THE RELATIONSHIP OF STREPTOCOCCI TO SCARLET FEVER.

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This report is a contribution to the joint investigation, organised by the Ministry of Health, into the bacteriology of Scarlet Fever. It has been carried out with the aid of a grant from the Ministry to whom I am indebted for permission to publish these results.

The bulk of the work has been concerned with a serological classification of β-haemolytic streptococci[1] isolated from the throats of patients suffering from scarlet fever. In addition, the toxins of several serologically distinct types of haemolytic streptococci have been compared by means of skin reactions on human volunteers, and, thirdly, the wider subject—the serological classification of haemolytic streptococci in general and their relation to the streptococci from scarlatinal sources—has been opened, partly because it would serve as a control of the major portion of the work, and also because the results obtained with the several toxins used pointed to the importance of this aspect of the subject.

Isolation of Strains.

Material has been obtained through the courtesy of the Medical Superintendents and Resident Medical Officers of the Fazakerley and Netherfield Road City Hospitals, the Royal Infirmary, the Maternity Hospital and the Brownlow Hill Institution. In the early stages of the investigation the tonsils, peri-tonsillar fossa, the posterior pharyngeal wall and the naso-pharynx of less sensitive patients were accurately swabbed. Similar results were obtained from each site and it was therefore decided to confine the examination to the tonsils.

Within 2 hours after examining the throat the swabs have been "plated" on four 5 per cent. horse blood agar plates. After 18 to 24 hours' incubation, haemolytic colonies were picked off and sown in 15 per cent. defibrinated rabbit's blood broth. After 24 hours' incubation the blood broth cultures were replated. The character of the colonies and the degree of haemolysis on these plates were noted, and two or three tubes of blood broth sown with representative colonies. After 18 hours' incubation these cultures were stored in the ice chest.

[1] "Haemolytic" means "β-haemolytic" throughout this report. The nomenclature of Smith and Brown has been adopted, β-haemolytic streptococci signifying streptococci which when grown on 5 per cent. horse blood agar plates produce clear, transparent, completely haemolysed, colourless zones from 2 to 4 mm. in diameter after 24 to 48 hours' incubation.

Material has been examined from numerous sources, and 124 strains have been collected and stored. These have been isolated from four types of case:

(1) The major group—scarlet fever throats—the diagnosis in each case has been confirmed by experienced clinicians.

(2) Inflamed throats not accompanied by a rash.

(3) Normal throats.

(4) Sources other than throat. Careful enquiry has been made in these cases to exclude any possible association with scarlet fever.

In addition, several strains have been received from Dr F. Griffith, and five strains of non-scarlet haemolytic streptococci from Dr J. F. D. Shrewsbury.

Table I.

Scarlet fever strains.

Case No.	Age	Day of disease	β-haemolytic streptococci	Strain No.	Type
Dochez				31	
Dr F. Griffith's		S.F. 130/2		86	1
,,	,,	S.F. 22		87	2
,,	,,	U.S. 55/4		88	3
,,	,,	S.F. 14/2		119	3
Zingher				112	
1	$3\frac{3}{12}$	5	+	1	1
2	2	2	+	2	?
3	.	3	+	3	?
4	.	5	+	4	3
8	11	3	−	.	.
9	9	4	−	.	.
10	$7\frac{1}{2}$	4	−	.	.
11	9	4	−	.	.
12	$6\frac{10}{12}$	5	−	.	.
13	$7\frac{4}{12}$	5	−	.	.
14	$3\frac{3}{12}$	12	+	6	1
15	10	10	+	7	2
16	5	3	+	8	2
17	$7\frac{1}{2}$	9	+	9	3
19	11	4	+	10	?
20	28	6	+	11	3
21	$2\frac{1}{2}$	4	+	21	?
22	12	6	+	13	−
23	10	6	+	14	2
30	15	6	+	17	?
31	$4\frac{1}{2}$	4	+	18	?
32	18	3	−	.	.
34	8	4	+	19	?
35	$5\frac{1}{2}$	5	+	20	?
36	11	3	+	21	?
37	13	6	+	22	?
38	45	7	+	23	3
39	$2\frac{8}{12}$	18	+	24	?
41	5	16	+	25	2
42	7	4	+	−	.
43	12	6	+	26	3
165	.	.	+	37	?
48	9	4	+	38	?
49	6	6	+	39	1
50	$10\frac{10}{12}$	5	+	40	?
51	14	4	+	41	?
52	$7\frac{1}{2}$	2	+	42	—
53	11	4	+	43	2
54	11	2	+	44	?
55	$2\frac{1}{2}$	6	+	45	?
56	$11\frac{1}{2}$	.	+	46	3
57	12	2	+	47	?
58	14	5	+	48	3
59	$10\frac{1}{2}$	7	+	49	?
60	$14\frac{1}{2}$	6	+	50	?
61	$6\frac{1}{2}$	6	+	51	3
62	3	2	+	52	?
65	30	3	+	55	1
66	6	2	+	56	?
72	$9\frac{1}{2}$	5	+	70	—
73	$10\frac{1}{2}$	4	+	71	1
74	$4\frac{10}{12}$	4	+	72	2
76	$7\frac{1}{2}$	4	+	74	1
77	$8\frac{9}{12}$	4	+	75	—
79	$6\frac{3}{12}$	.	+	79	2
80	$5\frac{1}{2}$	6	+	85	1
97	9	.	+	91	?
99	$2\frac{1}{2}$	3	+	89	1
113	28	3	+	96	3
114	21	3	+	94	2
119	$1\frac{4}{12}$	10	+	97	2
93 (*a*)	$2\frac{6}{12}$	4	+	98	1
126	14	18	+	100	2
127	25	4	+	101	?
130	30	10	+	102	3
153	42	7	+	117	—
174	13	4	+	—	.
33	12	6	−	.	.
		Additional scarlet fever material			
5	S.F. + Cellulitis			5	?
6	S.F. + Otitis media			−	.
18	S.F. + Otitis media			−	.
40	S.F. + Cervical adenitis			−	.
67	S.F. + Meningitis			57	?
112	S.F. + Scalds			92	? 2

Note. ? = Strain tested with available sera but not yet identified.
− = Strain not yet tested.

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Table I gives particulars of the 68 cases of scarlet fever which have been examined. Haemolytic streptococci were isolated from 60 of these cases, that is, from 88·2 per cent.

Tables II and II A contain particulars of the "control" strains. Only those strains which have been isolated from authentic non-scarlet lesions can be regarded as true controls, and as many of these as time would permit have

Table II.

Inflamed "non-scarlet" throats.

