STREPTOCOCCUS S.B.E.: IMMUNOLOGICAL CHARACTERISTICS

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A detailed description of the physiological characteristics of *Streptococcus* s.b.e., a hitherto unrecognized group of streptococci associated with subacute bacterial endocarditis, has been given in a previous publication (White and Niven, 1946). Since these streptococci seemed to comprise a relatively homogeneous group as judged by physiological tests, it seemed desirable to determine their serological properties.

The results of this study have shown that 37 of the 42 cultures studied fall into one serological type, whereas the remaining 5 strains fall into a second type. The interrelationships existing between these two types, as determined by the precipitin and agglutinin techniques, will be discussed.

METHODS

Antisera were prepared by injecting heat-killed cells intravenously into rabbits in accordance with the technique of Lancefield (1933). The cells were harvested from 18-hour meat infusion broth cultures containing 0.1 per cent glucose and 0.2 per cent K_2HPO_4, resuspended in $\frac{1}{2}$ volume of saline, and killed by heating to 56 C for 1 hour. One to two ml. of this suspension was injected daily for 7 days, followed by a week's rest period. Usually 3 series of injections were sufficient for obtaining satisfactory serum.

In the precipitin experiments the procedure of Lancefield (1933) was followed with respect to the preparation of cell extracts and the technique of testing. When absorption procedures were used, the technique of Lancefield (1938) was employed.

The agglutination experiments were performed by using cellular antigens prepared in the same manner as for animal inoculation. All cell suspensions were adjusted to approximately uniform turbidities before testing.

In studying the immunological characteristics of "Streptococcus MG," a streptococcus recovered from cases of primary atypical pneumonia, Mirick *et al.* (1944*a*) reported that washed cells of these organisms showed a much higher agglutinative titer than unwashed cells. In preliminary experiments no effect was observed in the titer by washing the cellular antigens of *Streptococcus* s.b.e. Therefore, the washing procedure was not followed throughout this study. After mixing the cells with an equal volume of the various serum dilutions, the vials were incubated for 8 to 12 hours at 37 C before the final observations were made.

RESULTS

Preliminary results with a group of 8 cultures of *Streptococcus* s.b.e. indicated a marked degree of serological homogeneity. Extracts of all cultures gave

positive precipitin tests with an antiserum prepared against one strain of this group. However, as more cultures were collected, an occasional strain was found which failed to react with the serum. In all, 5 such cultures have been encountered thus far.

Upon injecting a rabbit with one of the nonreacting cultures, a serum was obtained which reacted with extracts of the remaining 4 cultures. For the sake of convenience we have arbitrarily designated the larger group of the serologically specific *Streptococcus* s.b.e. cultures as type I; the 5 remaining cultures are referred to as type II.

Upon testing the entire collection with the type II serum, we made an interesting observation. As shown in table 1, 5 of the cultures which had shown positive precipitin tests with the type I serum also reacted with the type II serum. Thus it would appear that these 5 cultures contained both types I and II antigens. Accordingly, they have been labeled type I-II, *Streptococcus* s.b.e.

A logical explanation of this occurrence would be that the 5 type I-II cultures were actually impure and composed of a mixture of both types I and II strains.

TABLE 1

Precipitin reactions of Streptococcus s.b.e. extracts with sera prepared against three representative cultures

EXTRACTS TESTED	SERUM USED		
	P5 (type I)	JH49 (type II)	P25 (type I-II)
32	+	−	+
5	−	+	+
5	+	+	+

In order to test this hypothesis, one of these cultures was plated out on an agar medium by the loop dilution technique. Eighteen isolations were made from as many well-separated colonies. Extracts of all these freshly isolated strains reacted positively with both type-specific sera. Therefore, it would seem highly probable that the type I-II cultures possess both type-specific antigens.

