VOLUME XXXIX JANUARY, 1939 No. 1

THE STREPTOLYSINS OF VARIOUS GROUPS AND TYPES OF HAEMOLYTIC STREPTOCOCCI; A SEROLOGICAL INVESTIGATION

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IT has been demonstrated (Todd, 1938) that two distinct serological varieties of streptolysin may be prepared from group A haemolytic streptococci. They have been named streptolysin O to indicate sensitivity to oxygen and streptolysin S to indicate extractability in serum. Reduced filtrates of cultures grown in Hewitt's glucose-bicarbonate broth contain streptolysin O free from streptolysin S. On the other hand, serum streptolysin and Weld's haemotoxin are mixtures of both forms of streptolysin; and attempts to prepare streptolysin S free from streptolysin O have not been successful. Streptolysin O is antigenic; streptolysin S apparently ceases to be an antigen when separated from streptococci. Consequently when animals are inoculated with haemolytic streptococcal filtrates prepared from group A strains they produce antistreptolysin O but no antistreptolysin S; and when living cultures of group A strains are used as the antigen both antistreptolysin O and antistreptolysin S are produced. These two different kinds of sera have been prepared from a single group A strain of haemolytic streptococcus and they have been used in the following serological investigation of the streptolysins of various groups and types of haemolytic streptococci.

THE EFFECT OF GROUP A ANTISTREPTOLYSIN S ON SERUM STREPTOLYSINS DERIVED FROM STRAINS BELONGING TO DIFFERENT SEROLOGICAL GROUPS AND TYPES

The neutralizing action of group A antistreptolysin S on serum streptolysins, prepared from strains belonging to each of Lancefield's groups and to each of Griffith's types, has been determined. The various strains were cultivated for 12 hr. in 20% serum broth which was then passed through Seitz filters. The streptolysin O in the filtrates was neutralized by adding an excess of antistreptolysin O; the residual streptolysin S was then titrated against rabbit sera of known antistreptolysin S titre to detect serological differences between the lysins. The neutralization tests were set up in the same way as antistreptolysin S titrations (Todd, 1938). They consisted of two sets of six tubes containing respectively increasing dilutions of normal rabbit serum and the same dilutions of antistreptolysin S serum. The serum dilutions were made up to

0·5 c.c. with borate buffer solution at *p*H 8·0; and control tubes containing borate buffer solution, without any serum, were also included in the tests. Each tube then received 1 M.H.D. of the serum streptolysin to be examined made up to 1·0 c.c. with borate buffer solution, excess of antistreptolysin O being supplied by adding 0·1 c.c. of concentrated antistreptolysin O to 10·0 c.c. of diluted serum streptolysin. The concentrated serum contained 50,000 units of antistreptolysin O per c.c. and only 2·0 units per c.c. of antistreptolysin S. Each tube in the titrations therefore received an additional dose of 500 units of antistreptolysin O and only the negligible quantity of $\frac{1}{250}$ of a unit of antistreptolysin S. Any haemolysis occurring under these conditions is not due to streptolysin O. The tubes were shaken and incubated in a water-bath at 37° C. for 15 min. during which time the streptolysins were neutralized by their antibodies. Each tube then received 0·5 c.c. of a 5% suspension of washed rabbit's blood cells in normal saline; and after 2 hr. incubation, the presence or absence of haemolysis was noted.

In a number of instances the M.H.D. was greater than 0·5 c.c.; in these cases 0·5 c.c. or 1·0 c.c. of serum streptolysin was used in neutralization tests instead of 1 M.H.D.