Case No.	Age	Diagnosis	β-haemolytic streptococci	Strain No.	Type
7	12	Diphtheria	−	.	.
24	9	,,	+	15	2
25	39	,,	+	16	?
26	18½	,,	−	.	.
27	7	,,	−	.	.
29	50	Influenza tonsillitis	−	.	.
44	30	Tonsillitis	+	27	?
47	7	,, ? S.F., ? Diph.	+	36	1
63	14	,,	+	53	?
64	17	,,	+	54	?
75	38	,,	+	73	?
82	13	,,	−	.	.
83	19	,,	−	.	.
86	7	,,	−	.	.
87	12	,,	+	77	?
88	28	,,	+	80	?
89	5	,,	+	81	?
90	5	,,	−	.	.
91	3	,,	+	82	1
92	1 7/12	,,	−	.	.
128	9 days	"Congested throat"	−	.	.
129	22	Tonsillitis	−	.	.
148	8	,,	+	113	?
150	26	,,	+	115	−
154	.	,,	+	120	3
155	23	,,	+	121	3
157	20	,,	+	123	?
161	38	,,	+	32	2
162	38	Otitis media	+	33	2
172	31	Tonsillitis	−	—	.
173	10	,,	−	—	.

Note. ? = Strain tested but not identified.
− = Strain not yet tested.
S.S. 64 to 69 Dr F. Griffith's strains.
S.S. 106 to 111 Dr J. F. D. Shrewsbury's strains.

Table II A.

Non-scarlet strains.

Case No.	Diagnosis	Strain No.	Type
45	Fornix vaginae	28	−
160	Ovarian abscess	30	?
151	Septicaemia, endocarditis	34	?
164	Cellulitis	35	?
68	Septicaemia	59	?
69	Pemphigus	60	?
70	,,	61	?
71	Septicaemia	62	−
166	,,	64	−
167	Uterus	65	−
168	Septicaemia	66	?
169	,,	67	−
170	,,	68	−
171	Meningitis	69	−
78	Normal vagina	78	?
81	Septicaemia	84	A
118	Puerperal septicaemia	95	B
125	,, ,,	99	C
139	Peritonitis	106	?
140	Subcutaneous abscess. Arm	107	A
144	,, ,, Horse	109	?
145	Osteo-myelitis	110	?
146	Empyema	111	−
152	Puerperal septicaemia	116	−
156	,, ,,	122	?
157	Osteo-myelitis	124	?
46	Post-scarlatinal osteo-myelitis	29	2
100	Normal throat	90	?
107	,, ,,	93	?
131	,, ,,	103	?
133	,, ,,	104	?
136	,, ,,	105	?
142	,, ,,	108	?
149	,, ,,	114	3

Number of normal throats examined, 36.

been examined. But it must be admitted that strains isolated from normal throats and from cases of angina in which a rash was never recognised do not serve as rigid controls of the scarlatinal strains, since it is impossible in such an enquiry to disregard the possible relationship of such conditions to scarlet fever.

The incidence of haemolytic streptococci in the present short series of inflamed and clinically non-scarlet throats, and in normal throats, is of interest. Haemolytic streptococci were isolated from 58 per cent. of ordinary sore throat and from 19·4 per cent. of the normal throats.

Cultural Characteristics.

Several types of colony have been observed among the strains examined, though it has not been possible to correlate the differences in the appearances of the colonies with other biological reactions. Close attention has also been paid to the character of the growth in liquid media of the first sub-culture after isolation; several differences in detail occur, but, though of considerable importance in relation to the problem of auto-agglutination, they are of no practical value in differentiating these organisms.

Fermentation Reactions.

A peptone-sugar-water has been used; 2 per cent. solutions of the carbohydrates and a 2 per cent. peptone water solution have been made up separately and stored in flasks, equal parts of the stock sugar solutions and peptone water being mixed and tubed when required. One drop of litmus, added after incubation, has been used as the indicator. Tests have also been made with phenol red as the indicator, in which case reactions were tested on a carefully cleaned glazed white tile, one or two drops of the culture being transferred to the tile with a large sterile platinum loop to which a small drop of phenol red was added. The carbohydrates used were glucose, lactose, mannite, salicin, maltose, saccharose, raffinose, and inulin. The majority of the strains, irrespective of their source, ferment glucose, lactose, salicin, maltose, and saccharose, the only exceptions encountered being a few strains (as S.S. 36 and 78) which do not ferment lactose. None of the strains examined ferment mannite, raffinose, or inulin. The Dochez strain, unlike the scarlatinal strains in this series, does not ferment salicin.

These reactions are of no value in differentiating between the streptococci of scarlet fever and those from other sources, neither do they give any indication of the existence of serological groups among the scarlet fever strains, to which I have mainly given my attention. It may be, however, that the differences exposed by serological methods are superficial and perhaps of less importance than the relationship which the fermentation reactions indicate.

Serological Types.

Agglutinating sera have been made with twelve strains, from which seven types of serum, including the Dochez serum, have been obtained. Of these the Dochez serum together with the sera made with strains 4, 71 and 72, have claimed most of my attention.

S. 4 was chosen more or less arbitrarily, while S.S. 71 and 72, isolated from cases 73 and 74, were chosen as a result of experiments carried out with an antitoxic serum made in the laboratory. The filtrate of cultures of S. 4 in peptone broth was found to be toxic in high dilutions as judged by the intradermal test on human volunteers. Graduated doses of this toxin were given intravenously to a sheep. At the end of three months the sheep's serum had

acquired definite "blanching" properties in low dilutions, 0·2 c.c. of the serum in a dilution of one-tenth giving a well-marked Schultz-Charlton reaction when injected intradermally. In June and July, Dr C. Rundle, Medical Superintendent of the Liverpool City Hospital, Fazakerley, therefore made a series of tests with this serum and it was found that while the majority of rashes were blanched, a small proportion were unaffected. In attempting to interpret these reactions it was thought that the irregularities in the results of the Schultz-Charlton tests might be explained on the ground that "positive blanchers" and "negative blanchers" were infected by different types of streptococcus. Samples of the serum of a case whose rash was blanched by the sheep serum and of a case whose rash was unaffected were therefore taken during the fourth week of the illness. The blanching properties of the sheep serum and of these two sera—called "convalescent positive blancher's serum" and "convalescent negative blancher's serum"—were compared in the hope that the reactions obtained would throw some light upon the problem of typing. Among the cases so examined, Nos. 73 and 74, from which S.S. 71 and 72 were isolated respectively, gave particularly clear-cut results. The details of these two cases and the cultural characteristics of the strains which were isolated from them are given below:

