THE SPECIFIC TOXIGENIC PROPERTIES OF HAEMOLYTIC STREPTOCOCCI FROM SCARLATINA AND OTHER SOURCES.

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Introduction.

Since Dick and Dick, in 1924, demonstrated the formation by haemolytic streptococci from scarlatina of an extracellular toxic principle capable of producing a cutaneous reaction in non-immune persons, further work on the etiology of scarlet fever has added the strongest support to the view that the disease is streptococcal in origin. Thus, scarlatina has come to be regarded as primarily an infection by a haemolytic streptococcus, the general manifestations being due to a specific diffusible toxin (see Dick and Dick, 1923, 1924 *a*, *b*, 1925 *a*; Zingher, 1924 *a*; Dochez, 1925; O'Brien, 1926). It is unnecessary in this paper to review the data on which these conclusions have been based, but attention may be drawn to certain aspects of the etiological problem with which the investigation to be recorded is more immediately concerned.

Haemolytic streptococci constitute a group of organisms etiologically related to various pathological conditions, and also occur as commensals in the throats of healthy persons (Kerr, 1908; Eves and Watson, 1925; and others). The question has therefore arisen regarding the specificity of the streptococci so constantly associated with scarlatina. The tendency recently has been to regard the disease as due to a particular type of haemolytic streptococcus —"*Streptococcus scarlatinae*"—characterised by its capacity to produce the specific toxic principle referred to, and possibly distinct from the ordinary pyogenic strains. Efforts to identify scarlatinal streptococci by general biological characters and biochemical reactions have been unsuccessful, and in these respects strains show no essential difference from similar organisms isolated from various suppurative lesions and from non-scarlatinal inflammations of the throat.

The experimental inoculation of animals with scarlatina strains has failed to elicit any definite differences from other haemolytic streptococci, and experimental work with filtrates from cultures has also yielded negative results, owing apparently to the uniform insusceptibility of various animals even to large amounts of the filterable principle which is toxic to non-immune persons (see Dochez and Sherman, 1924; Dick and Dick, 1924 *c*; Rosenow, 1924; Okell and Parish, 1925). Kirkbride and Wheeler (1924, 1926) have reported, however, that fully-grown young goats are definitely susceptible to toxin preparations. These findings have not yet been confirmed by other workers.

On the other hand, some information regarding the specificity of "*Strepto-*

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coccus scarlatinae" and its products has been elicited from serological reactions, from the therapeutic results obtained by means of antitoxic sera prepared by "immunising" animals with culture filtrates, and from observations on the toxigenic properties of scarlatina strains, as evidenced by cutaneous tests in the human subject.

The serological relationship of scarlatina strains to other haemolytic streptococci has been extensively investigated. Early work indicated that strains from scarlatina cases formed a fairly well-defined serological group, differentiated from other strains by agglutination reactions. Thus, Bliss and Dochez (1920) claimed that about 80 per cent. of scarlatina strains could be classified in one group. Bliss (1922), Tunnicliff (1920, 1922), Eagles (1924), Dochez and Sherman (1924), and Stevens and Dochez (1924 *a*, *b*), reported similar results. Stevens and Dochez (1926), Eagles (1926), and Tunnicliff (1926) have more recently published observations supporting their previous findings. Other workers have stated that more than one serological type may be recognised by agglutination and agglutinin-absorption tests. Williams and Hussey (1924) found that only 35 per cent. of strains from scarlatina cases could be classified in one serological type, and Dick and Dick (1924 *c*) recorded that there were at least two serological varieties. Smith (1926) has identified two serological types of scarlatinal streptococci comprising more than 80 per cent. of the strains investigated by him. Observations by different workers on the serological grouping of these organisms are therefore contradictory, and it is still uncertain whether scarlatinal streptococci can be separated etiologically into a group or groups entirely distinct from other haemolytic streptococci as has been evidenced in recent work by Mackie and McLachlan (unpublished).

The only experimental criterion, therefore, of the identity of a scarlatina strain might appear to depend on the demonstration of its specific toxic principle by intradermal tests with a culture filtrate in known susceptible and non-susceptible persons. While such tests with the majority of strains from cases of scarlet fever have yielded results indicative of the formation of a specific toxin (Dick and Dick, 1924 *b*, 1925 *a*, *b*; Zingher, 1924 *b*), there has been a certain amount of evidence to show that the property is not strictly limited to scarlatina strains (Rosenow, 1924, 1926; Paraf, 1925; Kirkbride and Wheeler, 1926). This question is of obvious importance in relation to the whole etiological problem.