Table I records a few typical neutralization tests, and it shows that 1 M.H.D. of group A serum streptolysin was neutralized by a dose of immune serum ten times smaller than the neutralizing dose of normal serum. When less than 1 M.H.D. of group A lysin was used, the range of neutralization by immune serum was extended (strain N.W. 28). On the other hand, the lysins of groups B, C, D and G were neutralized by a dose of immune serum either equal, or almost equal, to the neutralizing dose of normal serum. The most pronounced inequality, observed in the whole experiment, between the neutralizing doses of normal and immune sera for heterologous group lysins is shown in Table I under the heading strain "Niel". In this case 0·5 c.c. of normal serum partially neutralized a dose of group C lysin which was completely neutralized by 0·5 c.c. of immune serum. An additional immune serum containing 313 antistreptolysin S units per c.c. was therefore included in this neutralization test to investigate the degree of serological overlapping; and it was found that although the second immune serum contained sufficient antistreptolysin S in 0·004 c.c. to neutralize 1 M.H.D. of group A serum streptolysin, yet 0·2 c.c. failed to neutralize 1 M.H.D. of the group C serum streptolysin. The degree of serological overlapping is therefore very small. A still smaller difference between the neutralizing doses of normal and immune sera for heterologous group lysins is seen in Table I under the heading strain "Harrison".

Table II summarizes the results of neutralization tests and it shows that antistreptolysin O and antistreptolysin S acting together neutralized all the group A lysins but failed to neutralize any of the lysins of groups B, C, D, E, F, G, H, or K. Three different immune sera with antistreptolysin S titres of 50, 30 and 25 units per c.c. were used in the neutralization tests. All the

Table I. *Neutralization of serum streptolysins of haemolytic streptococci belonging to various groups and types by the combined action of both antistreptolysins*

Character of strains			Number of M.H.D.s used in titrations	Character of serum	Volumes of sera used in neutralization tests (c.c.)						
Group	Type	Name			0·5	0·2	0·1	0·05	0·02	0·01	Control
A	8	S.F. 4	1	Normal	0	+ + +	+ + + +	C	C	C	C
			1	Immune	0	0	0	0	+ +	+ + +	C
A	9	Symons	1	Normal	0	+ + +	+ + + +	C	C	C	C
			1	Immune	0	0	0	0	+ + +	+ + + +	C
A	11	Blackmore	1	Normal	0	+ +	+ + +	+ + + +	C	C	C
			1	Immune	0	0	0	0	+ +	+ + +	C
A	4	N.W. 28	<1	Normal	0	+	+ +	+ + +	+ + +	+ + +	+ + +
			<1	Immune	0	0	0	0	0	+	+ + +
B	I*a*	O. 90	<1	Normal	+ +	+ +	+ +	+ +	+ +	+ +	+ +
			<1	Immune	+ +	+ +	+ +	+ +	+ +	+ +	+ +
B	II	Lewis	<1	Normal	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
			<1	Immune	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
C	20	Niel	1	Normal	+	+ + +	+ + + +	C	C	C	C
			1	Immune	0	+ +	+ + +	+ + +	C	C	C
C	B2	Chestle	1	Normal	+	+ + +	+ + + +	C	C	C	C
			1	Immune	+	+ + +	+ + + +	C	C	C	C
D	—	Heazman	<1	Normal	+ +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
			<1	Immune	+ +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
G	16	Harrison	1	Normal	0	+ +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
			1	Immune	0	+	+ +	+ + +	+ + + +	+ + + +	+ + + +

C = complete haemolysis. + + + +, + + +, + +, +, = degrees of partial haemolysis. 0 = no haemolysis.
Normal serum contained 5·0 units of antistreptolysin S per c.c. Immune serum contained 50·0 units of antistreptolysin S per c.c.

Table II. *Neutralization of the serum streptolysins of strains belonging to different groups and types by the combined actions of both antistreptolysins*