From these findings it would appear likely that an antiserum prepared against one of the type I-II cultures would react with the entire collection of *Streptococcus* s.b.e. organisms. As shown in table 1, such a serum has been produced. The serum, however, reacted noticeably stronger with type I extracts than with those prepared from type II cultures.

The presence of both type-specific antigens in one culture was further confirmed by absorption experiments. Both a type I and a type II serum were absorbed with a concentrated cell suspension prepared from a culture representing the so-called type I-II. Table 2 shows that both sera were entirely voided of their respective antibodies, and that they no longer reacted with any of the cultures tested.

Another means of demonstrating the presence of dual antigens in these cul-

tures was accomplished by a second absorption experiment. Two portions of a serum prepared against one of the type I-II cultures were absorbed by type I and type II cells, respectively. The serum absorbed with type I cells no longer reacted with any type I extracts tested, but continued to show positive precipitin

TABLE 2

Absorption experiment showing the removal of precipitin antibodies from types I and II sera with one culture

RABBIT SERUM	CULTURE EXTRACT AND TYPE REPRESENTED	PRECIPITIN REACTION
Type I, unabsorbed	P5(I)	++
	P24(I-II)	++
	JH49(II)	−
Type I, absorbed with P25 (type I-II) cells	P5(I)	−
	P24(I-II)	−
Type II, unabsorbed	P5(I)	−
	P24(I-II)	++
	JH49(II)	++
Type II, absorbed with P25 (type I-II) cells	P24(I-II)	−
	JH49(II)	−

TABLE 3

Absorption experiment demonstrating the presence of two type-specific antibodies in a serum produced against one culture

RABBIT SERUM AGAINST P25 (TYPE I-II)	CULTURE EXTRACT AND TYPE REPRESENTED	PRECIPITIN REACTION
Unabsorbed	P5(I)	++
	JH40(II)	+
	P24(I-II)	++
Absorbed with P5 (type I cells)	P5(I)	−
	JH40(II)	+
	P24(I-II)	+
Absorbed with JH49 (type II) cells	P5(I)	+
	JH40(II)	−
	P24(I-II)	++

tests with the type II extracts. The opposite was true when the serum was absorbed with type II cells (table 3).

The type-specific sera prepared against *Streptococcus* s.b.e. strains appear to be specific for this group of organisms when tested by the Lancefield precipitin technique. A number of streptococci representing the various Lancefield groups, A through G, have been tested with negative results. The entire col-

lection of streptococci from subacute bacterial endocarditis has been tested, with no single culture showing cross reactions; the only reactions obtained were with those streptococci which had been previously identified as *Streptococcus* s.b.e. by virtue of their physiological characteristics. Also included in the survey were various members of the well-defined species of the viridans group of streptococci, including *Streptococcus salivarius* and *Streptococcus mitis* from the human throat. Again no cross reactions were obtained.

Because of the likelihood that the aggulutination technique may be more convenient to perform than the precipitin test in some laboratories, the possibility of identifying *Streptococcus* s.b.e. by this method was determined. The results of this study have revealed the presence of a multiplicity of agglutinative

TABLE 4

Absorption experiments demonstrating complexity of agglutinative antibodies in a type 1 Streptococcus s.b.e. serum

RABBIT SERUM	CELL SUSPENSION AND TYPE REPRESENTED	SERUM DILUTION								
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Type I, unabsorbed	P5(I)	3*	4	4	4	4	4	4	4	2
	JH49(II)	3	4	4	4	3	1	0	0	0
	P25(I-II)	4	4	4	3	2	1	0	0	0
Type I, absorbed with JH49 (type II) cells	P5(I)	4	4	4	4	4	4	4	4	4
	JH49(II)	0	0	0	0	0	0	0	0	0
	P25(I-II)	4	4	4	4	3	2	0	0	0
Type I,† absorbed with P24 (type I-II) cells	P5(I)	4	4	4	4	3	3	1	0	0
	JH49(II)	0	0	0	0	0	0	0	0	0
	P25(I-II)	0	0	0	0	0	0	0	0	0

* 0 to 4 indicates degree of agglutination.

