtained from the pus obtained at operation from the mastoid cells. In a scarlet fever ward there is doubtless every opportunity for the spread of the various types of streptococci.

Strains obtained from members of the same family. In nine instances the strains found in the throat cultures from members of the same family suffering from scarlet fever have shown agreement in their serological classification. In one family the second case 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.

Relapse in scarlet fever. That patients under treatment for scarlet fever do occasionally have a true relapse of the disease is admitted by all authorities. This usually occurs in about 1 per cent. of cases in the third or fourth week of the illness, and during the course of this investigation three such cases have been observed. From these throat cultures were obtained on admission to hospital, and again when the relapse occurred, two strains being obtained on each occasion. The serological classification of these strains is given in Table VI.

Table VI. *Relapse in scarlet fever.*

Case No.	Type of streptococcus on admission	Type of streptococcus on relapse
279	IV	I
283	IV	I
298	I	I

This small series of cases shows, therefore, that a relapse need not necessarily indicate an infection with another type of streptococcus. Since it is generally admitted that scarlet fever is, in the main, due to absorption of the toxin of the streptococcus this would appear to be due to lack of response or insufficient stimulation of the patient's immunity mechanism. All these cases showed a positive Dick test when the relapse occurred, and when two were tested during convalescence negative reactions were obtained. The relapse would therefore appear to be due to a comparative absence of protective antitoxin in the body fluids.

Toxic scarlet fever. During the course of this investigation one case of toxic scarlet fever has been encountered.

Case No. 90. Female aged 11 years. Admitted to hospital on 13. 3. 25. History: Patient taken suddenly ill on day previous to admission. The chief symptoms were headache and rigors, and later vomiting and diarrhoea. On admission the child was unconscious, vomiting and diarrhoea and slight head retraction were the most marked clinical features. There was no evidence of rash. Death took place 13 hours after admission to hospital or 41 hours after the commencement of the illness. A blood culture made during life remained sterile and the examination of the cerebro-spinal fluid showed no abnormality whatsoever. On the day following admission of this case to hospital two members of the same family developed scarlet fever and this gave a clue to the cause of death in the above case.

At autopsy the small intestines showed enlarged and injected Peyer's patches and follicles, the spleen was slightly enlarged, otherwise no marked pathological change was noted. Cultures made from the heart blood, from the liver, spleen and kidneys remained sterile. Examination of the intestinal contents showed no pathogenic organisms. From the fauces and tonsils cultures were made and a practically pure culture of a haemolytic streptococcus was obtained.

Serological examination showed that the strains isolated belonged to Type I, and further, the strains isolated from the other two members of the family also belonged to Type I. Death was therefore due to an intense toxæmia resulting from an infection of the tonsils and fauces with a Type I haemolytic streptococcus. Actual systemic invasion with the streptococcus did not take place since a blood culture taken during life and the various cultures made *post mortem* remained sterile.

SEROLOGICAL TYPES IN RELATION TO NON-SCARLATINAL
STREPTOCOCCAL INFECTIONS.

Since it has been shown that seven serological types of streptococci have been obtained amongst the scarlatinal throat strains, it has been of interest to examine the non-scarlatinal strains in relation to their serological classification. The distribution of the various types in relation to disease processes is given in Table VII. Thus Type I strains were obtained from normal throats,

Table VII. *The distribution of the serological types in non-scarlatinal infections.*