Lancefield group	Type	Strain	Minimal haemolytic dose c.c.	Neutralizing dose of immune serums c.c.
A	1	S.F. 130	>0·5	0·05
A	2	S.F. 22	>0·5	0·05
A	3	Richards	0·1	0·05
A	4	N.W. 28	>0·5	0·02
A	5	Franklin	0·05	0·05
A	6	283 T	0·05	0·05
A	6	S. 43	0·1	0·05
C	7	Azgazardah	0·05	0·5
C	7	E. 3	0·05	0·5
A	8	S.F. 4	0·2	0·05
A	9	Symons	0·2	0·05
A	10	E. 14 M.V.	0·01	0·05
A	11	Blackmore	0·2	0·05
A	12	S.F. 42	0·1	0·05
A	13	Anderson	0·5	0·1
A	14	Williams	0·05	0·1
A	15	J.S. 5	>0·5	0·05
A	15	Gay	>0·5	0·05
G	16	Harrison	0·01	0·5
A	17	Gillard	0·05	0·1
A	18	Hutchinson	0·2	0·05
A	19	S.F. 73/4	0·05	0·1
C	20	Niel	0·01	0·5
C	21	Angel	0·05	>0·5
A	22	63 T	0·02	0·1
A	23	King	>0·5	0·05
A	24	G. 54	0·1	0·1
A	25	Matthews	0·05	0·1
A	26	S.F. 13	>0·5	0·1
A	27	S.F. 40	0·5	0·1
A	28	Small	0·05	0·1
A	—	O. 89	0·05	0·05
A	—	C. 203	0·05	0·05
B	Ia	O. 90	>0·5	>0·5
B	Ib	Greenaway	>0·5	>0·5
B	II	Lewis	>0·5	>0·5
B	III	Futterman	>0·5	>0·5
C	A	Thompson	0·05	>0·5
C	A	K. 64	0·05	0·5
C	—	L.O.	0·01	0·5
C	—	L.M.	0·01	0·5
C	B2	Chestle	0·05	>0·5
C	B2	Austin	0·01	0·5
D	—	Heazman	>0·5	>0·5
E	—	K. 128	0·1	0·5
F	—	H. 60 R.	0·5	>0·5
G	—	Valenti	0·05	0·5
H	—	Challis	>0·5	0·5
K	—	D. 34 E.	>0·5	0·5

The neutralizing dose of normal rabbit serum for the various lysins was never less than 0·5 c.c.

Column 1. Groups described by Lancefield (1933), Lancefield & Hare (1935) and Hare (1935).

Column 2. Types 1–28 described by Griffith (1935). Types I*a*–III of group B strains described by Lancefield (1934, 1938). Types A and B2 of group C strains described by Ogura (1929).

Strains Azgazardah, E. 3, Harrison, Niel and Angel are placed in the order of Griffith's types, although they do not belong to group A.

Strains L.O. and L.M. are the O and M variants of Loewenthal (1934) which he isolated from highly contagious septicaemia in mice.

Serum streptolysin was obtained from the group D strain by cultivation in 20 % serum broth, containing 1·0 % of sodium bicarbonate, for 12 hr. at 30° C.

https://doi.org/10.1017/S0022172400011645 Published online by Cambridge University Press

group A serum streptolysins were neutralized by the antistreptolysin S sera to full titre; six of the heterologous group lysins showed the small degree of serological overlapping which has already been described; the remaining fifteen heterologous group lysins were not neutralized by group A antistreptolysin S. These experiments show that group A antistreptolysin S, prepared from a single strain, neutralizes streptolysin S prepared from all types of group A strains; but it does not neutralize the serum streptolysins of heterologous groups although a small degree of serological crossing between the groups may be observed with individual strains. Group A streptolysin S is therefore group-specific but not type-specific.

THE EFFECT OF GROUP A ANTISTREPTOLYSIN O ON SERUM-FREE STREPTOLYSINS DERIVED FROM STRAINS BELONGING TO DIFFERENT SEROLOGICAL GROUPS AND TYPES

Strains belonging to each of Lancefield's groups and to each of Griffith's types were cultivated in Hewitt's glucose-bicarbonate broth and the haemolytic titres of the cultures were determined at intervals. When satisfactory rapid haemolysis occurred, indicating the probable presence of streptolysin O, the cultures were filtered through Maasen candles. The filtrates were reduced with sodium hydrosulphite and stored under vaseline seal at 2° C. for about 10 days to get rid of any streptolysin S which might be present. They were then titrated against antistreptolysin O prepared from a group A strain. Table III summarizes the results of these neutralization tests.

The streptolysins which were formed in serum-free broth by strains belonging to various groups had the following characteristics.

