

TWO SEROLOGICAL TYPES OF GROUP B HEMOLYTIC
STREPTOCOCCI WITH RELATED, BUT NOT
IDENTICAL, TYPE-SPECIFIC SUBSTANCES

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The object of this investigation was to study the cross immunological relationship that exists between two closely related types of group B hemolytic streptococci.

Overlapping serological reactions have recently been shown by the work of many investigators to occur with somewhat more frequency than was formerly supposed. They have been observed between both related and unrelated bacterial species since the early investigations of Gruber and Durham and others (1), who studied particularly the Gram-negative bacilli. Among the Gram-positive cocci, however, knowledge of such complicating immunological relationships is more recent, while chemical studies, necessary for a true understanding of the mechanism underlying these serological reactions, are still in the initial stages. Since Avery's (2) observations on the serological cross reactions of typical and atypical Type II pneumococci,¹ reports have appeared which show that substances responsible for such cross reactions occur not only in bacteria but also in other natural sources widely separated generically. A few of the specific polysaccharides concerned have been analyzed sufficiently to establish a chemical basis for the serological relationships, although the inherent experimental difficulties have more often caused investigators to employ serological methods only and to infer a chemical basis by analogy.

Chemical studies of great importance and interest have been made in connection with cross reactions associated with pneumococcus polysaccharides:

¹ The atypical Type II pneumococci, subgroups II A and II B, described by Avery, have since been given separate type numbers, namely, Types V and VI, respectively (3).

for example, the close relationship between the type-specific polysaccharides of Pneumococcus Type II, and Friedländer's bacillus type B, which parallels the serological cross relationships (4), and the similar cross relationship between Types III and VIII pneumococci (5-7). Goebel (8, 9) showed that the Type III pneumococcus capsular polysaccharide is built up of molecules of glucose and glucuronic acid in a ratio of 1:1, while the Type VIII pneumococcus polysaccharide is composed of the same substances in the ratio of 7:2.² He believes that the basis for the immunological crossing resides in the identity of the configuration of the aldobionic acid nucleus common to both carbohydrates. Heidelberger, Kabat, and Shrivastava (10) conclude, from quantitative estimations of the homologous and cross reacting antibodies in antisera for Types III and VIII pneumococci, that it is too soon to attempt to localize the cross reaction to a definite portion of the common configuration of these molecules.

The chemical and immunological relationship of the acetylated and the deacetylated forms of Pneumococcus Type I polysaccharide (11) was further evidence of the nature of the chemical basis of cross reactions.

Immunologically interrelated polysaccharides have also been found from widely divergent sources: the blood group A substance of man and Pneumococcus Type I polysaccharide (the acetylated form only, although the failure of the deacetylated form to cross react was not due to the acetyl radical (12)); gum arabic and Types II and III pneumococci (13); other plant gums which cross react with Pneumococcus Type II (14). These gums contain uronic acid, indicating that glucuronic acid or a related substance is the common determinant group. Less complete data indicate a serological relationship between Type I Pneumococcus and a mouse virulent strain of *Escherichia coli*, probably dependent upon capsular polysaccharides. Similar polysaccharides existing in *Bacillus proteus* X-19 and *Rickettsia prowazeki* explain the Weil-Felix reaction in the serum of patients with typhus fever (16).

The production *in vitro* of antigens containing known chemical structures has thrown considerable light on these cross reactions among natural antigens (14, 17-22) and, as a result, it has been concluded that a complex chemical substance may induce more than one kind of antibody in the animal body, depending upon determinant factors such as the presence of different chemical radicals, the position of these radicals in reference to others, and the presence of highly polar groups; but the underlying mechanism is far from being completely understood.

In still other recently recorded instances,³ where bacterial cross relationships

² Heidelberger, Kabat, and Shrivastava (10) recently gave this ratio as about 3:1.

³ Other examples in which the serological relationships have been worked out, but in which the chemical information is less complete or lacking, are the following. (a) Additional subtypes of Pneumococcus Type II (23). (b) The relationship between yeast and Pneumococcus Type II (24). (c) Cross reactions of the polysaccharide of Type II Pneumococcus with antiserum for *Pasteurella cuniculicida* (25). (d) The relationship of *Bacterium aerogenes* to Pneu-

involving Gram-positive cocci have been explained on the basis of related polysaccharides, the analysis has been entirely serological. Of special interest in connection with the present communication is the report by Bliss that the immunological relationship between one type of group F hemolytic streptococci and one of group G is due to identical type-specific substances, probably polysaccharides (29).