Source	Total No. isolated	Number of strains belonging to the various types										No. unclassified
		I	II	III	IV	V	VI	VII	VIII	IX	X	
Normal throat	17	2	4	1	1	0	0	0	0	0	7	2
Tonsillitis	15	1	3	0	0	0	1	0	0	1	6	3
Puerperal septicaemia (blood)	9	1	0	0	1	0	0	0	0	0	7	0
Puerperal sepsis (lochia)	1	0	0	0	0	0	0	0	0	0	1	0
Erysipelas	4	1	0	0	0	0	1	1	0	0	0	1
Otitis	2	1	0	0	0	0	0	0	0	0	1	0
Mastoiditis	2	0	0	0	0	0	0	0	0	0	2	0
Broncho-pneumonia	4	0	0	0	0	0	0	0	0	0	2	2
Rhinitis	6	1	0	0	0	0	0	0	1	0	2	2
Cervical abscess	3	1	0	0	0	0	0	0	0	0	2	0
Septicaemia non-puerperal	1	0	0	1	0	0	0	0	0	0	0	0
Osteomyelitis	3	0	0	0	0	0	0	0	0	0	3	0
Empyema	3	0	0	0	0	0	0	0	0	0	3	0
Meningitis	1	0	0	0	0	0	0	0	0	0	1	0
Wound	1	0	0	0	0	0	0	0	0	0	1	0
Total	72	8	7	2	2	0	2	1	1	1	38	10

tonsillitis, puerperal septicaemia, facial erysipelas and suppurative processes. Type II strains were obtained from cases of tonsillitis and normal throats only; Type III strains from a throat culture of a case of diphtheria and by blood cultures from a case of septicaemia following an injury to a hand. Type IV strains from a normal throat and from a case of puerperal septicaemia, but not a single homologue of Type V could be found in the non-scarlatinal group. Type VI strains were isolated from a case of erysipelas and from an acute tonsillitis. Types VII, VIII, and IX strains being the only examples of their respective types were obtained from erysipelas, mastoiditis, and tonsillitis. Type X strains were obtained from normal throats and many types of septic

processes. The ten unclassified strains were obtained from cases of tonsilitis, rhinitis, broncho-pneumonia, and from normal throats.

DISCUSSION.

The whole of the results therefore indicate that at the present time certain types of streptococci are more likely to be the cause of scarlet fever than others. This may be due to a greater biological activity of these strains, and this may be, to some extent, associated with a greater capacity to produce toxin. It is seen, however, that Type I strains have been isolated from normal throats, from cases of tonsilitis, from puerperal septicaemia and even erysipelas, and it may be argued if strains belonging to one serological type are capable of producing these various diseases then strains belonging to other types should be capable of doing the same, and this has been found to be the case. It has been shown that of the nine strains isolated by blood culture from puerperal septicaemia one strain belonged to Type I, another to Type IV, and the remaining seven to Type X, and all these types have also been found in the throat cultures from patients suffering from scarlet fever. Further, although only four strains from cases of erysipelas have been examined one was found to belong to Type I, one to Type VI and Type VII respectively, while the fourth strain was unclassified. These results were obtained after agglutinating sera were prepared for two of these strains. The only conclusion that can be made from the above findings is that the haemolytic streptococci causing scarlet fever cannot be distinguished by serological methods from the haemolytic streptococci causing other disease processes.

It may be contended that these findings afford some support to the view that the haemolytic streptococcus is not the cause of scarlet fever. Some evidence rebutting this contention is available. When scarlet fever, puerperal fever and erysipelas are considered from the point of view of past outbreaks there seems to be a definite connection. Scarlet fever has been found to become epidemic in wards into which cases of puerperal fever were admitted, and further, clinicians have long recognised that puerperal septicaemia may be attended with a rash which cannot be distinguished from that found in scarlet fever. On the other hand, when this occurs the throat symptoms are not so marked as in the ordinary case.

Again, the physicians of the eighteenth century were impressed with the relationship between puerperal septicaemia and erysipelas as it occurred in the wards of maternity hospitals. Further, there are numerous instances recorded in which newly delivered women have contracted puerperal fever from a nurse infected with mild erysipelas, and of cases in which a nurse has developed erysipelas following upon her association with a case of puerperal fever.

