THE SOLUBLE SPECIFIC SUBSTANCE OF FRIEDLÄNDER'S BACILLUS.

PAPER II. CHEMICAL AND IMMUNOLOGICAL RELATIONSHIPS OF PNEUMOCOCCUS TYPE II AND OF A STRAIN OF FRIEDLÄNDER'S BACILLUS.

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The preceding paper (1) records the initial attempts to isolate and purify the soluble specific substance of the so called E strain of a bacillus of the Friedländer group. The organism was originally isolated from a spontaneous epidemic of pneumonia among stock guinea pigs. The specifically reactive substance which has been isolated appears to be a nitrogen-free polysaccharide differing in many respects from the soluble specific substances recovered from the three fixed types of Pneumococcus (2). However, as in the case of the soluble specific substance of Pneumococcus Type II, only glucose could readily be identified among the products of hydrolysis. Moreover, the percentage of reducing sugars on hydrolysis is almost the same in both substances, and it therefore seemed possible that both of them might in reality be identical and that the differences observed might be due to impurities. In the preceding paper procedures were outlined which were designed to eliminate any impurities present in the Friedländer polysaccharide, but the employment of these methods only resulted in recovery of the original substance practically unaltered.

A comparison of the specific substance obtained from Type II pneumococcus with that isolated from the E strain of Friedländer's bacillus shows that both polysaccharides rotate the plane of polarized light to the right. In the case of the Friedländer substance the specific rotation is $+100°$, while the Type II pneumococcus sub-

stance rotates the plane of polarized light about +74°. Both substances have acidic properties, but the Friedländer specific substance has an acid equivalent approximately one-half that of the Type II polysaccharide. Neither of the two products gives glucuronic acid tests as do the specific substances of Types I and III pneumococcus. Both polysaccharides fail to form precipitates when treated with solutions of silver nitrate, copper sulfate, or phosphotungstic acid; both are precipitated by solutions of uranium nitrate and basic lead acetate. Whereas the Type II pneumococcus specific substance gives no precipitate with either barium hydroxide or neutral lead acetate, the Friedländer polysaccharide is precipitated by both of these reagents.

Because the two specific substances, although of widely different biological origin, resemble each other so closely in some of their chemical properties, the Friedländer polysaccharide was tested with Type II antipneumococcus serum, and a precipitin reaction was found to occur. On the other hand, there was absence of precipitation when this substance was tested with antipneumococcus serum of the other two fixed types. It then became necessary to determine as far as possible the immunological relationships not only of the soluble substances of the E strain of the Friedländer bacillus and of Type II pneumococcus, but of the microorganisms themselves. The present paper deals with the facts so far ascertained in this study.

EXPERIMENTAL.

Microorganisms.—The strain of Gram-negative bacillus from which the specific polysaccharide was derived corresponds in its cultural and biological reactions to organisms of the *encapsulatus* group, and for laboratory purposes is referred to as the E strain. Five other strains, four of which, as will be shown later, are similar in their serological reactions to the original organism, have been used in this study. It does not seem essential at this time to enter into a detailed description of the individual cultural characteristics of each strain;—they all possess in common the property of luxuriant growth on ordinary media with the elaboration of the abundant gummy, mucoid material characteristic of the group.

Methods.—Rabbits were immunized by the intravenous injection of suspensions of heat-killed organisms; *small* doses were administered every day for 6 days, and they were repeated after an interval of a week. Three courses of injections were given, and the animals were bled 9 days following the last inoculation.

The agglutination and precipitin tests were done as previously described for Pneumococcus. The protective power of type sera against Friedländer's bacilli and pneumococci was tested by injecting white mice intraperitoneally with varying doses of the virulent organisms and at the same time injecting a fixed quantity (0.2 cc.) of immune serum. All animals were observed for at least 10 days following injection, and those surviving this period were considered effectively protected.

Agglutination.—Immune rabbit sera were prepared against two strains of Friedländer's bacillus. One of these was the culture E from which the specific polysaccharide was isolated. The second culture (Sc) was isolated from the blood of a patient suffering from pneumonia. The study of the agglutinin reactions of these two immune sera afforded an opportunity, first, to establish the specific relationship of these two strains to each other and to other organisms of the group; and second, to confirm the immunological relationships between Type II pneumococcus and the E strain.