Group A. With two exceptions all the group A strains formed serum-free streptolysins which were neutralized by antistreptolysin O. The exceptions were both type 11 strains which produced insufficient haemolysin for neutralization tests. It has been noticed that certain group A strains, on continued subcultivation, gradually lose their power of producing streptolysin O while retaining the power of producing streptolysin S; it is possible that the strains "Blackmore" and "Lang" are extreme examples of this gradual decline. The streptolysins formed by three additional type 11 strains in glucose-bicarbonate broth all had low haemolytic titres; one of them was completely neutralized, and two were partially neutralized, by antistreptolysin O. The tests, in which partial neutralization occurred, showed traces of haemolysis in tubes containing an excess of antistreptolysin O; this haemolytic action was completely neutralized by antistreptolysin S. The filtrates evidently still contained traces of streptolysin S after storage under reducing conditions for 10 days.

Group B. The strains which were isolated from normal women and which were apparently non-pathogenic failed to produce streptolysin. Three strains, from fatal human infections, which were described by Fry (1938), all produced

Table III. *Neutralization by antistreptolysin O of reduced serum-free streptolysins prepared from strains belonging to different groups and types*

(1) Group	(2) Type	(3) Name of strain	(4) Minimal haemolytic dose c.c.	(5) Neutralizing dose of A.S.O. c.c.	(6) Origin of culture
A	1	S.F. 130	2·0	0·00002	Human. Scarlet fever
A	2	S.F. 22	0·5	0·00005	,, Scarlet fever
A	3	Richards	0·02	0·00003	,, Puerperal fever
A	4	N.W. 28	0·05	0·00002	,, Scarlet fever
A	5	Franklin	0·5	0·00003	,, Puerperal fever
A	6	283 T.	0·05	0·00001	,, Scarlet fever
A	6	S. 43	0·05	0·00002	,, Broncho-pneumonia
C	7	Azgazardah	0·4	0·00002	,, Puerperal fever
C	7	E. 3	1·2	0·00002	,, Erysipelas
A	8	S.F. 4	0·2	0·00003	,, Scarlet fever
A	9	Symons	0·9	0·00002	,, Sore throat
A	10	E. 14 M.V.	0·05	0·00003	,, Scarlet fever
A	11	Blackmore	>5·0	—	,, Scarlet fever
A	11	Adams	1·2	0·00003*	,, Scarlet fever
A	11	Kemball	2·0	0·00003*	,, Scarlet fever
A	11	Lang	>5·0	—	,, Scarlet fever
A	11	Dobson	1·2	0·00002	,, Scarlet fever
A	12	S.F. 42	0·5	0·00003	,, Scarlet fever
A	13	Anderson	0·2	0·00004	,, Tonsillitis
A	14	Williams	0·05	0·00003	,, Puerperal fever
A	15	J.S. 5	0·05	0·00002	,, Scarlet fever
A	15	Gay	0·12	0·00004	,, Erysipelas
G	16	Harrison	0·16	0·00002	,, Puerperal fever
A	17	Gillard	0·05	0·00003	,, Puerperal fever
A	18	Hutchinson	0·1	0·00003	,, Throat infection
A	19	S.F. 73/4	0·2	0·00004	,, Scarlet fever
C	20	Niel	0·6	0·00001	,, Throat infection
C	21	Angel	0·6	0·00002	,, Scarlet fever
A	22	63 T.	1·0	0·00002	,, Scarlet fever
A	23	King	0·2	0·00002	,, Scarlet fever
A	24	G. 54	1·2	0·00003	,, Tonsillitis
A	25	Matthews	0·8	0·00002	,, Normal throat
A	26	S.F. 13	0·2	0·00002	,, Scarlet fever
A	27	S.F. 40	1·6	0·00002	,, Scarlet fever
A	28	Small	0·3	0·00003	,, Tonsillitis
A	—	O. 89	0·1	0·00002	,, Scarlet fever
A	—	C. 203	2·0	0·00002	,, Scarlet fever
B	Ia	O. 90	>5·0	—	? Human. ? Scarlet fever
B	Ib	Greenaway	>5·0	—	Human, vagina. Non-pathogenic?
B	II	Lewis	>5·0	—	,, ,, Non-pathogenic?
B	III	Futterman	>5·0	—	,, ,, Non-pathogenic?
B	—	Cole	1·2	>0·1	,, Fatal subphrenic and liver abcesses with pleural effusion
B	—	Brownett	1·2	>0·1	,, Fatal endocarditis
B	—	Shipley	0·6	>0·1	,, Fatal endocarditis
C	A	Thompson	>1·0	>0·1†	Guinea-pig
C	A	K. 64	>1·0	>0·1†	,, Lymphadenitis
C	—	L.O.	>5·0	—	Mouse. Contagious septicaemia
C	—	L.M.	>5·0	—	,, Contagious septicaemia
C	B2	Chestle	0·04	0·00001	Human. Vagina. Non-pathogenic
C	B2	Austin	0·1	0·00003	,, Vagina. Non-pathogenic
D	—	Heazman	>5·0	—	,, Vagina. Non-pathogenic
E	—	K. 128	>5·0	—	Certified milk
F	—	H. 60 R.	>5·0	—	Human. Pleural fluid
F	—	Pargeter	>5·0	—	,, Normal throat
F	—	Mahoney	>5·0	—	,, Normal throat
G	—	Sears	0·5	0·00002*	,, Normal throat
G	—	Valente	0·7	0·00002	,, Vagina. Non-pathogenic?
G	—	50	1·0	0·00002*	Bitch. Abortion
G	—	51	1·2	0·00002*	,, Abortion
G	—	Mac	5·0	0·00002*	Dog. Discharging ear
G	—	53	>5·0	—	Bitch. Abortion
H	—	Challis	>5·0	—	Human. Normal throat
K	—	D. 34 E.	>5·0	—	,, Normal throat