The records of the past do not, however, show clearly that a relationship exists between scarlet fever and erysipelas. Accordingly, the incidence of scarlet fever and erysipelas in Aberdeen, in Scotland, and in England and

Wales has been examined. The relative incidence of these diseases in Aberdeen indicates that when scarlet fever becomes epidemic there is a marked increase in the number of cases of erysipelas. Further, the case rates of scarlet fever, erysipelas and puerperal fever per 10,000 of the population for Scotland and for England and Wales seen in Table VIII indicate that there is a marked increase in the number of cases of erysipelas when scarlet fever is epidemic. On the other hand, the case rate for puerperal fever shows no such fluctuations.

Table VIII. *Case rate of scarlet fever, erysipelas, and puerperal fever per 10,000 of population.*

Year	SCOTLAND. 1911-1924. Cases per 10,000 of population			ENGLAND AND WALES. Cases per 10,000 of Population		
	Scarlet fever	Ery- sipelas	Puerperal fever	Scarlet fever	Ery- sipelas	Puerperal fever
1911	41.4	10.1	0.7	29.0	6.9	0.6
1912	42.1	9.8	0.7	29.8	6.3	0.6
1913	49.0	10.1	0.6	36.2	6.4	0.5
1914	57.3	11.8	0.8	45.7	7.5	0.6
1915	56.0	10.3	0.7	35.2	6.5	0.6
1916	41.3	7.7	0.7	21.0	5.1	0.6
1917	20.2	5.6	0.6	13.5	3.7	0.4
1918	14.8	4.7	0.5	13.3	3.5	0.3
1919	27.0	6.3	0.7	22.8	4.4	0.6
1920	36.6	7.6	1.2	33.1	4.4	0.8
1921	34.9	7.5	1.3	36.2	3.5	0.6
1922	29.9	6.9	1.2	28.6	3.5	0.6
1923	34.3	6.9	1.2	22.6	3.3	0.6
1924	37.0	6.0	1.1	22.3	3.4	0.6

The human inoculation experiments of G. H. Dick and G. F. Dick (1923) have been confirmed by Nicolle (1926) and the whole of the evidence obtained from Dick tests, Schultz-Charlton reactions, and treatment with the specific antitoxin goes to show that a haemolytic streptococcus is the cause of scarlet fever. Again, in the course of the work on the preventive aspects of this disease it has been clearly and repeatedly shown (Kinloch, Smith and Taylor, 1927) that the complete clinical picture of scarlet fever can be produced by the subcutaneous injection into susceptible individuals of the exo-toxin of a haemolytic streptococcus (the Dochez strain of *S. scarlatinae* being used for the production of a high titre toxin), and takes place a few hours after the inoculation. It has been suggested by certain individuals that a virus might be transmitted when the injection of the filtrate is made. The filtrate containing 0.4 per cent. phenol has been stored in the refrigerator for months, and further, it has been shown that it may be heated at 55° C. for 1 hour and yet when injected into susceptible individuals in suitable dosage may produce typical scarlet fever.

It has been shown in the case of diphtheria, cerebrospinal meningitis, and pneumonia, that there are many serological types of the respective causative organisms. If it could be demonstrated that the haemolytic streptococci from various disease processes were capable of producing toxins which could be neutralised equally well by a monovalent antitoxin, then there would be no

necessity to assume that special serological types caused various diseases. So far, the skin tests with a series of toxins from various strains of haemolytic streptococci have given discrepant results, and the neutralisation tests carried out on human beings and especially on goats have given equivocal findings.

It is believed that scarlet fever is simply one symptom of a streptococcal infection and that the occurrence of the exanthem depends on the actual amount of toxin which is absorbed into the tissues and which accumulates to the extent of being capable of producing the rash. It has been shown for instance, that a Type I streptococcus has been isolated from the blood of two cases of scarlet fever, and that this blood invasion must have followed upon the infection of the fauces. In the routine examination by blood culture it has been found that a case may have a sterile blood culture at the commencement of the disease and later develop septic complications and general systemic infection. This case does not develop another exanthem when the blood infection occurs, presumably on account of having sufficient antitoxin to prevent its reappearance or because the streptococcus is unable to flood the tissue with free toxin. Further, the three cases in which a relapse occurred all showed a markedly positive Dick reaction indicating a comparative absence of antitoxin in the tissues, and in a small series of cases which have been Dick tested previous to or on the occurrence of such complications as otitis, mastoiditis and tonsillitis, the majority have shown a negative Dick test or only a slightly positive reaction. Again, it has been the experience of the physicians of this hospital that since the nursing staff have been immunised with scarlatinal toxin, no actual case of scarlet fever has occurred in those who become Dick negative, but this has not modified the incidence of streptococcal throat infections. All that immunisation has done is to prevent the occurrence of the rash, since the serological type of haemolytic streptococcus found in these cases has been frequently either Type I or Type II.