The investigation recorded in this paper was undertaken in order to ascertain the specific toxigenic power of a large number of scarlatina strains and at the same time to compare with them strains of haemolytic streptococci from other sources. Enquiry was also made as to whether the "toxins" showed evidence of any immunological differences and whether there was exact correspondence in the reactions among various individuals with preparations from different strains. The results with the scarlatina strains have shown considerable uniformity in these respects, while it has been clearly demonstrated that many non-scarlatina strains exhibit an analogous toxigenic

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property, though often quantitatively less than in the case of the former; further, anomalies have been elicited as regards the cutaneous reactions of different individuals compared with those produced by toxins from scarlatina strains.

Methods.

The specific toxigenic power of the various strains was ascertained by skin tests carried out according to the principle and technique of the Dick reaction in (*a*) known positive, (*b*) known negative reactors.

Neutralisation experiments with antitoxic sera were performed as a test for any specific difference in the character of toxins produced by strains from non-scarlatina sources compared with those from scarlet fever.

A standard toxin, prepared from a strain originally isolated in Edinburgh by Dr J. E. McCartney, was employed for purposes of comparison. This toxin had been extensively tested and was known to yield valid results in early scarlet fever cases and convalescents respectively. Toxin preparations from the strain used had been applied previously by Joe (1925) and had been found to give positive results in 95 per cent. of early cases of scarlet fever and positive results in 4·7 per cent. of convalescents.

At first, through the consent of Dr W. T. Benson, Medical Superintendent of the Edinburgh City Fever Hospital, it was possible to test the preparations from various strains on scarlet fever cases. Later, as the investigation became extended and a larger number of positive and negative reactors was required, it was necessary to perform the tests on healthy adults. By the courtesy of Colonel Ryan, A.M.S., it was possible to utilise soldiers in Edinburgh Military Hospital. Every individual on whom the various preparations were tested had been tested beforehand with the standard toxin. Positive and negative reactors identified by preliminary tests with the standard toxin were selected for the tests with the preparations from various strains.

The strains of "*Streptococcus scarlatinae*" were obtained from scarlet fever patients during the first week of the illness, generally about the 4th day. Throat swabs taken from the patients were plated on blood agar, and after incubation overnight, a single colony of the haemolytic streptococcus present was picked off and subcultured by successive strokes on a second blood agar plate. An isolated colony was subcultured from this second plate on a slope of coagulated sheep's blood medium. After adaptation to artificial culture the organisms remained viable on this medium for several weeks.

Strains of streptococci from other sources were obtained by plating out on blood agar the pathological material in which they occurred, and purification by replating was carried out as in the case of the scarlatina strains.

Toxins were prepared according to the method described by Mackie and McLachlan (1926). The streptococci were grown in a medium consisting of bouillon standardised to *p*H 7·6 with 5 per cent. sterile defibrinated rabbit's blood added. After incubation at 37° C. for 5 days, the blood broth cultures were heated to 57° C. for 1 hour. This procedure sterilises the culture and pre-

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cipitates a large amount of inactive material which can be deposited by centrifuging. The supernatant fluid (designated "57°-soluble fraction"), which contains the active toxic principle if present, can be removed and diluted to the appropriate degree for the purpose of the skin test. In a few instances filtrates of bouillon cultures were used similar to the preparation described by Ker, McCartney and McGarrity (1925).

The toxins (57°-soluble fraction) prepared from the scarlatinal streptococci were used in a dilution of 1 in 1000, and the control consisted of a similar dilution heated to 100° C. for $1\frac{1}{2}$ hours. The amount injected intradermally was 0·2 c.c. Nearly every toxin produced from scarlatinal streptococci proved to be active in this dilution when tested on susceptible subjects.

It was intended to test each toxin on at least three positive and three negative reactors, but owing to difficulty in obtaining a sufficient number of positive reactors the number of tests, in the case of some strains, had to be restricted.

The 57°-soluble fraction was obtained in a similar way from the cultures of streptococci isolated from non-scarlatina sources, and tested in various dilutions (1 in 100 to 1 in 1000).

Little trouble was experienced from pseudo-reactions. Persons who, in the preliminary test with the standard toxin and heated control, showed any degree of reaction to the control injection were not utilised for further tests.

The skin reactions were read 24 hours after the injections and the minimum reaction regarded as positive was an erythematous area 10 mm. by 10 mm. in diameter. Further, such minimum reactions were not accepted unless the corresponding control test was entirely negative.

Tests were also performed to ascertain whether the toxins were neutralised by a scarlatina antitoxic serum. A Parke-Davis concentrated serum was at first employed and the amount was ascertained which, when injected with one skin test dose of the standard toxin (0·2 c.c. of a 1 in 1000 dilution, or the equivalent of that amount), prevented the development of an erythematous area within 24 hours of the injection of the mixture. It was found that, if equal quantities of a 1 in 500 dilution of the standard toxin and a 1 in 2000 dilution of the Parke-Davis serum were mixed and incubated for 1 hour at 37° C., no reaction developed within 24 hours of injecting 0·2 c.c. of the mixture. A small erythematous patch occasionally developed subsequently, presumably owing to dissociation of the toxin-antitoxin mixture (see Zingher, 1924 *a*).