† A different lot of type I serum with lower titer was used for this absorption.

antigens among these streptococci, a condition which reminds one of similar situations existing in certain groups of the *Enterobacteriaceae.*

Rabbit sera with relatively high titers of agglutinins for homologous cellular antigens could be obtained with 2 or 3 series of injections. For example, the type I serum used throughout most of this study agglutinated all type I cells tested in a serum dilution as high as 1:10,240. In addition, this serum also agglutinated both types II and I-II cells, but at a much lower dilution (1:640). Since types II and I-II cells were agglutinated at approximately the same titer, it might be assumed that they contained similar, or identical, antigens. That this is not the case is shown in table 4. The type I serum absorbed with type II cells continued to agglutinate type I *Streptococcus* s.b.e. and also all members in type I-II. On the other hand, a type I serum absorbed with type I-II cells would no longer agglutinate type II cells. Unfortunately for the latter absorption experiment, another lot of type I serum had to be used because of the depletion of the original lot. Consequently, the results tabulated in table 4 make

it appear that absorption with type I-II cells reduced the titer of the serum for type I cells also. This was not the case.

Table 5 demonstrates a similar situation when a type II serum was absorbed with type I and type I-II cells, respectively. In harmony with reports from

TABLE 5

Absorption experiments showing complexity of agglutinative antibodies in a type II Streptococcus s.b.e. serum

RABBIT SERUM	CELL SUSPENSION AND TYPE REPRESENTED	SERUM DILUTION						
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280
Type II, unabsorbed	JH52(II)	4*	4	4	4	2	1	0
	JH58(I)	4	4	3	0	0	0	0
	P25(I-II)	4	4	3	2	2	1	0
Type II, absorbed with P5 (type I) cells	JH49(II)	4	4	4	4	3	3	3
	JH44(I)	1	0	0	0	0	0	0
	P24(I-II)	3	2	2	1	0	0	0
Type II, absorbed with P25 (type I-II) cells	JH49(II)	4	4	4	3	3	2	1
	P5(I)	1	0	0	0	0	0	0
	JH19(I-II)	0	0	0	0	0	0	0

* 0 to 4 indicates degree of agglutination.

TABLE 6

Absorption experiments demonstrating complexity of agglutinative antibodies in a type I-II Streptococcus s.b.e. serum

RABBIT SERUM	CELL SUSPENSION AND TYPE REPRESENTED	SERUM DILUTION							
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
Type I-II, unabsorbed	P25(I-II)	4*	4	4	4	4	3	2	1
	P5(I)	3	2	2	2	2	1	0	0
	JH40(II)	4	4	4	4	3	2	1	1
Type I-II, absorbed with P5 (type I) cells	P24(I-II)	3	3	3	3	3	3	2	1
	JH44(I)	0	0	0	0	0	0	0	0
	JH40(II)	3	3	3	2	2	2	1	0
Type I-II, absorbed with JH49 (type II) cells	P25(I-II)	4	4	4	4	4	2	0	0
	P5(I)	2	2	2	1	0	0	0	0
	JH40(II)	1	0	0	0	0	0	0	0

* 0 to 4 indicates degree of agglutination.

other laboratories, we have observed that absorption of a serum with a heterologous type may actually increase its titer for organisms of the homologous type.

Another series of agglutination experiments was performed on the serum against a type I-II *Streptococcus* s.b.e. As shown in table 6, the titer of the serum for type II cells was as high as it was with the homologous culture. Type I cells agglutinated in somewhat lower serum dilutions. Absorption with either type

I or type II cells removed the agglutinative antibodies for the respective type only.