Materials and Methods

The present report, based chiefly on immunological data, concerns the cross reactions of two closely related serological types of group B hemolytic streptococci. The indications are that their type specificity is determined by polysaccharides (30). Group B includes the strains usually responsible for bovine mastitis, and also certain strains of human origin which are not ordinarily pathogenic for man. These microorganisms are designated by some authors as *Streptococcus mastitidis* or as *S. agalactiae*; Stableforth (35 a) calls them "Group I mastitis streptococci." Originally most of the group B streptococci encountered in this laboratory were shown to be included within three serological types. In the first series of strains studied, one somewhat atypical member of the so called Type I was found, and it was doubtful whether this strain, K 127, really belonged in this type. Finally, in spite of incomplete cross agglutination and precipitin reactions, it was assigned to Type I on account of satisfactory cross protection experiments in mice. Later, however, other strains were found which had the same peculiarities as K 127, and this necessitated further study to classify these streptococci properly. In Table I are listed the strains so far found which belong in these two related types. The relative distribution as to origin is probably of no significance, since a fair sampling from the different sources in which they occur has not been attempted.

Antisera and extracts were prepared as previously described (30 b). The specific methods for each experiment are given under the tables.

Cross Reactions between Types Ia and Ib

Extracts of all the strains which reacted in antisera for Type I (hereafter called Type Ia⁴) and in antisera for the atypical Type I mococcus Type II, and Friedländer's bacillus type B (26). (e) The reactions between the species-specific polysaccharides of gonococci and meningococci and antiserum for Type III Pneumococcus (27). (f) The report of two strains of *S. viridans*, not identical serologically, which reacted in antiserum for Pneumococcus Type XXIX (28).

⁴ Stableforth (35 a) has reported the existence of main types of *S. agalactiae* within which he distinguishes subtypes by reciprocal agglutinin absorption tests. Some of these are related to the Types II and III, group B, previously described by us. In view of uncertainty as to the exact relationship of types defined by Stableforth (35 a) and by Stewart (35 c) to those described in this country (30 b), the nomenclature previously used by us has been retained for the present.

TABLE I
Source of Group B Strains, Types I a and I b

Strain	Animal origin	Source of culture	Isolated
Type I a			
O 90	Human	Scarlet fever	Aronson (Wamoscher) (31), Germany
K 107	Bovine	Udder: mastitis	*Dr. F. S. Jones, Princeton, N. J.
K 158 A	Rabbit	Vaccinia	*Dr. Frieda Fraser, Toronto, Canada
Kaufman-0	Human	?	Loewenthal (32)
Human-29-0	"	Derived from strain isolated from septicemia following mastitis	"
H 69 B 8	"	Vagina: normal maternity case	Hare and Colebrook (33), London, England
" B 13	"	"	"
H 93 B 4	"	"	"
" B 8	"	"	"
H 132 C	"	"	"
S 117	Bovine	Udder: mastitis	Sloane Hospital, New York *Dr. A. W. Stableforth, London, England
Type I b			
K 127	Bovine	Certified milk	Brown, Frost, and Shaw (34), Chicago, Illinois
H 36 B	Human	Blood: new-born infant	*Dr. J. H. Brown, Baltimore, Maryland
H 132 A	"	Vagina: normal maternity case	Sloane Hospital, New York
" E	"	"	"
" G	"	"	"
F 4 A	"	Throat (slight angina) rheumatic fever	Hospital of The Rockefeller Institute, New York
H 69 B 1	"	Vagina: normal maternity case	Hare and Colebrook (33), London, England
" B 4	"	"	"
" B 12	"	"	"
H 93 B 6	"	"	"
" B 10	"	"	"
" B 12	"	"	"
" B 15	"	"	"

*Personal communication.