	Scarlet fever	
	S. 71 from Case 73 L. G., aged 10½ 4th day—fatal	S. 72 from Case 74 N. W., aged 4 11/12 4th day
Schultz-Charlton reactions		
Serum 4191. Dr O'Brien preparation ...	+ + +	−
Sheep serum 4	+ + +	−
Convalescent Positive Blancher's serum	−	+ + +
„ Negative „ „	−	+ + +
Colonies on 5 % Horse blood agar plates		
Number of haemolytic colonies	About 1000	About 500
"Glass-clear" zone of haemolysis ...	Wide	Narrow
Colonies	Convex, surface granular, centre dark; firm, can be lifted entire from the plate	Flat, surface smooth, soft, 2–3 times size of S. 71
Growth in Peptone Broth		
Supernatant fluid	Clear	Uniformly turbid
Deposit	Heavy, coarse, granular	Light, slight, diffuses readily on shaking
Agglutination with Dochez serum (titre 1/15000)	+ + + 1/100 − 1/200	Negative

In view of their interest, both of these strains were used to make agglutinating sera, but I am not inclined now to attach much importance to their differences in cultural characteristics. The Dochez strain, S.S. 4, 71 and 72 were subsequently found to be serologically distinct types.

Some considerable time before the sera which I was preparing had attained to any practical value, Dr F. Griffith had obtained sera of high titre, and kindly provided me with a supply of these. Among the strains typed with his sera, strains 55 and 79, homologues respectively of his type strains S.F. 130/2 and S.F. 22, were used to make sera in case the strains which I had chosen proved to be serologically identical. The sera made with strains 84, 95 and 99

have been used as controls. S. 29 was originally made for this purpose, but it was subsequently found to be an homologue of Dr Griffith's S.F. 22 and our S. 72. A serum has also been made with the S. 21, a scarlet fever streptococcus which does not fall into any of the types so far recognised, but up to the present a few tests only have been made with this serum and they have not been included in the present report.

The scarlatinal sera used are described in the tables as Sera: Dochez 1, 2 and 3, and the non-scarlet sera as A, B and C. The following table gives the relationship of these sera to Dr F. Griffith's sera, together with the strains used in their preparation.

	Scarlet fever sera				Non-scarlet sera			
	Dochez	1	2	3	A	B	C	S. 84. Isolated from a superficial wound of the leg
Dr F. Griffith's sera	—	S.F. 130/2	S.F. 22	U.S 55/4 S.F. 14/2	—	—	—	S. 95 Isolated from the blood of cases of puerperal septicaemia
Liverpool sera	—	S. 71 S. 55	S. 72 S. 79 S. 29	S. 4	84	95	99	S. 99

Absorption Experiments.

The results of the absorption tests up to date are recorded in Table III. There is room for considerable difference of opinion as to the exact technique and scope of this test. Many strains examined appear to possess a very complex antigenic structure, which would require for its detailed analysis a more exact technique than has been employed. Attention has been confined for the most part to the recognition of specific antigens, care only being taken to ensure the use of an "efficient dose" of absorbing culture. In all the experiments recorded the absorbing dose has been the centrifuged deposit from 90 c.c. of a heavy, 18-hour trypsinised broth culture. The doses have probably varied within fairly wide limits and in any case they have frequently been considerably larger than the minimum amount required to exhaust the various sera by their homologous strains; moreover, the amount of agglutinin present in the different sera, and in different batches of the same sera, has varied considerably. To standardise these factors would have necessitated a lengthy preliminary research into the technique of the absorption test as applied to haemolytic streptococci. Since it does not seem necessary, however, to adopt a more refined technique than that which I have used to define the major antigens, it was decided to adhere to the method detailed below, the feeling being that with a more extended experience of the test and a number of records available one would be in a better position to correct any errors in the technique which might be exposed.

Method of absorption. The absorbing cultures (90 c.c. of broth, heavily sown, and incubated overnight) have been made in batches and stored for short periods with the addition of 1·0 c.c. of chloroform. When required for use they are centrifuged, and the supernatant fluid removed with the aid of

a double bulb filter pump attached to a water-tap. Usually about 0·5 c.c. of the fluid remains with the undisturbed deposit; this is made up to 1·25 c.c. with normal saline. The culture is thoroughly emulsified and transferred to a small centrifuge tube.

The serum has been used in different dilutions, allowance being made for its titre. 0·25 c.c. of suitably diluted serum is added to the absorbing culture, the two being thoroughly mixed to ensure contact. One control containing the same quantities of diluted serum and normal saline is put up with each batch. The tubes are securely stoppered to prevent evaporation. The absorption time has been fairly constant throughout—from 16 to 18 hours. The mixtures have been left on the bench overnight, with or without a preliminary 2 hours' incubation; in a few instances they have been left in the incubator overnight at 37° C. After centrifuging, sufficient absorbed serum is available for titration.

The serum is titrated in a series of agglutination tubes each of which contains the 0·5 c.c. of normal saline; 0·5 c.c. of the test suspension is then added to the tubes of diluted absorbed serum. The tubes are incubated at 52° C. for 5 hours. Readings have been taken at 1, 3 and 5 hours in most cases and these may be checked after standing on the bench or in the ice chest overnight.

Table III.

Absorption and agglutination reactions with Scarlet Fever Sera. Dochez, 1, 2, and 3.

(*a*) *Sera absorbed by each of the marginal strains and tested against their homologous strains for the presence of agglutinin.*

(*b*) *Direct agglutination reactions of those strains which have also been tested by this method.*

A. *Scarlatinal Strains.*

Strain No.		Sera			
		Dochez	1	2	3
Dochez	(*a*)	+	−	−	−
,,	(*b*)	40·000/40·000	640/20·000	0/20·000	0/20·000
S.F. 130/2 (S. 86)	(*a*)	−	+	−	−
	(*b*)	320/40·000	20·000/20·000	160/20·000	0/20·000
S.F. 22 (S. 87)	(*a*)	−	−	+	−
U.S. 55/4 (S. 88)	(*a*)	−	−	−	+
Zingher	(*a*)	+	−	−	−
	(*b*)	15·000/20·000	0/10·000	0/10·000	0/7·500
4	(*a*)	−	−	−	+
	(*b*)	2·500/40·000	1·280/20·000	640/20·000	20·000/20·000
71	(*a*)	−	+	−	−
	(*b*)	80/40·000	20·000/20·000	40/20·000	0/20·000
72	(*a*)	−	−	+	−
	(*b*)	0/10·000	0/20·000	20·000/20·000	0/10·000
1	(*a*)	−	+	−	−
	(*b*)	640/10·000	20·000/20·000	160/20·000	80/20·000
2	(*a*)	−	−	−	−
	(*b*)	160/40·000	0/20·000	40/20·000	0/20·000
3	(*a*)	−	−	−	−
	(*b*)	320/20·000	80/20·000	0/20·000	0/20·000