A Burroughs-Wellcome serum (unconcentrated) was also used for neutralisation experiments. It was necessary to add an equal volume of a 1 in 50 dilution of this serum to a 1 in 500 dilution of toxin in order to obtain neutralisation.

In making neutralisation tests with other toxins, the antitoxic serum was employed in the concentration found to neutralise the 1 in 500 dilution of the standard toxin.

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General Biological Characters of the Strains Employed.

All the strains employed were haemolytic, but varied quantitatively to a considerable extent in their haemolytic power. The colony appearances also varied widely, some organisms forming pin-point shining colonies after 24 hours' incubation at 37° C. on blood agar plates, other strains forming larger, flat colonies. Neither colony appearances nor haemolytic power afforded a means of distinguishing scarlatinal from other strains. None of the streptococci appeared to produce methaemoglobin when grown on blood media.

Biochemical tests showed the existence of three fermentative groups among the scarlatina strains, and of five fermentative groups among the non-scarlatinal streptococci in the series tested. The medium used in determining the fermentative powers of the organisms was a serum-smeared agar slope containing 1 per cent. of the sugar with litmus as indicator, the agar being made up without meat extract. After being inoculated, the sugar media were incubated for 7 days at 37° C., and the results were then read. This was the medium found most uniformly satisfactory for testing the fermentative reactions of streptococci.

Scarlatina Strains.

	Glucose	Lactose	Saccharose	Mannite	Raffinose	Salicin	Inulin
89 strains (including the standard strain)	⊥	⊥	⊥	–	–	⊥	–
4 strains (15, 34, 53, 57) ...	⊥	–	⊥	–	–	⊥	–
6 ,, (17, 19, 32, 49, 72, 80)	⊥	⊥	⊥	⊥	–	⊥	–

Non-Scarlatina Strains.

21 strains	⊥	⊥	⊥	–	–	⊥	–
6 ,, (A, F, G, S, T, T_2) ...	⊥	–	⊥	–	–	⊥	–
2 ,, (X, T_3)	⊥	⊥	⊥	⊥	–	⊥	–
2 ,, (C, T_1)	⊥	⊥	⊥	–	–	–	–
1 strain (U)	⊥	–	⊥	⊥	–	⊥	–

⊥ signifies fermentation of the sugar. – signifies absence of fermentation.

It has been stated (O'Brien, 1926) that certain mannite-fermenting streptococci, after isolation, may gradually lose their power of fermenting this sugar. The fermentative reactions of many of the strains employed were not tested till the organisms had been in artificial culture for some time. It is therefore possible that some mannite-fermenting strains may not have been recognised.

The biochemical reactions of some strains have been re-tested after an interval of several months and have proved to be stable.

Cutaneous Tests with "Toxin" Preparations.

Scarlatina Strains.

The general results are illustrated in Table I. Ninety-eight scarlatina strains were tested on known positive and negative reactors. As many of the results were similar and uniform, only illustrative cases are given. All exceptional results are, of course, quoted.

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From these results it is evident that the great majority of strains of haemolytic streptococci obtained from the throat in early cases of scarlet fever produce a toxic substance analogous to the standard toxin. However, three strains (68, 71 and 89) did not yield a toxic product capable of evoking a skin reaction in a known susceptible subject when the preparation was used in a dilution of 1 : 1000. Preparations from these strains, however, when tested in a higher concentration, gave rise to cutaneous reactions.

The "toxins" produced by the different strains varied somewhat in activity, but, for the most part, a toxin that gave a pronounced reaction in one susceptible individual led to a similar effect in another equally susceptible individual. This, however, was not always the case.

The reactions of two susceptible individuals, Reactor I and Reactor XII, are shown in the case of toxins 69, 70, 72, 78, 83, 89. Both reactors showed approximately equal reactions with the standard toxin. Comparison of the dimensions of the erythematous areas produced in the two reactors shows that there is considerable difference in the degree of reaction to toxins 69 and 70, more than is explicable by experimental error. Toxin 78, also, produced a weakly positive reaction in the one reactor but was nearly inactive in the other. Toxin 89 failed to produce a reaction in both individuals in a dilution of 1 : 1000. There was little difference in the activity of toxins 72 and 83, as judged by the degree of the reactions. Table I also shows the reactions of another susceptible individual (No. IX) to toxins 21, 23, 24, 25, 26, 27, 28, 29 and 49. In this case, the reactions are parallel to those in the positive reactor No. 1 column, but in most cases slightly weaker.

One toxin (17) produced a definitely positive reaction in a child on the 5th day of the illness, whereas the standard toxin produced only a faint evanescent reaction that had faded before 24 hours elapsed. Other tests with toxin 17 served to show that it was similar to the standard toxin but more powerful.