Although the antigenic complex of the different serological types of *Streptococcus* s.b.e. appears to be somewhat involved, the picture may be visualized in the following manner. Both types (as well as type I-II) appear to have a minimum of three agglutinative antigens. The three agglutinative antigens of type I *Streptococcus* s.b.e. might be labeled A, B, and C; type II would have antigens A, D, and E; and type I-II, antigens A, C, and D. All *Streptococcus* s.b.e. cultures, then, have one common agglutinative antigen, whereas those cultures in type I-II would have two agglutinative antigens common to either type I or type II.

A number of other streptococci from subacute bacterial endocarditis have been tested for their ability to cross-agglutinate with the various *Streptococcus* s.b.e. type sera. Although the majority showed no evidence of agglutination, an occasional culture of *Streptococcus mitis* was found which agglutinated with the sera in relatively high dilutions.

Mirick *et al.* (1944*b*) reported that type I *Streptococcus salivarius* cultures cross-reacted with their sera prepared against *Streptococcus* MG, as demonstrated by both the agglutination and precipitin techniques. It is interesting to note that *Streptococcus salivarius* also cross-agglutinates with the *Streptococcus* s.b.e. sera in relatively high dilutions. *Streptococcus salivarius* cells representing three serological (precipitin) types cross-agglutinated with all three *Streptococcus* s.b.e. sera used in this study, positive results sometimes occurring in serum dilutions as high as 1:320. Conversely, types I and II *Streptococcus* s.b.e. cultures cross-agglutinated with types I and II *Streptococcus salivarius* sera. It should be recalled, however, that *Streptococcus salivarius* does not show a positive precipitin test with the *Streptococcus* s.b.e. sera.

The cross reactions with *Streptococcus salivarius* should by no means imply that *Streptococcus* MG and *Streptococcus* s.b.e. are related organisms. They are known to be two serologically and physiologically distinct groups of streptococci.

These cross agglutinations with *Streptococcus salivarius* and occasional strains of *Streptococcus mitis* would tend to detract from the usefulness of the agglutination technique as a means of identifying *Streptococcus* s.b.e. As reported previously (Niven and White, 1946), *Streptococcus mitis* was recovered from approximately the same number of cases of endocarditis as was *Streptococcus* s.b.e. No *Streptococcus salivarius* cultures were encountered, however.

DISCUSSION

In harmony with many other studies upon the viridans streptococci in this and other laboratories, *Streptococcus* s.b.e. appears to have no group-specific antigen that would correspond to the "C" substance found in the hemolytic and other groups of streptococci. From the results with the type I-II streptococcus it might appear that this organism possesses a group-specific antigen, since a serum prepared against one of these cultures shows positive precipitin tests with all *Streptococcus* s.b.e. extracts. However, the absorption experiments would seem to indicate the presence of two type-specific antigens rather than a group antigen.

Since two serological types among the 42 cultures of *Streptococcus* s.b.e. have been found, it would be natural to expect that more types exist and will be found as more cultures are studied. In spite of this probability, serological methods should prove to be useful in the study of streptococci recovered from endocarditis. A serum prepared against a type I-II strain might be of value from the practical standpoint.

There seems to be no tendency for individual strains of *Streptococcus* s.b.e. to lose the type-specific antigens. Some of the cultures have been transferred at occasional intervals in the laboratory for over two years with no evidence in loss or change of type antigen. One type I strain was serially cultured 40 times in a broth containing 50 per cent homologous serum with no success in inducing loss of the type-specific antigen.

SUMMARY

The 42 cultures of *Streptococcus* s.b.e. recovered from cases of subacute bacterial endocarditis fall into two serological types as determined by the precipitin technique.

Five cultures were found which gave positive precipitin tests with both type I and type II antisera. A serum prepared against one of these strains reacted with all 42 cultures. This appears to be due to the presence of antigens of both types in the cells of these strains, rather than to the presence of a group-specific antigen.

There appears to be a number of agglutinating antigens in the individual strains of *Streptococcus* s.b.e. The agglutination test, however, is not suitable for the identification of this organism.

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