Table III A—*continued.*

Strain No.	Sera			
	Dochez	1	2	3
6 (*a*)	–	+	–	–
(*b*)	0/10·000	8·000/10·000	0/20·000	0/10·000
7 (*a*)	–	–	+	–
8 (*a*)	–	–	+	–
9 (*a*)	–	–	–	+
(*b*)	320/10·000	640/20·000	160/8·000	5·000/10·000
10 (*a*)	–	–	–	–
11 (*a*)	–	–	–	+
12 (*a*)	–	–	–	–
14 (*a*)	–	–	+	–
(*b*)	0/10·000	0/10·000	5·000/7·500	0/10·000
17 (*a*)	–	–	–	–
18 (*a*)	–	–	–	–
19 (*a*)	–	–	–	–
20 (*a*)	–	–	–	–
21 (*a*)	–	–	–	–
(*b*)	320/10·000	640/20·000	80/6·000	160/10·000
23 (*a*)	–	–	–	+
24 (*a*)	–	–	–	–
25 (*a*)	–	–	+	–
26 (*a*)	–	–	–	+
37 (*a*)	–	–	–	–
38 (*a*)	–	–	–	–
39 (*a*)	–	+	–	–
(*b*)	80/10·000	10·000/10·000	80/7·500	0/4·000
40 (*a*)	–	–	–	–
41 (*a*)	–	–	–	–
43 (*a*)	–	–	+	–
44 (*a*)	–	–	–	–
45 (*a*)	–	–	–	–
46 (*a*)	–	–	–	+
47 (*a*)	–	–	–	–
48 (*a*)	–	–	–	+
(*b*)	640/10·000	320/12·000	160/6·000	5·000/5·000
49 (*a*)	–	–	–	–
50 (*a*)	–	–	–	–
(*b*)	0/8·000	0/10·000	0/8·000	0/6·000
51 (*a*)	–	–	–	+
52 (*a*)	–	–	–	–
55 (*a*)	–	+	–	–
(*b*)	640/10·000	20·000/20·000	320/5·000	80/6·000
56 (*a*)	–	–	+	–
74 (*a*)	–	+	–	–
79 (*a*)	–	–	+	–
(*b*)	0/20·000	0/10·000	10·000/20·000	0/10·000
85 (*a*)	–	+	–	–
(*b*)	640/10·000	20·000/30·000	0/5·000	40/10·000
89 (*a*)	–	+	–	–
91 (*a*)	–	–	–	–
94 (*a*)	–	+	–	–
96 (*a*)	–	–	–	+
97 (*a*)	–	+	–	–
(*b*)	20/8·000	15·000/20·000	0/8·000	0/5·000
98 (*a*)	–	+	–	–
(*b*)	20/8·000	10·000/10·000	40/10·000	0/5·000
100 (*a*)	–	–	+	–
(*b*)	0/8·000	0/15·000	5·000/5·000	0/5·000
101 (*a*)	–	–	–	–
102 (*a*)	–	–	–	+
(*b*)	640/10·000	640/15·000	320/8·000	2·500/4·000

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Table III—*continued.*

B. *Non-Scarlatinal Strains.*

(1) Non-scarlet throat strains

Strain No.	Sera: Dochez	1	2	3	Diagnosis
15 (*a*)	–	–	+	–	Diphtheria
16 (*a*)	–	–	–	–	,,
27 (*a*)	–	–	–	–	Tonsillitis
32 (*a*)	–	–	+	–	,,
33 (*a*)	–	–	+	–	Otitis media
36 (*a*)	–	–	? +	–	Tonsillitis
53 (*a*)	–	–	–	–	,,
54 (*a*)	–	–	–	–	,,
73 (*a*)	–	–	–	–	,,
80 (*a*)	–	–	–	–	,,
81 (*a*)	–	–	–	–	,,
82 (*a*)	–	+	–	–	,,
113 (*a*)	–	–	–	–	,,
120 (*a*)	–	–	–	+	,,
(*b*)	1·280/10·000	80/20·000	320/12·500	5·000/5·000	,,
121 (*a*)	–	–	–	+	,,
(*b*)	80/10·000	320/20·000	160/6·000	2·500/4·000	,,
90 (*a*)	–	–	–	–	Normal throat
93 (*a*)	–	–	–	–	,, ,,
103 (*a*)	–	–	–	–	,, ,,
(*b*)	0/20·000	0/20·000	0/20·000	0/10·000	,, ,,
104 (*a*)	–	–	–	–	,, ,,
(*b*)	0/20·000	0/20·000	0/20·000	0/20·000	,, ,,
105 (*a*)	–	–	–	–	,, ,,
108 (*a*)	–	–	–	–	,, ,,
114 (*a*)	–	–	–	+	,, ,, (post-scarlatinal)
123 (*a*)	–	–	–	–	Tonsillitis

(2) Authentic non-scarlet strains

30 (*a*)	–	–	–	–	Pus. Ovarian abscess
34 (*a*)	–	–	–	–	Septicaemia
(*b*)	0/10·000	0/20·000	0/5·000	0/7·500	,,
59 (*a*)	–	–	–	–	,,
60 (*a*)	–	–	–	–	Pemphigus
66 (*a*)	–	–	–	–	Septicaemia
(*b*)	1·280/10·000	1·280/20·000	160/5·000	160/7·500	,,
78 (*a*)	–	–	–	–	Normal vagina
84 (*a*)	–	–	–	–	Septic wound
(*b*)	160/30·000	0/20·000	0/5·000	40/7·500	,,
95 (*a*)	–	–	–	–	Puerperal septicaemia
(*b*)	160/20·000	0/15·000	0/20·000	0/10·000	,, ,,
99 (*a*)	–	–	–	–	,, ,,
(*b*)	320/20·000	0/15·000	40/20·000	0/10·000	,, ,,
106 (*a*)	–	–	–	–	Peritonitis
107 (*a*)	–	–	–	–	Abscess. Arm
(*b*)	40/10·000	0/10·000	0/5·000	0/8·000	,,
109 (*a*)	–	–	–	–	Subcutaneous abscess. Horse
110 (*a*)	–	–	–	–	Osteo-myelitis
111 (*a*)	–	–	–	–	Empyema
122 (*a*)	–	–	–	–	Puerperal septicaemia
124 (*a*)	–	–	–	–	Osteo-myelitis

(3) Additional strains

5 (*a*)	–	–	–	–	Scarlet fever + cellulitis
(*b*)	80/20·000	40/20·000	0/5·000	0/10·000	,, ,, ,,
29 (*a*)	–	–	+	–	Post-scarlatinal cellulitis
(*b*)	0/20·000	0/5·000	7·500/10·000	0/7·500	,, ,, ,,
57 (*a*)	–	–	–	–	Scarlet fever, meningitis
92 (*a*)	–	–	? +	–	Scarlet fever, scalds

Note. Numerator of the fraction = Highest dilution in which complete agglutination occurred.
Denominator ,, ,, = Titre of the serum at the time of the experiment.