The results, therefore, with the scarlatina strains have been remarkably uniform with the exceptions quoted. A small minority of the strains (68, 71 and 89) exhibited a weaker toxigenic effect according to the method used for testing this property, and certain strains, *e.g.* 69 and 70, produced toxins which varied in their effect on different individuals in whom the standard toxin yielded identical results.

Strains from Sources other than Scarlatina.

The results are illustrated in Table II and have been particularly interesting. The sources of the various strains (designated A, B, C, etc.) are shown in the table, and their biochemical reactions have been indicated above. In many cases, even where high concentrations of the preparations were tested on known positive reactors, negative results were obtained (*e.g.* strains A, B, E, F, G, etc.), but in some instances, as in the cases of scarlatina strains, positive reactions were obtained in known positive reactors and negative results in known negative reactors.

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The toxins prepared from the non-scarlatina strains could be classed into five categories as a result of the intradermal tests:

I. Preparations resembling the toxin of scarlatina strains and effective in the same high dilution—strains K, N, R, U, Z.

II. Preparations similar to the toxin of scarlatina strains but quantitatively weaker. Thus, a reaction was elicited in the case of D in a dilution of 1 in 100, and in the case of O, P, T, X, T_2 in a dilution of 1 in 250, but not in higher dilutions.

III. Preparations failing to produce a reaction even in susceptible individuals in a dilution of 1 in 100—strains A, B, E, F, G, I, L, M_a, Q, V.

IV. Preparations that produced positive reactions in known positive reactors and, in addition, positive results in persons who did not react to the standard toxin. Such preparations were derived from

H, tested in a dilution of 1 in 1000,
M, ,, ,, ,, 1 in 1000,
Y, ,, ,, ,, 1 in 1000.

In the case of two of these preparations, M and Y, the reaction in insusceptible individuals was not surprising in view of the intensity of the effect produced in persons susceptible to the standard toxin (*vide* Table IV). In the case, however, of the other preparation, the reaction in known positive individuals was comparatively mild.

V. In the case of a certain number of strains, the reactions obtained appeared to bear no parallel to the tests with the standard toxin, reactions occurring irregularly in both known positive and known negative reactors. These results were yielded by preparations derived from C, S, T_3 and T_5 tested in a dilution of 1 in 100 and W tested in a dilution of 1 in 250.

The number of individuals tested with strains in this category was, however, small.

It is to be noted that in all cases where tests were carried out with high concentrations of the toxin preparation, the corresponding heated control gave a negative result.

While the vast majority of strains associated with scarlatina produce some similar toxic principle detectable by cutaneous tests, only a percentage of strains from non-scarlatinal sources possess this property. As judged by these intradermal tests, however, certain of the non-scarlatina strains form a toxic principle as active as that from scarlet fever strains, and indistinguishable from it by simple tests in known positive and negative reactors.

There appears to be no relationship between the biochemical reactions and the toxigenic properties of scarlatina and non-scarlatina strains.

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Table I. *Results of tests with "toxins" from scarlatinal streptococci.*