A.S.O. = antistreptolysin O serum containing 20,000 units per c.c.

* = partial neutralization.

† = fresh filtrates tested as reduction with sodium hydrosulphite caused disappearance of haemolysin.

https://doi.org/10.1017/S0022172400011645 Published online by Cambridge University Press

streptolysins which did not disappear when mixed with sodium hydrosulphite and stored for 10 days in the cold. These lysins were not neutralized by antistreptolysin O.

Group C. Six group C strains, which were isolated from human sources, all produced streptolysins which were neutralized by antistreptolysin O. On the other hand, four group C strains which were isolated from animal infections either failed to produce streptolysin or they produced weak streptolysins which were not preserved by reduction. Fresh haemolytic filtrates prepared from these strains were not neutralized by antistreptolysin O; and the haemolysin disappeared after reduction with sodium hydrosulphite followed by cold storage under vaseline seal for 10 days. This seems to show that the haemolysins of group C strains derived from human infections differ both chemically and serologically from the haemolysins of group C strains isolated from animal infections. It is known that human and animal group C strains differ in other respects. Hare (1935) pointed out that group C strains derived from human infections or from human carriers ferment trehalose but not sorbitol, they are fibrinolytic and belong to type B2 of Ogura (1929). On the other hand, group C strains derived from animal infections either fail to ferment both trehalose and sorbitol or they ferment sorbitol but not trehalose. They do not produce fibrinolysin and they are either *Streptococcus equi* or type A of Ogura.

Group G. Three strains from human sources all produced streptolysins which were neutralized by antistreptolysin O. Three strains, which were isolated by Hare & Fry (1938) from dogs, also formed streptolysin O. In neutralization tests with four of the group G lysins there were two zones of haemolysis. The zone of complete haemolysis included all tubes containing less than 0·00002 c.c. of serum; while tubes containing an excess of antistreptolysin O showed only traces of haemolysis. This partial neutralization indicates that a second form of streptolysin, which was not neutralized by antistreptolysin O, remained in the filtrates in an active state after 10 days under reducing conditions.

Groups D, E, F, H and K failed to produce streptolysin in glucose-bicarbonate broth.