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Agglutination Experiments.

Most investigators have experienced considerable trouble in applying the technique of agglutination tests to this group of organisms, and, before any progress could be made, it was necessary to overcome this difficulty. The initial stages of the research were therefore concerned with an attempt to evolve a method of making stable suspensions of those strains which agglutinate spontaneously. The difficulty might have been avoided by relying upon the absorption of agglutinin test, provided one's type strains happened to produce a uniform turbidity in liquid media, in which case no difficulty would be experienced in making the necessary test suspensions for the titration of absorbed sera. Since, however, all the strains in my series, with three or four exceptions, exhibit the phenomenon of auto-agglutination in a marked degree, this has not been possible, and the problem had to be faced. For some time the technique evolved was applied to test suspensions of type strains only, attention being confined to absorption experiments. But after experience of the absorption test, when it became apparent that haemolytic streptococci comprise a wide group containing several specific antigens, agglutination experiments were commenced, and numerous strains were suspended, in the hope that by combining the results of the two tests more accurate information would be obtained concerning the antigenic structure and inter-relationship of different types of haemolytic streptococci than if attention were confined to the absorption tests alone. It was also desirable to decide the vexed question of whether the direct agglutination test can be successfully applied to this group of organisms.

The following is the method employed.

Strains are sown from blood broth to 3·0 c.c. of glucose trypsin broth and after three or four passages through this medium at 2-, 3- or 4-hourly intervals, they are passed through trypsin broth[1]. Those strains which suspend slowly in these media are passed through salt-free, peptone, meat infusion broth. Inoculations are made with a teat pipette instead of a platinum loop, 0·5 to 1·0 c.c. being passed over each time. A few strains can be suspended within two days by frequent subcultivation and heavy sowing at short intervals, but the majority of strains take from four to ten days to suspend, while occasional strains take longer. Any hastening of the process has resulted in unsatisfactory suspensions. When the culture stands up for 18 to 24 hours, at least 10·0 c.c. are transferred to 100·0 c.c. of trypsinised broth. This is incubated for 5 to 8 hours until sufficiently dense for use. It is then heated for 20 minutes at 45° C. to 50° C., 1·0 c.c. of chloroform being added as a preservative. If shaken up daily, such suspensions can be stored for several weeks; several have been kept for 5 months, and were quite suitable for use at the end of this period.

It was thought at first that haemolytic streptococci were peculiarly sensi-

[1] Douglas's Trypsin Broth without the added salts. *Lancet*, 10th October, 1914, p. 891.

tive to the action of electrolytes; for this reason suspensions were centrifuged, washed, and finally re-suspended in distilled water. Though the suspensions so obtained were smooth and stable, the resulting agglutination tests were unsatisfactory. Not only was the titre of the serum for these suspensions appreciably lowered, but agglutination was delayed and the resulting clumps of agglutinated organisms were small and soft. Some factor essential to the mechanism of agglutination was evidently absent. Further tests showed that, provided the strains had been satisfactorily suspended in the first place, they remained stable when mixed with sodium chloride in dilutions up to 1·0 per cent. Centrifuging and washing were therefore discontinued and sera were diluted with 0·85 per cent. sodium chloride.

Adopting this technique, sharp results are obtained, complete agglutination being characterised, when a high titre serum is employed, by the rapid appearance of large and fairly firm clumps which settle to the bottom of the tube, leaving the supernatant fluid clear. After standing overnight a fine light powdery deposit may settle in the control tube, though the supernatant fluid is not cleared. This deposit is rapidly dispersed by shaking the tube slightly and cannot be confused with the clumps which settle when agglutination has occurred.

Suspensions have been titrated against normal rabbit serum and also against pneumococcal and *Bacillus coli* agglutinating sera. As all the control tests were negative, they have not been recorded. Experiments with sera 1, 2 and 3 and the Dochez serum are recorded in Table III. In addition to the four scarlet fever sera, three non-scarlet sera—A, B and C—have been used in a number of experiments which are recorded in Table IV.

As in the case of the absorption tests, homologous strains are clearly defined; subject to minor variations in the sensitiveness and concentration of suspensions, the reactions of homologues keep pace, both as regards time and quantity, with the control titrations. With a good serum, homologues can usually be recognised within an hour, and after 5 hours' incubation at 52·5° C. complete agglutination has occurred either to titre or almost to titre. Comparing the two tests, there is a complete agreement, so far as homologous strains are concerned, and, judging from my experience with the forty strains tested by both methods, it is a matter of choice which of the two methods be employed; both are equally reliable when dealing only with specific antigens. When a large number of strains is being examined, probably the direct agglutination test is the least troublesome.

ANALYSIS OF RESULTS.

One hundred and one strains have been examined; 5 of these were received from Dr F. Griffith, namely the Dochez strain, a Zingher strain (U.S. 45/2), and his three type strains S.F. 130/2, S.F. 22, and U.S. 55/4. Of the remaining 96 strains, 53 were isolated from scarlet fever throats while 43 were isolated from other sources; 40 of these 96 strains absorb the agglutinins from one