Strain of strepto-coccus	Day of illness on which isolated	Dilution of "toxin" (57°-soluble fraction of blood bouillon culture)	Known positive reactors tested			Known negative reactors tested			Dimensions of skin reactions		
			Total	With positive results	With negative results	Total	With positive results	With negative results	Positive reactor No. I (in all cases the same person) mm.	Another positive reactor	
										Reactor	mm.
Standard	—	1 : 1000	—	—	—	—	—	—	20 × 25	IX	20 × 22
21	4th	,,	4	4	0	3	0	3	16 × 15	IX	14 × 11
23	4th	,,	4	4	0	3	0	3	14 × 14	IX	11 × 12
24	4th	,,	3	3	0	4	0	4	10 × 12	IX	15 × 12
25	4th	,,	3	3	0	2	0	2	14 × 12	IX	12 × 10
26	5th	,,	3	3	0	3	0	3	13 × 14	IX	10 × 11
27	4th	,,	3	3	0	4	0	4	14 × 14	IX	12 × 12
28	3rd	,,	3	3	0	4	0	4	14 × 13	IX	13 × 13
29	5th	,,	3	3	0	4	0	4	14 × 13	IX	12 × 12
49	4th	,,	3	3	0	5	0	5	21 × 16	IX	17 × 19
Standard	—	—	—	—	—	—	—	—	—	XII	20 × 25
69	4th	,,	2	2	0	2	0	2	15 × 15	XII	25 × 29
70	5th	,,	2	2	0	3	0	3	10 × 16	XII	30 × 30
72	5th	,,	2	2	0	2	0	2	20 × 20	XII	15 × 15
78	6th	,,	2	1	1	2	0	2	6 × 6	XII	12 × 12
83	4th	,,	2	2	0	1	0	1	12 × 12	XII	18 × 19
89	5th	,,	2	0	2	1	0	1	Negative	XII	Negative
		1 : 100	1	1	0	1	0	1	11 × 11	—	—
Standard	—	1 : 1000	—	—	—	—	—	—	—	VIII	16 × 16
18	4th	,,	2	2	0	4	0	4	13 × 13	VIII	10 × 10
30	4th	,,	2	2	0	2	0	2	13 × 12	VIII	15 × 17
31	4th	,,	2	2	0	3	0	3	13 × 15	VIII	12 × 11
32	4th	,,	2	2	0	2	0	2	16 × 17	VIII	11 × 14
33	3rd	,,	2	2	0	3	0	3	15 × 18	VIII	13 × 10
34	5th	,,	2	2	0	3	0	3	15 × 16	VIII	11 × 11
35	4th	,,	3	3	0	2	0	2	18 × 20	VIII	12 × 13
38	3rd	,,	2	2	0	2	0	2	13 × 12	VIII	13 × 12
39	4th	,,	2	2	0	2	0	2	20 × 21	VIII	14 × 14
40	4th	,,	2	2	0	4	0	4	18 × 18	VIII	13 × 12
41	4th	,,	2	2	0	4	0	4	15 × 16	VIII	12 × 12
42	3rd	,,	2	2	0	2	0	2	18 × 18	VIII	15 × 16
50	3rd	,,	2	2	0	2	0	2	20 × 22	VIII	12 × 10
Standard	—	—	—	—	—	—	—	—	—	VII	11 × 11
17	5th	,,	3	3	0	4	1	3	20 × 25	VII	18 × 15
19	3rd	,,	2	2	0	3	0	3	15 × 22	VII	12 × 13
68	5th	,,	2	0	2	2	0	2	Negative	VII	Negative
		1 : 250	1	1	0	1	0	1	10 × 10	—	—
Standard	—	1 : 1000	—	—	—	—	—	—	—	XV	13 × 15
71	5th	,,	2	0	2	3	0	3	5 × 5	XV	Negative
		1 : 250	1	1	0	1	0	1	15 × 15	—	—

Filtrates of blood bouillon cultures of strains 1, 2, 3 and 5, prepared according to the method of Ker, McCartney, and McGarrity (1925), were tested by Dr Benson, City Fever Hospital, Edinburgh, and were reported as yielding results similar to those given by the standard toxin in early cases of scarlet fever and in convalescents respectively.

The results of the control tests are not shown in the table, as they were uniformly negative, *i.e.* no reaction occurred, or, as in a few instances, there was only a small papule at the site of injection.

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Table II. *Results of tests with preparations of strains from sources other than scarlatina.*

Strain of strepto-coccus	Source of strain	Dilution of 57°-soluble fraction	Tests on positive reactors			Tests on negative reactors		
			Number tested	With positive results	With negative results	Number tested	With positive results	With negative results
A	Cervical abscess: case of diphtheria ...	1 : 100	2	0	2	1	0	1
B	Abscess of finger in child	1 : 100	2	0	2	1	0	1
C	Erysipelas	1 : 100	2	1	1	2	0	2
D	Peritonitis	1 : 100	2	2	0	2	0	2
E	Infection of knee joint	1 : 100	1	0	1	1	0	1
F	Simple abscess	1 : 100	2	0	2	1	0	1
G	Simple abscess in leg	1 : 100	1	0	1	1	0	1
H	From nose	1 : 1000	2	2	0	2	1	1
I	Nasal catarrh	1 : 100	1	0	1	2	0	2
K	Mastoiditis	1 : 1000	2	2	0	2	0	2
L	Tonsilitis	1 : 100	1	0	1	1	0	1
M	Septicaemia following tonsilitis ...	1 : 1000	2	2	0	4	1	3
N	Otitis media	1 : 1000	2	2	0	1	0	1
O	Simple abscess	1 : 250	2	2	0	2	0	2
P	Mastoiditis	1 : 250	2	2	0	2	0	2
Q	Purulent conjunctivitis	1 : 100	2	0	2	1	0	1
R	Abscess of finger	1 : 1000	2	2	0	2	0	2
S	Cervical abscess: case of diphtheria ...	1 : 100	2	1	1	2	1	1
T	Throat of healthy person	1 : 250	2	2	0	2	0	2
U	,, ,, ,, ...	1 : 1000	2	2	0	2	0	2
V	,, ,, ,, ...	1 : 100	2	0	2	3	0	3
W	Tonsilitis	1 : 250	2	1	1	1	0	1
X	Subcutaneous abscess of foot	1 : 250	2	2	0	3	0	3
Y	Empyema	1 : 1000	2	2	0	2	1	1
Z	Tonsilitis	1 : 1000	2	2	0	2	0	2
M_a	Tonsil of case from which M was obtained	1 : 100	1	0	1	2	0	2
T_1	Throat of healthy person	1 : 100	2	0	2	2	0	2
T_2	,, ,, ,, ...	1 : 250	2	2	0	2	0	2
T_3	,, ,, ,, ...	1 : 100	2	1	1	2	1	1
T_4	,, ,, ,, ...	1 : 100	1	0	1	1	0	1
T_5	,, ,, ,, ...	1 : 100	2	0	2	2	1	1
T_6	,, ,, ,, ...	1 : 100	2	0	2	2	0	2

In the case of strains A, B, C and D the ordinary filtrate of blood broth cultures was used as toxin.