Table III gives in column 5 the volumes of antistreptolysin O serum required to neutralize 1 M.H.D. of the various reduced serum-free streptolysins; the neutralizing dose varies from 0·00005 c.c. to 0·00001 c.c. This degree of variation is probably due to the different quantities of irreversibly oxidized streptolysin O in the various filtrates and it is probably within the limits of experimental error.

The neutralization tests show that strains belonging to groups A, C (human) and G all produced streptolysin O while strains belonging to the other groups, including group C (animal) did not form this variety of streptolysin.

A comparison of Tables II and III shows that strains which produced powerful streptolysins in serum broth did not necessarily produce equally

powerful streptolysins in serum-free broth; in fact there was no correlation between the haemolytic titres of the streptolysins which were formed in the two different culture media by individual strains. This is evidently due to independent variation of ability to produce streptolysin O and streptolysin S.

Discussion

Group A haemolytic streptococci produce two distinct serological varieties of streptolysin which have been named respectively streptolysin O to indicate sensitivity to reversible oxidation and reduction, and streptolysin S to indicate solubility in serum. Antibodies to these two forms of streptolysin have been prepared from group A strains; but no attempt has yet been made to prepare antibodies to the streptolysins of the other groups of haemolytic streptococci. The serological analysis of the streptolysins is therefore incomplete; the only sera available being group A antistreptolysin O and group A antistreptolysin S. Neutralization tests with these antibodies show that group A antistreptolysin S neutralizes group A streptolysin S but does not neutralize the serum-soluble streptolysins of strains belonging to other groups. Group A streptolysin S is therefore group-specific; and its neutralization by homologous serum provides a simple test for the recognition of group A strains which can be applied in conjunction with the usual test for "soluble haemolysin".

On the other hand, group A antistreptolysin O neutralizes the streptolysin O which is formed not only by strains belonging to group A but also by group G strains, and by those strains belonging to group C which are isolated from human infections. The streptolysin O of groups C (human) and G is serologically indistinguishable from that of group A. The group C strains which are isolated from animal infections do not produce streptolysin O.

There is reason to suppose that group C (human) strains and group G strains may each form group-specific streptolysins, similar to those of group A, since they both produce, in addition to streptolysin O, serum-soluble streptolysins which differ serologically from either of the group A streptolysins. Antibodies to these serum-soluble streptolysins have not yet been prepared but if they are eventually found to be group-specific it will be necessary to refer to group C streptolysin S and group G streptolysin S.

Group A antistreptolysin O and group A antistreptolysin S do not neutralize either the serum-free streptolysin or the serum streptolysin of group B strains; it is therefore uncertain whether these organisms produce one or two varieties of streptolysin. The same uncertainty applies to group C (animal) strains and it is increased by the fact that glucose-bicarbonate broth appears to provide optimal conditions for the production of streptolysin O by strains belonging to groups A, C (human) and G. Lowenthal & Pradhan (1935) found that a group C (animal) strain did not produce streptolysin in glucose-bicarbonate broth but they were able to prepare serum-free streptolysin from this strain

by adding cysteine hydrochloride to anaerobic cultures in O'Meara's (1934) broth which does not contain added glucose. They found that serum-free streptolysin prepared in this way from a group C (animal) strain lost its haemolytic activity after aerobic storage in the cold for 48 hr. and that although the haemolytic activity of comparatively fresh filtrates could be fully restored by reduction, yet older filtrates which had been stored in the cold for about a fortnight were not restored to haemolytic activity by reduction. This observation, which has been confirmed in the present work, shows that group C (animal) strains do not produce a serum-free streptolysin having the same characteristics as streptolysin O; additional evidence on this point is now supplied by the observation that the serum-free streptolysin of group C (animal) strains is not neutralized by antistreptolysin O. Lowenthal & Pradhan found that serum streptolysin prepared from a group C (animal) strain was reactivated by reduction in the same way as their serum-free streptolysin so that there is no evidence that these strains produce two varieties of streptolysin.

It is possible that, if suitable culture media were employed, strains belonging to groups B, C (animal), D, E, F, H and K might each produce two varieties of streptolysin but in the absence of serological evidence there is no reason to suppose that haemolysis by these strains is due to multiple lysins.