Table IV. *Agglutination reactions with Sera A, B and C.*

Agglutinated strain	Agglutinating sera	Serum dilutions 1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5000	1/10000	1/20000	1/40000	Control	Diagnosis
84 (A)		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	−	−	"Septic wound"
Dochez (S. 31)		+++	+++	++	+	+	±	−	−	−	−	−	−	−	Scarlet fever
S.F. 130/2 (S. 86)		+++	+++	+++	+++	+++	+	±	−	−	−	−	−	−	,, ,,
Zingher (S. 112)		+	+	+	+	±	−	−	−	−	−	−	−	−	,, ,,
4		+++	+++	+++	+++	+++	+++	+++	+	−	−	−	−	−	,, ,,
9		++	++	++	++	++	+	−	−	−	−	−	−	−	,, ,,
14	A	−	−	−	−	−	−	−	−	−	−	−	−	−	,, ,,
24	(S. 84)	±	+	+++	+++	±	±	−	−	−	−	−	−	−	,, ,,
48		+++	+++	+++	+++	+++	++	±	−	−	−	−	−	−	,, ,,
50		−	−	−	−	−	−	−	−	−	−	−	−	−	,, ,,
34		−	−	−	−	−	−	−	−	−	−	−	−	−	Septicaemia
95 (B)		+	+	−	−	−	−	−	−	−	−	−	−	−	Puerperal septicaemia
99 (C)		+++	+++	+++	+++	+++	+	−	−	−	−	−	−	−	,,
107		+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	−	−	Abscess. Arm
95 (B)		+++	+++	+++	+++	+++	+++	+++	+++	++	−	−	−	−	Puerperal septicaemia
99 (C)		+	+	+	±	−	−	−	−	−	−	−	−	−	,, ,,
Dochez (S. 31)		−	−	−	−	−	−	−	−	−	−	−	−	−	Scarlet fever
4		+++	+++	+++	+++	++	−	−	−	−	−	−	−	−	,, ,,
9		−	++	+++	+	±	−	−	−	−	−	−	−	−	,, ,,
14	B	+++	+++	++	−	−	−	−	−	−	−	−	−	−	,, ,,
24	(S. 95)	±	±	±	−	−	−	−	−	−	−	−	−	−	,, ,,
48		+++	+++	+++	+++	−	−	−	−	−	−	−	−	−	,, ,,
50		−	−	−	−	−	−	−	−	−	−	−	−	−	,, ,,
71		+	+	+	±	−	−	−	−	−	−	−	−	−	,, ,,
72		+++	+++	+++	+++	+++	+	−	−	−	−	−	−	−	,, ,,
99 (C)		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	−	−	−	Puerperal septicaemia
95 (B)		+	++	++	+	±	−	−	−	−	−	−	−	−	,, ,,
122		−	−	−	−	−	−	−	−	−	−	−	−	−	,, ,,
Dochez (S. 31)		+	+	+	+	−	−	−	−	−	−	−	−	−	Scarlet fever
4		+++	+++	+++	+++	+++	+++	+++	−	−	−	−	−	−	,, ,,
9	C	−	±	+	+	+++	+++	+	−	−	−	−	−	−	,, ,,
14	(S. 99)	−	−	+	+	++	−	−	−	−	−	−	−	−	,, ,,
24		−	−	−	+	+	+	−	−	−	−	−	−	−	,, ,,
48		−	±	++	+++	+++	++	+	+	−	−	−	−	−	,, ,,
50		−	−	−	−	−	−	−	−	−	−	−	−	−	,, ,,
71		±	±	++	+	−	−	−	−	−	−	−	−	−	,, ,,

Note. +++ = Complete agglutination: firm deposit with clear supernatant fluid.
++ = Partial agglutination: marked granular deposit with light flocculi floating in almost clear supernatant fluid.
+ = Partial agglutination: less marked deposit with small granules floating in turbid supernatant fluid.
± = Little or no deposit; small clumps seen in turbid supernatant fluid when examined with a hand lens.

The titre of the sera at the time of the experiments is indicated by the vertical line.

or other of the three sera 1, 2 and 3; 30 of these identified strains were isolated from scarlet fever throats, so that 56·6 per cent. of the scarlatinal strains examined have been identified serologically. A homologue of the Dochez strain has not been encountered in my series, and this type must be very rare in Liverpool and district. The Zingher strain is evidently a homologue of the Dochez strain. Types 1, 2 and 3 occur in much the same proportion. The incidence of the four types in my series of scarlet fever strains is as follows:

	Dochez	1	2	3
Homologues	0	11	9	10
Percentage of scarlet fever strains identified	0	36·6 %	30·0 %	33·3 %
Percentage of scarlet fever strains examined	0	20·9 %	16·97 %	18·8 %

Ten of the 40 identified strains have still to be considered; 8 of these were isolated from inflamed throats, S. 29 from a case of osteo-myelitis following scarlet fever, and S. 92 from the discharge from an infected scald in a fatal case of scarlet fever. None of them were obtained from authentic non-scarlet sources. Particulars of these strains are given below:

S. 15 (*Type* 2) isolated from the throat of a girl, aged 9 years, while in hospital suffering from diphtheria.

S. 29 (*Type* 2) isolated from a case of osteo-myelitis. The patient, a girl aged 7 years, had scarlet fever in September, 1924, followed by pyaemia. Three septic foci were recognised, one of which was incised. S. 29 was isolated in pure culture from the pus obtained at the operation.

S. 32 *and* 33 (*Type* 2) isolated respectively from the throat and aural discharge of a woman aged about 40, suffering from a severe tonsillitis, complicated by a bilateral suppurative otitis media; a rash was not seen.

S. 36 (*Type* 1). The report of the medical attendant was as follows: G. R., aged 7 years, transient atypical rash confined to the right arm and shoulder. Temperature 100·0 degrees, slight malaise, tonsils enlarged and ulcerated, diagnosis uncertain, "tonsillitis (?) scarlet fever." Klebs-Loeffler bacilli were also recognised and the case was regarded as diphtheria. Four days later this boy's brother developed typical scarlet fever, S. 56 being isolated from his throat. (Case 66, S. 56.)

The following strains were isolated from swabs taken by Dr C. O. Stallybrass, Assistant Medical Officer of Health, Liverpool, who has been keenly interested in the question of the practical value of throat swabs as an aid in the diagnosis of angina of doubtful nature. Dr Stallybrass kindly supplied particulars of these cases. In each instance he was consulted because there was a suspicion of scarlet fever.

S. 82 (*Type* 1). A girl, W. W., aged 3 years, who, with her two brothers—cases 90 and 92—contracted sore throats. Haemolytic streptococci were not isolated from the two boys' throats. Though there was a suspicion of scarlet fever when the children were examined, the diagnosis was returned later as "tonsillitis, definitely not scarlet fever."

S. 114 (*Type* 3). A boy, H. D., aged 9 years, 6 xii. 25, onset of scarlet fever, which followed an uncomplicated course, the boy being discharged from hospital on 19. i. 26. Four weeks after his return home his mother developed scarlet fever (S. 117 was isolated from her throat but has not yet been tested), and one week later a brother, aged 8 years, also developed scarlet fever. H. D. was therefore suspected to be a "carrier." His throat when examined was normal, there were no "cracks" about his mouth or nose, and no nasal or aural discharge. A swab of the tonsils was taken and S. 114 isolated.

S. 120 *and S.* 121. Several cases of scarlet fever and tonsillitis of doubtful nature occurred

in the Royal Infirmary, Liverpool, during the winter months of 1925–26. Dr Stallybrass was consulted and obtained swabs from the following cases:

Case	Date	Ward	Diagnosis	β-haemolytic streptococci	Type	Strain No.
114. Nurse T.	Dec. 18	1	Scarlet fever	+	1	94
113. Miss E. T.	Dec. 23	1	Scarlet fever	+	3	96
129. Nurse T.	Jan. 4	A	Tonsillitis	−	.	.
130. Nurse L.	Jan. 4	B	Scarlet fever	+	3	102
154. Dr P., R.M.O.	Feb. 15	.	Tonsillitis, rheumatism	+	3	120
155. N. F., ward-maid	Dec. 18 / Feb. 15	1	Tonsillitis	+	3	121

Nos. 113, 114 and 130 were unmistakable cases of scarlet fever.