Neutralisation Tests with Antitoxic Sera.

Scarlatina Strains.

As was previously mentioned, neutralisation experiments were employed in order to test whether the toxins from the different strains were neutralised by a scarlatina antitoxic serum.

The results of certain of these neutralisation tests have been selected for illustration and are shown in Table III. The typical results are illustrated in the case of strains 1, 7, 41, and all the exceptional results are noted. Twenty-nine of the 98 toxin preparations from scarlatina strains were not neutralised by the Parke-Davis serum used in a dilution of 1 in 2000, *i.e.* the dilution that sufficed to neutralise the standard toxin. Even when a 1 in 200 dilution of the Parke-Davis serum was employed, twelve of the scarlatinal toxins were still unneutralised, as judged by the skin reaction. Two of these toxins, however, viz. 55 and 69, were neutralised by a Burroughs Wellcome serum employed in a dilution of 1 in 50.

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Non-Scarlatina Strains.

The toxic preparations from non-scarlatina strains were neutralised by scarlatina antitoxic serum with the exception of that produced by M. Selected illustrative results of these tests are shown in Table IV.

Table III. *Neutralisation of toxic preparations with antitoxic serum.*

			Scarlatina Strains.		Unneutralised toxin	
Strain	Dilution of toxin	Serum	Dilution of serum	Dimensions of reaction	Dilution of toxin	Dimensions of reaction
1	1 : 500	B.W.	1 : 50	Nil	1 : 1000	17 × 18 mm
7	,,	,,	1 : 50	,,	,,	14 × 12
10	,,	P.D.	1 : 200	10 × 10	,,	20 × 17
	,,	B.W.	1 : 50	15 × 15		
17	,,	P.D.	1 : 2000	Nil	,,	20 × 25
25	,,	,,	1 : 200	10 × 10 (faint)	,,	14 × 12
	,,	B.W.	1 : 50	12 × 12		
29	,,	P.D.	1 : 200	12 × 12	,,	14 × 13
	,,	B.W.	1 : 50	14 × 14		
31	,,	P.D.	1 : 2000	10 × 8 (faint)	,,	15 × 15
	,,	,,	1 : 200	Nil		
32	,,	,,	1 : 2000	12 × 12	,,	16 × 17
	,,	,,	1 : 200	Nil		
35	,,	,,	1 : 2000	10 × 10	,,	18 × 20
	,,	,,	1 : 500	Nil		
39	,,	,,	1 : 2000	10 × 10	,,	20 × 21
	,,	,,	1 : 200	Nil		
40	,,	,,	1 : 2000	10 × 10	,,	18 × 18
	,,	B.W.	1 : 50	15 × 15		
41	,,	P.D.	1 : 2000	Nil	,,	15 × 16
42	,,	,,	1 : 2000	13 × 13	,,	18 × 18
	,,	,,	1 : 200	Nil		
43	,,	,,	1 : 200	13 × 13	,,	14 × 16
	,,	B.W.	1 : 50	14 × 14		
44	,,	P.D.	1 : 200	15 × 15	,,	16 × 16
	,,	B.W.	1 : 50	13 × 13		
45	,,	P.D.	1 : 2000	12 × 12	,,	15 × 15
	,,	,,	1 : 200	Nil		
46	,,	,,	1 : 2000	13 × 13	,,	14 × 12
	,,	,,	1 : 200	Nil		
47	,,	,,	1 : 2000	10 × 10 (faint)	,,	18 × 18
	,,	,,	1 : 200	Nil		
50	,,	,,	1 : 2000	10 × 11 (faint)	,,	20 × 22
	,,	,,	1 : 200	Nil		
51	,,	,,	1 : 2000	14 × 14	,,	20 × 20
	,,	,,	1 : 200	Nil		
52	,,	,,	1 : 2000	13 × 13	,,	13 × 14
	,,	,,	1 : 200	Nil		
55	,,	,,	1 : 2000	13 × 13	,,	23 × 24
	,,	,,	1 : 200	10 × 10 (faint)		
	,,	B.W.	1 : 50	Nil		
58	,,	P.D.	1 : 2000	10 × 10	,,	15 × 15
	,,	,,	1 : 200	Nil		
60	,,	,,	1 : 2000	14 × 14	,,	19 × 20
	,,	,,	1 : 200	Nil		
61	,,	,,	1 : 2000	14 × 14	,,	18 × 17
	,,	,,	1 : 200	Nil		
62	,,	,,	1 : 2000	10 × 12 (faint)	,,	17 × 18
	,,	,,	1 : 200	Nil		
64	,,	,,	1 : 2000	10 × 10	,,	13 × 13
	,,	,,	1 : 200	10 × 10		
	,,	B.W.	1 : 50	12 × 12		
65	,,	P.D.	1 : 200	10 × 11	,,	12 × 12
	,,	B.W.	1 : 50	12 × 12 (faint)		
68	1 : 125	,,	1 : 50	Nil	1 : 250	10 × 10