Todd (1934) found that *Pneumococcus* haemolysin and tetanolysin, which are subject to reversible oxidation and reduction in the same way as streptolysin O, are both neutralized to a considerable extent by antistreptolysin O. This serological relationship between the haemolysins of such diverse organisms as streptococci and tetanus bacilli suggests the possibility that all those bacterial haemolysins which are preserved indefinitely by reduction may be serologically related. On the other hand, *Staphylococcus* haemolysin, which is not subject to reversible oxidation and reduction, is not neutralized by either of the antistreptolysins.

Hare (1937), in a table compiled from the work of several authors, gives the relative frequency of human infections by the various groups of haemolytic streptococci as follows: group A, 431 cases; group B, 3 cases; group C, 11 cases; group D, 1 case; and group G, 3 cases. Fry (1938) has described three fatal human infections due to group B haemolytic streptococci. It is interesting to note that three of the groups of haemolytic streptococci which most commonly infect man, namely groups A, C (human) and G, all produce streptolysin O; an increase in the antistreptolysin O titres of sera from patients infected with any of these groups may therefore be expected; but no increase can be expected in the group A antistreptolysin S titres of sera from patients infected with groups C and G. On the other hand, an increase in the antistreptolysin O titres of sera from patients infected with group B strains is not to be expected since these organisms do not produce streptolysin O *in vitro*.

Group F strains which are the "minute haemolytic streptococci" described by Long & Bliss (1934) have been found by Long *et al.* (1934) to occur

frequently in the throats of patients with glomerular nephritis. Longcope (1936) found that the sera of patients infected with "minute haemolytic streptococci" may have very high antistreptolysin O titres. He also found that high antistreptolysin O titres occurred regularly in a form of acute haemorrhagic nephritis which he named type A. In a second form of nephritis, also preceded by streptococcal infection, which he named type B the antistreptolysin O titres were rarely above the normal level. Long & Bliss (1938) isolated "minute haemolytic streptococci" from 96% of cases of type A glomerular nephritis and from 40% of type B glomerular nephritis. These observations seem to show that "minute haemolytic streptococci" produce streptolysin O *in vivo* and yet Table III shows that three group F strains all failed to produce streptolysin O in glucose-bicarbonate broth. This discrepancy indicates the need for further studies on the production of streptolysin O by group F strains.

In the work recorded in this paper only single strains belonging to groups D and E have been examined for streptolysin production. In an earlier paper on the formation of streptolysin in glucose-bicarbonate broth (Todd, 1934) it was reported that three group E strains produced weak haemolysins which were not neutralized by antistreptolysin O and two group D strains produced no haemolysin. It is therefore probable that group D and E strains do not produce streptolysin O and, if infections due to these groups occur, an increase in the antistreptolysin O titre of the serum would not be expected. Of the remaining groups, namely H and K, which are probably not pathogenic, only single strains have been examined and they both failed to produce streptolysin O.

When streptolysins, prepared by the methods described in this paper, are mixed with their respective antibodies to form neutral mixtures precipitation does not occur. This probably indicates that they are less complex substances than the "labile antigen" described by Mudd *et al.* (1938).

Summary

1. Group A haemolytic streptococci produce two distinct varieties of streptolysin:

(*a*) Streptolysin O which is oxygen sensitive.

(*b*) Streptolysin S which is serum extractable.

These two streptolysins are neutralized by separate antibodies which appear to be entirely unrelated.

2. Neutralization experiments *in vitro* with group A antistreptolysin O show that streptolysin O is formed by strains belonging to groups A, C (human) and G; it is not formed by strains belonging to groups B, C (animal), D, E, H and K. The relation of group F strains to streptolysin O is at present uncertain.

3. Group A strains of different serological types all produce streptolysin S neutralizable by group A antistreptolysin S. Strains belonging to groups B, C, D, E, F, G, H and K do not produce streptolysin S neutralizable by group A antistreptolysin S. The neutralization is therefore group-specific but not type-specific.

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(*MS. received for publication* 27. VIII. 38.—Ed.)