Nurse T. (Case 129) was carefully isolated and very closely watched, but no trace of a rash or desquamation was seen. The final diagnosis in her case was "tonsillitis."

Dr P. was seriously ill. His illness commenced on 13th February with a headache followed on 14th February by a sore throat, temp. 102·0° and a rigor. On 15th February there was a pultaceous deposit on both tonsils and a faint erythematous rash on the chest. There were no palpable lymphatic glands. Two weeks later a swelling of several joints occurred.

N.F. (Case 155). This girl had suffered from several attacks of "septic throat" since the commencement of the outbreak. She was suffering from sore throat at the time cases 113 and 114 occurred and was engaged as a maid in the ward in which Nurse T. was nursing and in which Miss E. T. was a patient. She also had another attack of tonsillitis at the time of Dr P.'s illness in February. It was therefore suspected that she might be a "carrier." It is significant that four of the strains isolated from these Royal Infirmary cases are of the same serological type.

S. 92 (? *Type* 2). Isolated from the discharge from an infected scald in a fatal case of scarlet fever.

The details of the above 10 strains have been given since they show that with most of them it has been impossible to exclude scarlet fever with certainty.

Group Reactions.

A consideration of the details of the titrations recorded suggests the existence of one or more secondary antigens. While homologous strains readily exhaust a serum of its agglutinins, many heterologous strains also lower the titre of the sera considerably. This is notably the case with serum 3 prepared from strain 4. Moreover, after absorption with these heterologous strains, the sera agglutinate the suspensions of the homologous strains more slowly than do the unabsorbed sera, and complete agglutination occurs in the lower and middle range of dilutions only, even after standing overnight. It may be that some of the strains exhibit both group and specific phases, which have been shown to exist within the Salmonella group by Andrewes and others. Many of the strains examined appear to possess a complex antigen, particularly the Dochez strain and S. 4, which appear to be more generalised strains than, for example, S. 72. The original colonies chosen from which to breed the type strains may have contained both the specific and unspecific elements, and the sera made with these mixtures must be correspondingly complex. Whether these elements, if they exist, can be separated is a matter for further research;

Table V. *Agglutination reactions of strains* 9, 14, 24 *and* 48.

Strain No.	Agglutinating serum	Serum dilutions 1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10000	1/20000	1/40000	Control
9	Normal serum	−	−	−	−	−	−	−	−	−	−	−	−	−
	B. coli	−	−	−	−	−	−	−	−	−	−	−	−	−
	Dochez	+	+	+ +	+ + +	+ + +	+ +	+	−	−	−	−	−	−
	1	+	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	−	−	−	−	−
	2	−	+	+ + +	+ + +	+	±	−	−	−	−	−	−	−
	3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	−	−	−	−
	A	+ +	+ +	+ +	+ +	+ +	+	−	−	−	−	−	−	−
	B	−	+ +	+ + +	+	±	−	−	−	−	−	−	−	−
	C	−	±	+	+	+ + +	+ + +	+	−	−	−	−	−	−
14	Normal serum	−	−	−	−	−	−	−	−	−	−	−	−	−
	B. coli	−	−	−	−	−	−	−	−	−	−	−	−	−
	Dochez	−	−	−	−	−	−	−	−	−	−	−	−	−
	1	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	−	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−
	A	−	−	−	−	−	−	−	−	−	−	−	−	−
	B	+ + +	+ + +	+ +	−	−	−	−	−	−	−	−	−	−
	C	−	−	±	+	+ +	−	−	−	−	−	−	−	−
24	Normal serum	−	−	−	−	−	−	−	−	−	−	−	−	−
	Dochez	−	−	−	−	−	−	−	−	−	−	−	−	−
	1	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	−	−	−	−	−	−	−	−
	3	−	−	−	−	−	−	−	−	−	−	−	−	−
	A	±	±	+	+ + +	+ + +	+	−	−	−	−	−	−	−
	B	±	±	±	−	−	−	−	−	−	−	−	−	−
	C	−	−	−	+	+	+	−	−	−	−	−	−	−
48	Normal serum	−	−	−	−	−	−	−	−	−	−	−	−	−
	B. coli	−	−	−	−	−	−	−	−	−	−	−	−	−
	Dochez	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	−	−	−	−	−	−
	1	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	−	−	−	−	−	−
	2	+ + +	+ + +	+ + +	+ + +	+	−	−	−	−	−	−	−	−
	3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	−	−	−
	A	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	±	−	−	−	−	−	−
	B	+ + +	+ + +	+ + +	+ + +	−	−	−	−	−	−	−	−	−
	C	−	±	+ +	+ + +	+ + +	+ +	+	+	−	−	−	−	−

Note. The titre of the sera at the time of the experiments is indicated by the vertical line.

up to the present I have failed to do so. Should it prove possible, more refined reagents could then be made.

The agglutination reactions of strains 9, 14, 24 and 48 are given for convenience of reference in Table V; they suggest considerable variation in the structure of individual strains. A number of experiments have also been carried out with the object of determining the nature and distribution of these secondary antigens. Up to the present, however, sufficient data have not been collected to enable one to draw any final conclusions; but, considered as a whole with the results of the absorption and direct agglutination tests, they appear to indicate the existence of one or more secondary antigens common to members of the genus isolated from widely different sources and not confined within the limits of a group such as a scarlatinal or puerperal group.

Toxin Reactions.

A number of tests have been made with the toxins of four serologically distinct types of streptococcus, namely, S.S. 4, 71, 72 and 34, none of which absorbed the agglutinin from the Dochez serum; S.S. 4, 71 and 72 were scarlet fever strains and S. 34 an authentic non-scarlet strain. S. 34 was isolated from the blood of a woman who died of a fatal streptococcal infection. The primary focus was a superficial wound of the right leg; a cellulitis followed by an arthritis of the right knee joint developed. At the post-mortem recent vegetations were found upon the aortic valve.

The technique described by Dick and Dick has been employed in making the toxins, which have been used in a dilution of 1/1000. The control solutions,

Table VI.

Reactions of toxins 4, 71, 72 *and* 34.