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Table III—*continued.*

			Scarlatina Strains.		Unneutralised toxin	
Strain	Dilution of toxin	Serum	Dilution of serum	Dimensions of reaction	Dilution of toxin	Dimensions of reaction
69	1 : 500	P.D.	1 : 2000	13 × 13	1 : 1000	15 × 15 mm.
	,,	,,	1 : 200	10 × 14		
	,,	B.W.	1 : 50	Nil		
70	,,	P.D.	1 : 2000	13 × 14	,,	16 × 17
	,,	B.W.	1 : 50	Nil		
71	1 : 125	,,	1 : 50	,,	1 : 250	15 × 15
73	1 : 500	P.D.	1 : 200	10 × 11	1 : 1000	14 × 15
	,,	B.W.	1 : 50	10 × 12		
77	,,	P.D.	1 : 2000	10 × 10	,,	13 × 12
	,,	,,	1 : 200	Nil		
80	,,	,,	1 : 200	10 × 10	,,	10 × 10
	,,	B.W.	1 : 50	12 × 12		
82	,,	P.D.	1 : 2000	11 × 11		
	,,	,,	1 : 200	Nil		
89	1 : 50	B.W.	1 : 50	,,	1 : 100	11 × 11

B.W. = Burroughs Wellcome. P.D. = Parke-Davis.

Table IV. *Neutralisation of toxic preparations with antitoxic serum.*

			Non-Scarlatina Strains.		Unneutralised toxin	
Strain	Dilution of toxin	Serum	Dilution of serum	Dimensions of reaction	Dilution of toxin	Dimensions of reaction
C	1 : 50	B.W.	1 : 50	Nil	1 : 100	15 × 15 mm.
D	1 : 50	,,	1 : 50	,,	1 : 100	13 × 14
H	1 : 500	,,	1 : 50	,,	1 : 1000	14 × 14
K	1 : 500	,,	1 : 50	,,	1 : 1000	21 × 20
M	1 : 500	,,	1 : 50	18 × 18	1 : 1000	22 × 24*
		,,	1 : 25	14 × 15		
		P.D.	1 : 2000	15 × 15		
N	1 : 500	B.W.	1 : 50	Nil	1 : 1000	15 × 15
O	1 : 125	,,	1 : 50	,,	1 : 250	15 × 16
P	1 : 125	,,	1 : 50	,,	1 : 250	20 × 20
S	1 : 50	,,	1 : 50	,,	1 : 100	13 × 14
Y	1 : 500	,,	1 : 50	18 × 20 (faint)	1 : 1000	20 × 22*
		,,	1 : 25	Nil		
T_2	1 : 125	,,	1 : 50	,,	1 : 250	12 × 14
T_3	1 : 50	,,	1 : 50	,,	1 : 100	12 × 12

* These areas were surrounded by a fainter erythematous area about $2\frac{1}{2}$ inches in diameter.

Discussion.

The results of the investigation have shown clearly that scarlatina strains resemble each other in their almost uniform power of producing a toxin that is active in a high dilution (*e.g.* 1 in 1000). Only five of the non-scarlatina strains were found to produce a toxin that acted in a similar manner and in an equally high dilution. It is to be noted that four of these five strains were derived from the throat or infections traceable to the throat. Several non-scarlatina strains, however, yielded a toxic substance which, in dilutions less than 1 in 1000, behaved like scarlatinal toxin. Toxic preparations from other non-scarlatina strains produced positive reactions in known negative reactors as well as in known positive reactors, and preparations from some non-scarlatinal streptococci failed to produce a skin reaction, even in a dilution of 1 in 100.

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Strains of haemolytic streptococci from sources other than scarlet fever, therefore, fail to show the uniform and marked toxigenic property of the scarlatinal streptococci.