(hereafter called Type Ib) still reacted in these antisera after the sera were absorbed with strains classified as Types II or III. The example, given in Table II, column 2, shows the cross precipitin reactions of the unabsorbed Type Ia serum used to illustrate this point. The cross reactions with crude extracts of strains belonging to Types II and III were due to the presence of the group-specific carbohydrate, C, in the extracts, and the corresponding anti-C precipitin in the serum. Absorption of the serum with bacteria of Type II removed

TABLE II
Precipitin Reactions to Show the Close Relationship between Types Ia and Ib

Crude extract from bacteria of	Type Ia antiserum	
	Serum untreated	Serum absorbed* with Type II bacteria
Type I a	+++	++
“ I b	++±	++
“ II	++±	—
“ III	++±	—

In all Tables: — indicates a negative reaction.

+ to ++++ indicate degrees of reaction.

Only one dilution of the various extracts is tabulated, although in most experiments several dilutions were employed.

In all precipitin tests, the dilutions of extract tested were contained in a volume of 0.4 cc. to which 0.2 cc. of undiluted serum was added. The readings were made first as ring-tests, then after thorough mixing and incubation at 37°C. for 2 hours, and the final reading, recorded here, after refrigeration overnight at 4°C.

* Two parts of undiluted serum were thoroughly mixed with one part of packed bacteria, previously killed by heating at 56°C. for 1 hour. The mixture was incubated in a water bath at 37°C. for 30 minutes and centrifuged at once. The clear supernatant serum was used in the precipitin reaction.

this group-specific antibody from the serum, as illustrated in column 3. Extracts from bacteria of both Types Ia and Ib continued to react in serum thus absorbed. Similar results were obtained when a Type III strain was used as the absorbing agent, or when a Type Ib antiserum was absorbed and tested in the same manner. Such experiments suggested that Types Ia and Ib might be identical.

Reciprocal Absorption Experiments

Absorption of Precipitins.—That Types Ia and Ib were not identical was demonstrated by reciprocal absorption experiments. Antisera

for Types Ia and Ib, which untreated showed reciprocal cross precipitin and protection reactions, lost all antibodies when absorbed with streptococci homologous to the respective type serum, but, on the other hand, lost only their ability to cross react when absorbed with a heterologous strain of the related type. Table III shows the results of precipitin tests with antisera treated in this way. The same relationship was made evident if the absorption was performed with

TABLE III
Precipitin Reactions
Reciprocal Absorption Experiment to Show That Types Ia and Ib Are Related but Not Identical*

†Extract from bacteria of	Type Ia antiserum		
	Serum untreated	Serum absorbed with (homologous) Type Ia bacteria‡	Serum absorbed with (heterologous) Type Ib bacteria‡
Type I a	++++	—	++±
Type I b	+++	—	—
†Extract from bacteria of	Type Ib antiserum		
	Serum untreated	Serum absorbed with (heterologous) Type Ia bacteria‡	Serum absorbed with (homologous) Type Ib bacteria‡
Type I a	+++	—	—
Type I b	+++±	++±	—

* See footnote to Table II.

† These extracts were partly purified chemically so that they were free of group-specific carbohydrate, C

‡ The same results were obtained when the absorption was carried out with the extracted and partially purified type-specific substances instead of the whole bacteria.

extracted, partially purified, type-specific polysaccharides from these two types of organisms. These results were confirmed with many different antisera.

Cross Protection between Types Ia and Ib.—This relationship was also established by protection tests in mice, since in the final analysis the best proof of immunological specificity is based upon protection conferred on animals, by passively immunizing them with the respective sera. Reciprocal protection tests with an excess of antiserum

titrated against decreasing amounts of culture showed equal cross protection (Table IV) with both strains. The Type Ia strain, O 90,

TABLE IV
Reciprocal Passive Protection Tests in Mice, Using an Excess of Unabsorbed Antiserum to Show the Cross Protection between Types Ia and Ib