It immediately becomes evident from Table I that, as others have previously observed, the *encapsulatus* group does not consist of organisms which are immunologically identical. The two strains (E and Sc) chosen at random are antigenically distinct one from the other. Observations by Julianelle (3) indicate, however, that these two strains are representatives of two distinct but not unusual immunological types, since of forty strains of Friedländer's bacillus which he has tested, seventeen were found to be immunologically identical with either Strain E or Strain Sc. It is not the purpose of this paper, however, to deal with the serological relationships within the Friedländer group. Of more immediate interest is the fact, as shown by the experiments recorded in Table I, that the Friedländer Type E strain reacts in antipneumococcus Serum II and that Type II pneumococcus is agglutinated in serum produced by immunization with the E strain of Friedländer's bacillus. An immunological similarity, therefore, exists between these two biologically remote organisms, and this relationship is reciprocal. The experiment also shows that there is no immunological relationship between Pneumococcus Type II and the other strain of Friedländer's bacillus tested (Sc), which, although a typical organism of the *encapsulatus* group, is immunologically distinct from Strain E.

TABLE I.

Agglutination Reactions of Two Strains of Friedländer's Bacillus and Pneumococcus Type II in Homologous and Heterologous Sera.

Culture.	Immune sera.														
	Anti-Friedländer Sc.*					Anti-Friedländer E.†					Antipneumococcus Type II.‡				
	1:5	1:10	1:20	1:40	1:80	1:5	1:10	1:20	1:40	1:80	1:10	1:20	1:40	1:80	1:160
Friedländer's bacillus (Sc)....	++++	++++	+	−	−	−	−	−	−	−	−	−	−	−	−
Friedländer's bacillus (E).....	−	−	−	−	−	++++	++++	++++	++	−	+++	+++	+	−	−
Pneumococcus Type II.....	−	−	−	−	−	++++	++++	++	++	−	++++	++++	++	++	±

++++ indicates complete, compact, disk-like agglutination; +++, marked, compact, disk-like agglutination; ++, clumping—more easily broken up; +, partial clumping; ±, slight clumping; −, negative.

* Rabbit 77 D.

† Rabbit 84 D.

‡ Horse 91 A.

That other strains of Friedländer's bacilli, which are immunologically identical with Strain E, also react with antipneumococcus serum Type II just as does the E strain is shown in Tables II, III, and IV, in which are presented the results of agglutination reactions of six

TABLE II.

Agglutination of Friedländer's Bacillus and Pneumococcus Type II in Anti-Freidländer Sera.

Friedländer's bacillus.	Anti-Friedländer Serum E.					Anti-Friedländer Serum Sc.				
	1:5	1:10	1:20	1:40	1:80	1:5	1:10	1:20	1:40	1:80
E	++++	++++	++++	+++	−	−	−	−	−	−
K	++++	++++	+++	+++	−	−	−	−	−	−
H_1	++++	++++	++++	++	−	−	−	−	−	−
H_2	++++	++++	++++	+++	−	−	−	−	−	−
F_6	++++	++++	++++	++++	++	−	−	−	−	−
Sc	−	−	−	−	−	++++	++++	+	−	−
Pneumococcus Type II.	+++	+++	++	±	−	−	−	−	−	−

TABLE III.

Agglutination of Friedländer's Bacillus in Antipneumococcus Serum Type II.

Friedländer's bacillus.	Antipneumococcus serum.						Normal horse serum.		Salt solution.
	Type I.		Type II.		Type III.				
	1:1	1:10	1:1	1:10	1:1	1:10	1:1	1:10	
E	−	−	++++	++++	−	−	−	−	−
K	−	−	++++	++++	−	−	−	−	−
H_1	−	−	++++	++++	−	−	−	−	−
H_2	−	−	++++	++++	−	−	−	−	−
F_6	−	−	++++	++++	−	−	−	−	−
Sc	−	−	−	−	−	−	−	−	−
G_9	−	−	−	−	−	−	−	−	−

additional strains. When the strains are immunologically related to Strain E they react with antipneumococcus serum Type II; when they are immunologically distinct (Sc and G_9, Table III) they fail to react. Moreover, Table III shows that the agglutination of Friedländer's bacilli of the Strain E group does not occur with anti-

pneumococcus sera of Types I and III, but only in that of Type II. This fact suggests that the immunological relationship between certain strains of the Friedländer bacillus and certain strains of Pneumococcus is dependent upon chemical similarities in the soluble specific substances of the organisms concerned. Table IV shows that while Friedländer strains of the E type all react about equally in Type II antipneumococcus serum, they do not react in as high dilution as does Pneumococcus Type II itself. This difference in the capacity of the two kinds of bacteria to react to the same degree in the same immune serum shows a lack of complete immunological identity between them, a fact presumably related to the minor chemical

TABLE IV.

Agglutination of Friedländer's Bacillus by Antipneumococcus Serum II.