Other observers have noted that culture filtrates of non-scarlatinal streptococci may produce reactions similar to those yielded by culture filtrates of scarlatina strains (Rosenow, 1924, 1926; Paraf, 1925; Williams, 1925). Birkhaug (1925) has obtained toxic filtrates from erysipelas strains, and Lash and Kaplan (1926) from puerperal septicaemia strains. Eagles (1926) has reported that strains from erysipelas, puerperal fever, and other conditions produced toxic filtrates differing apparently from filtrates of *S. scarlatinae* only in that they were weaker. Kirkbride and Wheeler (1926) found that 90 per cent. of scarlatina strains and 65 per cent. of non-scarlatina strains produced toxins, as judged by cutaneous tests in goats. Rosenow (1926) has also demonstrated the similarity of the toxins from scarlatina and certain non-scarlatina strains. He states that strains of haemolytic streptococci obtained in the acute stage of scarlet fever develop a more "highly irritant" toxin than strains isolated in the later stages of the illness. He also claims that it is possible to stimulate in a non-toxigenic strain the power of producing toxin by passage through animals. He believes, therefore, that this toxigenic power is an acquired property of haemolytic streptococci. It is evident, therefore, that the power of producing a toxic principle is not a specific characteristic of scarlatina strains, since some non-scarlatina strains form a toxin closely resembling scarlatinal toxin, though often to a lesser extent, while other strains yield substances that differ considerably from scarlatinal toxin in their action on known positive and known negative reactors.

Neutralisation tests with antitoxin revealed differences in the behaviour of the scarlatinal toxins. Of 98 scarlatinal toxins tested, 10 were not neutralised by an antitoxic serum. Similar results have been reported by Kirkbride and Wheeler (1926), who found that only 67 per cent. of a series of scarlatinal toxins were neutralised by antitoxic serum. Results such as these would suggest variation in the antigenic composition of the toxins of different scarlatina strains. Park and Spiegel (1925) have recorded results suggestive of the complex antigenic composition of scarlatinal toxin.

Similar tests, however, with the non-scarlatinal toxins showed that, with one exception, these were all neutralised by scarlatinal antitoxin. This has already been observed by other workers. Eagles (1926) has stated that all toxic filtrates, regardless of their source, are neutralised by antitoxin developed by immunisation of horses. He states that antitoxic sera obtained by the immunisation of rabbits are more specific, and suggests that the lengthy process of immunisation in horses may conduce to the development of group antibodies to other haemolytic streptococci. In this connection, it is of interest to note that Kirkbride and Wheeler, employing a scarlatinal antiserum prepared by immunisation of goats over a period of four months, have reported that only 67 per cent. of scarlatinal toxins and 70 per cent. of non-scarlatinal preparations are neutralised. Birkhaug (1925) has stated that toxic filtrates

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from erysipelas strains are neutralised by erysipelas antitoxin but not by scarlatinal antitoxin.

Neutralisation tests with horse antitoxic serum in this investigation have, however, demonstrated differences amongst the scarlatinal toxins, but have failed to distinguish scarlatinal toxins from those produced by streptococci from sources other than scarlet fever. Such results are difficult to explain and require further investigation.

These tests show how closely the scarlatinal streptococci are related to other haemolytic streptococci and the difficulty of demonstrating satisfactorily that "*Streptococcus scarlatinae*" is a distinct variety characterised by specific toxigenic properties. Conditions such as surgical scarlet fever and puerperal scarlet fever suggest that the power of producing a scarlatinoid condition may be widespread amongst these streptococci, and it is possible that strains of scarlatinal streptococci may differ from pyogenic strains in their invasive properties as well as in their toxigenic characteristics. The toxins produced by some of the non-scarlatina strains are identical, so far as can be judged by antitoxin neutralisation tests, with those produced by indubitable scarlatina strains, but the fact that, on the whole, scarlet fever strains are capable of producing "toxin" more active quantitatively than similar preparations of strains from other sources, would indicate that scarlet fever strains form a group, differentiated to some extent from other streptococci by their greater power of producing this extracellular toxic substance.

Summary and Conclusions.

1. Without exception, ninety-eight strains of haemolytic streptococci isolated from scarlet fever cases have been found to produce a toxic principle which in high dilutions is capable of evoking a cutaneous reaction in susceptible persons.

2. A certain proportion of haemolytic streptococci from other sources possess a similar toxigenic property. In the majority of such cases this property is only weakly developed as judged by quantitative tests. In a few instances a "toxin" is produced which is equally active with that of scarlatina strains.

3. Certain non-scarlatina strains produce "toxins" which evoke a cutaneous reaction both in known positive reactors and also in persons who do not react to a standard toxin from a scarlatina strain.

4. In the majority of cases the toxins produced by scarlatina strains are neutralised by the scarlatina antitoxic sera at present being used for therapeutic purposes, but a certain proportion of such toxins are not neutralised.

5. The "toxins" produced by non-scarlatina strains are in the majority of cases neutralised by the antitoxic sera.

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