Type Ia culture strain O 90	No serum	0.5 cc. undiluted antiserum	
	Virulence controls	(Homologous) Type Ia	(Heterologous) Type Ib
<i>cc.</i>			
10 ⁻⁸	D 48 hrs.	S	S
10 ⁻⁷	D 41 "	S	S
10 ⁻⁶	D 41 "	S	S
10 ⁻⁵	D 41 "	S	S
10 ⁻⁴	D 89 "	S	S
10 ⁻³	D 25 "	S	S
10 ⁻²	D 20 "	S	S
10 ⁻¹	D 17 "	S	S
Type Ib culture strain H 36 B	No serum	0.5 cc. undiluted antiserum	
	Virulence controls	(Heterologous) Type Ia	(Homologous) Type Ib
<i>cc.</i>			
10 ⁻⁶	S	S	S
10 ⁻⁵	D 26 hrs.	S	S
10 ⁻⁴	D 21 "	S	S
10 ⁻³	D 21 "	S	S
10 ⁻²	D 21 "	S	S
10 ⁻¹	D 21 "	S	S

In all tables, S indicates survived, and D indicates died within the number of hours or days stated.

The protection tests were performed as follows: Serial dilutions in broth were made of a fresh 16 hour broth culture, so that the amount to be inoculated was contained in 0.5 cc. These dilutions, simultaneously with 0.5 cc. of undiluted serum, were injected intraperitoneally into white mice, 18 to 20 gm. in weight. A series of controls received culture and no serum. Survivors were observed for 2 weeks before they were discarded. Blood agar plates were poured containing, respectively, 10⁻⁶ cc., 10⁻⁷ cc., and 10⁻⁸ cc. of culture, and colony counts were made to estimate the number of organisms injected.

was somewhat more virulent than the Type Ib strain, H 36 B, but both Type Ia and Ib antisera protected mice against infection with

TABLE V a

Reciprocal Passive Protection Tests in Mice, Using Varying Amounts of Absorbed and Unabsorbed Antiserum to Show That Types Ia and Ib Are Related but Not Identical

Type Ia culture (homologous) strain O 90	Type Ia antiserum (against strain O 90)			
	0.5 cc. of serum diluted	Serum untreated	Serum absorbed with (homologous) *Type Ia	Serum absorbed with (heterologous) Type Ib
0.5 cc. 1:200 dil.	1:1	S	D 1 day†	S
" " " "	1:2	S	D 1 "	S
" " " "	1:10	S	D 1 "	S
" " " "	1:50	S	D 1 "	S
" " " "	1:100	S	D 1 "	S
" " " "	1:250	S	D 1 "	S
" " " "	1:500	S	D 1 "	S
" " " "	1:1,000	S		S
" " " "	1:1,500	S		S
" " " "	1:2,000	S		D 3 days
" " " "	1:2,500	S		D 6 "
" " " "	1:3,000	D 6 days		S
" " " "	1:4,000	S		D 3 days
" " " "	1:5,000	D 6 days		S
" " " "	1:6,000	S		D 7 days
" " " "	1:7,000	D 3 days		
" " " "	1:8,000	S		
" " " "	1:9,000	D 3 days		
" " " "	1:10,000	D 4 "		
	‡Virulence control			
" " " "	No serum	D 1 day		

Protection tests recorded in Tables V a, b, c, and d were performed as follows:

Serial dilutions of serum in saline, contained in a volume of 0.5 cc., were injected intraperitoneally into white mice simultaneously with 0.5 cc. of a 1:200 broth dilution of a fresh 16 hour broth culture. Controls received this dilution of culture without serum. Additional controls which received from 10^{-1} cc. through 10^{-8} cc. were included to determine the minimal lethal dose of the culture. The technique of this determination and of the estimation of the number of organisms inoculated was that described under Table IV.

* Strains K 158 A and K 107 were used as typical Type Ia strains for absorption on account of the great difficulty of sedimenting strain O 90 in the centrifuge.

† Further tests with a larger amount of serum (0.5 cc. undiluted serum) and serial dilutions of culture, usually to a dilution containing 10^{-7} or 10^{-8} cc., resulted in the death of all mice.

‡ Additional virulence controls showed that 10^{-1} through 10^{-8} cc. of this culture killed mice.