These findings would appear to suggest that at least two immunity substances are required to give complete protection against streptococcal infections, namely, antitoxic and antibacterial properties, and that the antibacterial properties are probably more species specific than the antitoxic. Further, that the clinical manifestations of streptococcal infections vary, not according to the serological type of the haemolytic streptococcus causing the infection, but in relation to the biological activity of the type, in relation to the site of inoculation, in relation to the amount of toxin absorbed, in relation to the antibody content of the patient's tissues, and finally, in relation to the response of the patient's immunity mechanism to infection.

CONCLUSIONS.

The antigenic analysis has shown that strains of haemolytic streptococci obtained from scarlatinal and non-scarlatinal sources constitute a closely related group.

The strains obtained from 210 cases of scarlet fever were not found to conform to one specific type, but could be divided into many types; thus 119 cases gave Type I strains, 57 cases Type II, 3 cases Type III, 10 cases Type IV, 1 case Type V, 8 cases Type X, and from 5 cases the strains were not classified.

Strains conforming to Types I, II, III, IV, VI, and X were obtained from scarlatinal and non-scarlatinal sources. The single strains belonging to Types V, VII, VIII, and IX were the only examples of those respective types, Type V strain being obtained from a scarlatinal throat, and Types VII, VIII and IX strains from non-scarlatinal sources.

A homologue of the Dochez strain has not been encountered among either the scarlatinal or non-scarlatinal strains.

Scarlatinal strains from cases occurring in isolated outbreaks and from related cases have been found to belong to the same serological type.

The secondary pyogenic infections occurring in cases of scarlet fever may be caused by either the original infecting strain or by a secondary invader.

The haemolytic streptococci obtained from cases of scarlet fever in which a relapse occurs, may or may not conform to the serological type of the original infecting strain. The occurrence of relapse would appear to be determined by the comparative absence of specific antitoxin in the patient's tissues.

Strains belonging to the various types associated with scarlatina have been obtained from non-scarlatinal sources, Type I strains have been obtained from cases of otitis and cervical abscess following diphtheria, from normal throats, from cases of tonsillitis, puerperal septicaemia, and erysipelas, Type II strains from normal throats and cases of tonsillitis, Type III strains from the throat culture of a case of diphtheria, and from the blood in a case of septicaemia, Type IV strains from a throat culture and from a case of puerperal septicaemia, Type VI strains from a case of erysipelas, Type X strains from normal throats, from cases of puerperal septicaemia, tonsillitis, and many other infections.

REFERENCES.

- BROWN, H. C. and KIRWAN, E. W. O'G. (1915). *Indian J. Med. Research*, **2**, 763.
DE KRUIF, P. and NORTHROP, J. H. (1923). *J. Exp. Med.* **37**, 647.
DICK, G. H. and DICK, G. F. (1923). *J. Amer. Med. Assoc.* **81**, 1166.
GRIFFITH, F. (1926). *J. of Hygiene*, **25**, 385.
JAMES, G. R. (1926). *Ibid.* **25**, 415.
KINLOCH, J. P., SMITH, J. and TAYLOR, J. S. (1927). *Ibid.* **26**. (In the press.)
NICOLLE, C., CONSEIL, E. and DURAND, P. (1926). *Ann. Inst. Pasteur de Tunis*, **15**, 229.
SMITH, J. (1926). *J. of Hygiene*, **25**, 165.

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