Case	Age	Diagnosis	History of scarlet fever	Toxins					Controls
				Dick	4	71	72	34	
1	35	Normal throat	4 years ago	.	−	.	.	.	−
2	23	,, ,,	3 years ago	.	−	.	.	−	−
3	25	,, ,,	−	+ + +	+ + +	.	.	+ + +	−
4	31	,, ,,	20 years ago	−	−	−	−	−	−
5	12	Measles	−	+ + +	.	+ +	−*	.	−
6	13	Rubella	+	−	.	−	.	.	−
7	10	Diphtheria	−	+ +	.	.	+ +	.	−
8	7	,, (50th day)	−	+ +	.	+ +	+ +	.	−
9	8	,, (3rd day)	−	+ +	.	+ +	+ +	.	−
10	35	Normal throat	−	.	+ + +	.	.	.	−
11	12	Scarlet fever	14th day	+	.	+	.	.	−
12	5	,, ,,	5th ,,	.	.	.	.	−	−
13	4	,, ,,	4th ,,	.	.	.	.	+ + +	−
14	9	,, ,,	19th ,,	.	.	.	.	−	−
15	8	,, ,,	3rd ,,	.	.	.	.	+ + +	−
16	5	,, ,,	27th ,,	.	.	.	.	−	−
17	9	,, ,,	27th ,,	.	.	.	.	−	−
18	3	,, ,,	33rd ,,	.	.	.	.	+ + +	−
19	15	,, ,,	39th ,,	.		.	.	−	−
20	6	,, ,,	3rd ,,	.	.	.	.	+ + +	−
21	14	,, ,,	41st ,,	.	.	.	.	−	−

* The negative result with toxin 72 was probably due to an error in technique.

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used in the same dilution, consisted of toxins boiled for one and a half hours. The tests were made with great care by Dr Rundle and Dr Hodgson, who have had a very extensive experience of toxin work at the City Hospital, Fazakerley. They state that in their opinion the reactions obtained with these toxins are indistinguishable from those given by the Dick toxin.

The results of the tests are given in Table VI. It is difficult to find patients who will submit to several "prickings," but, few as the tests are, they indicate that the antigenic differences which exist among this group of organisms do not affect to any considerable extent their toxin radicles. Certainly the toxins of serologically distinct types of scarlatinal strains appear to be identical, as judged by the intradermal tests; and the tests with the toxin of S. 34 suggest that there is a close relationship between the toxins of some at least of the non-scarlet strains and the toxins of the Dick streptococcus and S.S. 4, 71 and 72.

DISCUSSION AND CONCLUSIONS.

The initial object of this research being to test the claim made by Dick and Dick that a specific streptococcus is the causal agent of scarlet fever, a search has been made among a considerable number of patients for homologues of the Dick streptococcus. Not a single homologue of this strain has been encountered. This fact, combined with our as yet limited experience with the several toxins used, indicates an absence of the specificity originally claimed by these authors. It may be, however, that scarlet fever is caused by several serologically distinct types of streptococcus, and the extreme rarity of the Dochez strain in Liverpool and district may be explained on the supposition that there is considerable variation in the incidence of types in different parts of the world.

Working on the assumption that several types of haemolytic streptococcus may be concerned in this disease, a serological classification of scarlatinal streptococci is being attempted, and, up to the present, three sub-groups or types have been recognised among the scarlet fever strains, 56·6 per cent. of which fall into one or other of these three types. Further work is required to complete the classification of the remaining 43·4 per cent. of strains. These three types are defined by the apparently specific antigens 1, 2 and 3, but the three types are not as fixed as, for example, the three fixed pneumococcal types, and there is evidence which suggests that they contain individuals of varying degrees of antigenic complexity, many of them being more or less generalised strains which possess antigenic properties common not only to one or other of the three scarlatinal types, but also to members of the species outside the scarlatinal range. The three types 1, 2 and 3 occur in approximately the same proportion in my series.

With reference to the problem of the classification of haemolytic streptococci as a whole, three additional major antigens—A, B and C—have been defined among the non-scarlet strains, and the evidence suggests that the

species contains a large number of sub-groups possibly linked together by one or more group antigens.

Among the "non-scarlet" strains examined, it is significant that none of the authentic non-scarlet strains absorb the agglutinins from the four scarlet fever sera used. On the other hand, several of the clinically non-scarlet throat strains are of the same serological type as the scarlatinal throat strains, and the correct interpretation of these results is doubtful, in view of the possibility that the orthodox clinical definition of scarlet fever may be too narrow. In most outbreaks of scarlet fever, cases of considerable epidemiological importance are recognised in which inflammatory conditions of the throat occur in varying degrees of severity, unaccompanied by a rash. In some of these cases no doubt the rash is evanescent or localised, as, for example, in case 47, S. 36, and is consequently overlooked. In any case, the sequence of events which follows the inflammation of the throat and the exanthem of typical scarlet fever is absent in these instances. It may, of course, be argued that the streptococcus infection accompanying scarlatina is a secondary infection, and that the way is prepared for these cocci by an as yet unrecognised factor. But, while this possible explanation of scarlet fever must be borne in mind, it nevertheless seems sufficient to assume variations in the antitoxic content of the patient's blood together with variations in the virulence and toxigenic properties of the infecting strain to bring these cases into line with typical cases of scarlet fever. In support of this point of view, Case 46 and the Royal Infirmary cases associated with strain 121 are of considerable interest.

In the absence of the necessary critical experiments of direct inoculation, it is not possible to arrive at a final conclusion concerning the alleged causal relationship of streptococci to scarlet fever, but certain facts have been collected which yield indirect evidence in favour of this claim. A number of children in the most susceptible age period, whose throats were shown by preliminary swabbing to be free from haemolytic streptococci, were kept under observation. Subsequently two of the children developed scarlet fever and haemolytic streptococci were obtained from their throats. In view of the number of types which exist within the species, it is significant that the strains (S.S. 97 and 98) concerned in these two cases were members of Type 1.

It seems reasonable to suppose that, if a streptococcus is the direct causal agent, the strains isolated from the throats of cases exposed to a common source of infection should belong to a single type. Although direct association could not be proved with absolute certainty in the Royal Infirmary series, the evidence was strongly in favour of a common source of infection, and it is significant that a type 3 streptococcus was isolated from four of these cases. On the other hand, different types were isolated from two brothers—Cases 47 and 66. It seemed safe to assume a common source of infection in their case, since only three days intervened between the commencement of the illnesses. A systematic examination of such groups of cases would probably throw considerable light upon this aspect of the problem.

In conclusion, no facts have been discovered which are at variance with the main proposition, and although the results indicate a lack of the specificity originally claimed by Dick and Dick, I am of opinion that the results recorded in this report tend to support the claim that streptococci, though not necessarily identical in type, are the direct etiological factor in scarlet fever.

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