TABLE Vb

Reciprocal Passive Protection Tests in Mice, Using Varying Amounts of Absorbed and Unabsorbed Antiserum to Show That Types Ia and Ib Are Related but Not Identical

Type Ib culture (heterologous) strain H 36 B	Type Ia antiserum (against strain O 90)			
	0.5 cc. of serum diluted	Serum untreated	Serum absorbed with (homologous) *Type Ia	Serum absorbed with (heterologous) Type Ib
0.5 cc. 1:200 dil.	1:1	S	D 1 day†	D 1 day†
“ “ “ “	1:2	S	D 1 “	D 1 “
“ “ “ “	1:10	S	D 1 “	D 1 “
“ “ “ “	1:50	S	D 2 “	D 1 “
“ “ “ “	1:100	S	D 1 “	D 1 “
“ “ “ “	1:250	S	D 2 “	D 1 “
“ “ “ “	1:500	S	D 1 “	D 2 “
“ “ “ “	1:1,000	S		
“ “ “ “	1:1,500	S		
“ “ “ “	1:2,000	S		
“ “ “ “	1:2,500	S		
“ “ “ “	1:3,000	S		
“ “ “ “	1:4,000	S		
“ “ “ “	1:5,000	D 1 day		
“ “ “ “	1:6,000	S		
“ “ “ “	1:7,000	S		
“ “ “ “	1:8,000	S		
“ “ “ “	1:9,000	D 1 day		
“ “ “ “	1:10,000	D 1 “		
	‡Virulence control			
“ “ “ “	No serum	D 1 day		

*† See Table Va for these footnotes.

‡ Additional virulence controls showed that 10^{-1} through 10^{-6} cc. of this culture killed mice.

as much as 10^{-1} cc. of either culture. In order, however, to detect quantitative differences in the protective value of the antisera, another test was made by injecting decreasing amounts of antiserum together with a constant, relatively large dose of culture.

Absorption of Protective Antibodies: Type Ia Antiserum.—Tables Va, Vb, Vc, and Vd show the results of such an experiment. Other similar, but less complete, experiments all gave consistent results.

For this experiment the same lots of antisera used in the precipitin absorption experiment recorded in Table III, were injected into mice in dilutions varying from 1:1 to 1:30,000. Simultaneously, the animals were inoculated intraperitoneally with 0.5 cc. of a 1:200 dilution of culture which had a minimal lethal dose for mice of 10^{-6} to 10^{-8} cc. Table Va shows the protective power of Type Ia antiserum for mice infected with Type Ia organisms. The high protective titer of the untreated serum for the homologous strain is recorded in column 3. Mice which received this serum survived infection even though the serum was diluted as much as 1 part in 2,500. With

TABLE Vc

Reciprocal Passive Protection Tests in Mice, Using Varying Amounts of Absorbed and Unabsorbed Antiserum to Show That Types Ia and Ib Are Related but Not Identical

Type Ia culture (heterologous) strain O 90	Type Ib antiserum (against strain H 36 B)			
	0.5 cc. serum diluted	Serum untreated	Serum absorbed with (heterologous) *Type Ia	Serum absorbed with (homologous) Type Ib
0.5 cc. 1:200 dil.	1:1	S	D 1 day†	D 1 day†
“ “ “ “	1:2	S	D 1 “	D 1 “
“ “ “ “	1:10	S	D 1 “	D 1 “
“ “ “ “	1:50	S	D 1 “	D 1 “
“ “ “ “	1:100	D 1 day	D 1 “	D 1 “
“ “ “ “	1:250	D 2 days	D 1 “	D 1 “
“ “ “ “	1:500	D 1 day	D 1 “	D 1 “

Virulence controls recorded in Table Va.

*† See Table Va for these footnotes.

higher dilutions the deaths were irregular, but still the animals survived for 2 to 3 days longer than the control mice, all of which died within 1 day. The animals which received serum absorbed with bacteria of the homologous type all died within 1 day. In further tests with larger amounts of this absorbed serum, it was found impossible to protect mice against even one minimal lethal dose of the homologous culture. When, on the other hand, the serum was absorbed with bacteria of the heterologous type, Ib, the protective capacity of the absorbed serum against Type Ia infection was not significantly reduced.

These same lots of Type Ia antisera were tested simultaneously for protective action against the heterologous Type Ib strain, H 36 B, as shown in Table Vb. Here again, the untreated serum protected

TABLE Vd
Reciprocal Passive Protection Tests in Mice, Using Varying Amounts of Absorbed and Unabsorbed Antiserum to Show That Types Ia and Ib Are Related but Not Identical

Type Ib culture (homologous) strain H 36 B	Type Ib antiserum (against strain H 36 B)			
	0.5 cc. of serum diluted	Serum untreated	Serum absorbed with (heterologous) *Type Ia	Serum absorbed with (homologous) Type Ib
0.5 cc. 1:200 dil.	1:1	S	S	
" " " "	1:2	S	S	
" " " "	1:10	S	S	D 1 day
" " " "	1:50	S	S	D 1 "
" " " "	1:100	S	S	D 1 "
" " " "	1:250	S	S	D 1 "
" " " "	1:500	S	S	D 1 "
" " " "	1:1,000	S	S	D 1 "
" " " "	1:1,500	S	S	
" " " "	1:2,000	S	S	D 1 "
" " " "	1:2,500	S	S	
" " " "	1:3,000	S	S	D 1 "
" " " "	1:4,000	S		
" " " "	1:5,000	S		
" " " "	1:6,000	S		
" " " "	1:7,000	S		
" " " "	1:8,000	S		
" " " "	1:9,000	S		
" " " "	1:10,000	S		
" " " "	1:15,000	D 3 days		
" " " "	1:20,000	D 1 day		
" " " "	1:25,000	D 1 "		
" " " "	1:30,000	D 3 days		

Virulence controls recorded in Table Vb.

* See Table Va for this footnote.

in high dilution even against the heterologous culture. The apparently greater protective value of the serum for the heterologous strain than for the homologous is probably of no significance because of the lower virulence of the heterologous strain. Its virulence for

mice varied on different occasions from a minimal lethal dose of 10^{-5} to 10^{-7} cc., while that of strain O 90 rarely fell below 10^{-8} cc. Tested against the heterologous strain, H 36 B, the Type Ia antiserum absorbed with either Type Ia or Type Ib bacteria failed entirely to protect mice against infection with this strain.

These absorption experiments demonstrate that the homologous Type Ia bacteria remove from Type Ia antiserum the antibodies which protect against either strain, while the heterologous Type Ib strain removes only that antibody which protects against itself. This agrees with the evidence afforded by the precipitin reaction.

Absorption of Protective Antibodies: Type Ib Antiserum.—Tables Vc and Vd show the results of similar experiments with Type Ib antiserum. In Table Vc there are shown the results of passive protection tests in mice injected with absorbed and unabsorbed Type Ib antiserum and inoculated with the heterologous Type Ia strain, O 90. This serum had a relatively low titer of protective antibody for the heterologous strain, although it protected mice against infection with the homologous strain, H 36 B, even when the serum was diluted 1:10,000 (*cf.* 3rd column of Table Vd). Absorption with the homologous Type Ib strain removed the protective antibodies for both Types Ia and Ib (column 5, Tables Vc and Vd), in marked contrast to the absorbing capacity of the heterologous Type Ia strain, which removed the antibody for itself only (column 4, Table Vc) and not that for the strain homologous to the serum (column 4, Table Vd). In this last titration the end-point for protection against the homologous strain, H 36 B, was not reached, but obviously the protective power of this lot of Type Ib serum absorbed with the heterologous Type Ia strain was still high.

DISCUSSION

The results of the cross precipitin and cross protection tests with these reciprocally absorbed antisera and the control untreated antisera prove that one of the serological types previously identified within group B streptococci really comprises two types, now designated as Types Ia and Ib.⁴ These are closely related, as shown convincingly by the large amount of cross protection with unabsorbed sera for the two types; but they are not identical, even though so closely related

as to give reciprocal protection, since, in each case, bacteria or extracts of the heterologous related type absorb only the antibody active against its own type. The homologous type organisms or their extracts, on the contrary, absorb all antibodies from a given serum, including those for the heterologous related strains.

The quantitative relationships are somewhat difficult of interpretation, since the relative antibody content of different antisera with respect to the two types varied considerably. For example, serum, taken at an earlier date from the rabbit that furnished the Type Ia serum used in the protection tests recorded in Tables *Va* and *Vb*, protected mice against both Types Ia and Ib when the serum was diluted 1:100; while the same dilution (1:100) of a Type Ia antiserum from another rabbit, immunized with the same strain, protected mice against infection with the homologous Type Ia organisms but afforded no protection against the heterologous Type Ib organisms. Nor was it possible to detect any precipitation of this serum with extracts of the heterologous Type Ib strain. These variable responses of individual animals in producing antibodies to the minor antigenic groupings are in accord with the findings of Sugg and Neill (24*a*) with respect to cross reactions between yeast and Pneumococcus Type II, and of the same authors and their associates (5, 6) with regard to Types III and VIII strains of pneumococci. Heidelberger, Kabat, and Shrivastava (10) also reported similar findings in their quantitative studies of the precipitin reaction with Types III and VIII pneumococcus polysaccharides.

The antigens under investigation in the present study were probably polysaccharides for a study of the chemical properties of the type-specific substance of a Type Ia strain, previously reported (30*b*), showed that this substance is, in fact, a polysaccharide; and incomplete investigation of the type-specific substance of a Type Ib strain indicates the probability that this is also a polysaccharide.

Although the cross reactions described in this paper might be explained as due to the presence in both Types Ia and Ib of a common antigen, and in addition of a separate type-specific antigen in each, the available evidence is insufficient to determine whether this is the case or whether the type-specific substances of the two types are distinct but, nevertheless, closely enough related to account for the

cross reactions. The latter viewpoint is probably the more tenable, since in neither case has it been possible to separate the type-specific substance chemically into two fractions, one common to the two types and one strictly specific for the type in question, or into fractions of different reactivities. Further study of these relationships is being made by growing each organism in media containing respectively antisera for each of these related types. The preliminary indications are that, under certain conditions, the Type Ib strain, H 36 B, can be made to produce an antigen which is almost strictly specific for that type, but the results with the Type Ia strain are somewhat different and too incomplete for inclusion in this report.

The foregoing observations are of interest in connection with a number of experiments reported recently in which it has been found that, although cross immunological reactions between bacterial and other naturally occurring substances usually indicate genetic relationships, as is true in the present instance, it also sometimes happens that no such consistent relationship is apparent. However, where fairly complete chemical data are available, it has been found that serological similarities are always based on chemical likenesses whether the determinant substances are derived from related or from unrelated sources.

A chemical and immunological investigation of particular importance as a pattern in determining the nature of such partial cross immune reactions among bacteria, was the formation of effective antigens from α and β glucosides by linking them to serum proteins (17 *a, b*). Avery, Goebel, and Babers were thereby enabled to furnish an example of substances of known structure which simulated the serological behavior of such bacterial polysaccharides as those derived from Friedländer's bacillus type B, and Pneumococcus Type II. The only difference in the two synthetic antigens studied was in the position of the glucoside linkages, but this was reflected in their serological reactions. Other studies which show a chemical basis for serological relationships have already been discussed, as well as the conclusions of several investigators that a single complex chemical substance may induce the formation in animals of more than one antibody.

With these facts in mind, it is not surprising to find another example

of closely related but not identical antigenic substances in bacteria of the same serological group. Although the chemical background has not been sufficiently developed to establish a chemical basis for the observed immunological relationship between Types Ia and Ib, group B hemolytic streptococci, it seems probable that each type is characterized by a specific polysaccharide which is chemically similar to, but not identical with, the type-specific polysaccharide of the related type.

SUMMARY

1. Among group B hemolytic streptococci one serological type previously described as homogeneous has been shown, instead, to contain two closely related types, distinguishable by reciprocal absorption experiments. These streptococci are designated Types Ia and Ib.⁴

2. Homologous organisms in each case absorb all antibodies from their respective antisera, while organisms of the heterologous related type absorb only the antibody responsible for the cross reactions. Group B streptococci of other types do not absorb the antibodies responsible for the cross reactions between these two related types. The precipitin reaction and passive protection tests in mice were employed in this analysis.

3. The type-specific substance of Type Ia is a polysaccharide. Preliminary study indicates that this is also true of Type Ib. While no data are available concerning the chemical relationships of these substances, it seems probable that the two types elaborate polysaccharides, related chemically as well as serologically.

BIBLIOGRAPHY

1. Gruber, M., and Durham, H. E., *Munch. med. Woch.*, 1896, **43**, 285. (Cf. Krumwiede, C., Cooper, G., and Provost, D. J., *J. Immunol.*, 1925, **10**, 55, for review of the early literature.)
2. Avery, O. T., *J. Exp. Med.*, 1915, **22**, 804.
3. Cooper, G., Edwards, M., and Rosenstein, C., *J. Exp. Med.*, 1929, **49**, 461.
4. Avery, O. T., Heidelberger, M., and Goebel, W. F., *J. Exp. Med.*, 1925, **42**, 709.
5. Sugg, J. Y., Gaspari, E. L., Fleming, W. L., and Neill, J. M., *J. Exp. Med.*, 1928, **47**, 917.
6. Harris, A. L., Sugg, J. Y., and Neill, J. M., *J. Exp. Med.*, 1928, **47**, 933.

7. Brown, R., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 859.
8. Goebel, W. F., *J. Biol. Chem.*, 1935, **110**, 391.
9. Goebel, W. F., *J. Bact.*, 1936, **31**, 66.
10. Heidelberger, M., Kabat, E. A., and Shrivastava, D. L., *J. Exp. Med.*, 1937, **65**, 487.
11. Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.
12. (a) Witebsky, E., Neter, E., and Sobotka, H., *J. Exp. Med.*, 1935, **61**, 703.
(b) Schiff, F., and Akune, M., *Münch. med. Woch.*, 1931, **78**, 657. (c) Schiff, F., and Weiler, G., *Biochem. Z.*, 1931, **235**, 454; **239**, 489.
13. Heidelberger, M., Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1929, **49**, 847.
14. Marrack, J. R., *Proc. Second Internat. Cong. Microbiol.*, London, 1936, 235.
15. Barnes, L. A., and Wight, E. C., *J. Exp. Med.*, 1935, **62**, 281.
16. Castaneda, M. R., *J. Exp. Med.*, 1934, **60**, 119; 1935, **62**, 289.
17. (a) Goebel, W. F., Babers, F. H., and Avery, O. T., *J. Exp. Med.*, 1932, **55**, 761. (b) Avery, O. T., Goebel, W. F., and Babers, F. H., *J. Exp. Med.*, 1932, **55**, 769. (c) Goebel, W. F., *J. Exp. Med.*, 1936, **64**, 29.
18. Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1936, **63**, 325.
19. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **61**, 563; 1935, **62**, 467, 697.
20. Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1936, **30**, 41.
21. Morgan, W. T. J., *J. Hyg.*, Cambridge, Eng., 1937, **37**, 372.
22. Burnet, F. M., *Brit. J. Exp. Path.*, 1934, **15**, 354.
23. Stillman, E. G., *J. Exp. Med.*, 1919, **29**, 251.
24. (a) Sugg, J. Y., and Neill, J. M., *J. Exp. Med.*, 1929, **49**, 183; 1931, **53**, 527.
(b) Sugg, J. Y., Richardson, L. V., and Neill, J. M., *J. Exp. Med.*, 1929, **50**, 579.
25. Dingle, J. A., *Am. J. Hyg.*, 1934, **20**, 148.
26. Julianelle, L. A., *J. Immunol.*, 1937, **32**, 21.
27. Miller, C. P., Jr., and Boor, A. K., *J. Exp. Med.*, 1934, **59**, 75.
28. Eyre, S., and Stovall, W. D., *J. Infect. Dis.*, 1936, **58**, 190.
29. Bliss, E. A., *J. Bact.*, 1937, **33**, 625.
30. (a) Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571; (b) 1934, **59**, 441. (c) Lancefield, R. C., and Hare, R., *J. Exp. Med.*, 1935, **61**, 335.
31. (a) Todd, E. W., *Brit. J. Exp. Path.*, 1930, **11**, 368. (b) Griffith, F., *J. Hyg.*, Cambridge, Eng., 1935, **35**, 23.
32. Loewenthal, H., *Brit. J. Exp. Path.*, 1934, **15**, 298.
33. Hare, R., and Colebrook, L., *J. Path. and Bact.*, 1934, **39**, 429.
34. Brown, J. H., Frost, W. D., and Shaw, M., *J. Infect. Dis.*, 1926, **38**, 381.
35. (a) Stableforth, A. W., *Proc. Second Internat. Cong. Microbiol.*, London, 1936, 80. (b) Stableforth, A. W., *J. Path. and Bact.*, 1937, **45**, 263. (c) Stewart, D. F., *J. Path. and Bact.*, 1937, **45**, 279.