Friedländer's bacillus (strains).	Antipneumococcus serum Type II.							Control.
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	
E	++++	++++	+++	+	−	−	−	−
K	++++	++++	++++	±	−	−	−	−
H_1	++++	++++	+++	±	−	−	−	−
H_2	++++	++++	++++	+++	−	−	−	−
F_6	++++	++++	++++	±	−	−	−	−
Sc.	−	−	−	−	−	−	−	−
G_9	−	−	−	−	−	−	−	−
Pneumococcus Type II.	++++	++++	++++	++++	++++	++	−	−

differences which appear to exist between the soluble specific substances of the two organisms.

Agglutinin Absorption.—Krumwiede and his associates (4) have recently published a critical and analytical review of the subject of agglutinin absorption in which they point out the significance of the method for bacterial identification. It will be important to apply this method with great exactness to the present problem, but at present we can only record the results so far obtained.

The results of observations as given in Tables V and VI indicate the failure of reciprocal absorption. Unabsorbed anti-Friedländer serum which agglutinates both the Strain E Friedländer bacillus and Pneumococcus Type II to about the same titer, loses its agglu-

TABLE V.

Agglutinin Absorption.

Culture.	Anti-Friedländer Serum E.												Normal rabbit serum control.			
	Unabsorbed.				Absorbed with Friedländer's bacillus (E).				Absorbed with Pneumococcus Type II.							
	1:5	1:10	1:20	1:40	1:5	1:10	1:20	1:40	1:5	1:10	1:20	1:40	1:5	1:10	1:20	1:40
Friedländer's bacillus (E).....	++++	++++	+++	++	−	−	−	−	++++	++++	+++	+	−	−	−	−
Pneumococcus Type II.....	++++	+++	+++	+	−	−	−	−	−	−	−	−	−	−	−	−

++++ indicates complete, compact agglutination; −, no reaction.

TABLE VI.

Agglutinin Absorption.

Heat-killed suspension of	Anti-Friedländer Serum E.											
	Unabsorbed.						Absorbed by Pneumococcus II.					
	1:5	1:10	1:20	1:40	1:80	1:160	1:5	1:10	1:20	1:40	1:80	1:160
Pneumococcus Type II.....	++++	++++	++++	+±	−	−	−	−	−	−	−	−
Friedländer's bacillus (E).....	++++	++++	+++	+	−	−	++++	++++	+±	±	−	−

Heat-killed suspension of	Antipneumococcus serum Type II.															
	Unabsorbed.								Absorbed with Friedländer's bacillus (E).							
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Pneumococcus Type II.....	++++	++++	++++	+++	++±	++	+	−	++++	++++	++++	+++	++	+	±	−
Friedländer's bacillus (E).....	++++	++++	+	±	−	−	−	−	−	−	−	−	−	−	−	−

tinins for both organisms when absorbed with the homologous strain of Friedländer's bacillus. On the other hand, absorption of the same serum with Pneumococcus Type II removes only the agglutinins for the absorbing organism and leaves the antibodies for the Friedländer strain only slightly reduced. Similarly, absorption of antipneumococcus serum Type II with Pneumococcus of the homologous type results in complete removal of agglutinins for both organisms, while absorption of the same serum with the E strain of Friedländer's bacillus takes out the antibodies for this organism and only slightly reduces the agglutinating power of the serum for Type II pneumococcus.

TABLE VII.

Precipitin Reactions: Anti-Friedländer Serum E against Carbohydrate Fraction Derived from Homologous Strain.

Anti-Friedländer Serum E.	Carbohydrate of Friedländer's bacillus (E).						
	1:50,000	1:100,000	1:250,000	1:500,000	1:1,000,000	1:2,000,000	1:4,000,000
0.2 cc.							
Rabbit 84	++++	++++	+++	+++	++	+±	±
" 83	++++	++++	+++	+++	+±	+	−
" 82	++++	++++	+++	++	+±	+	−
" normal	−	−	−	−	−	−	−
Antipneumococcus serum Type II	+++	+++	+++	+++	+++	++	±

Precipitin Reactions.—Immunization of rabbits with Friedländer's bacillus (Strain E) engenders antibodies which not only agglutinate organisms of the same type, but which precipitate the soluble specific polysaccharide derived from the bacterial cell. As in the case of the specific polysaccharide of Pneumococcus the reactivity of the Friedländer substance is exhibited in dilutions as great as 1:2 million. This fact is evident in Table VII, in which immune rabbit sera in quantities of 0.2 cc. are shown to react with minute amounts of the carbohydrate derived from the homologous strain of Friedländer's bacillus. Just as antipneumococcus serum has been found to agglutinate Friedländer's bacilli of the type represented by Strain E, so also it reacts in precipitin tests with the Friedländer specific substance in high dilution.

Cross-precipitin reactions between the pneumococcus and Friedländer polysaccharides are shown in Table VIII. The soluble specific substances of both organisms are reciprocally reactive with the antibacterial serum of each in dilutions of 1:2 million. The physical properties of the immune precipitate formed by the union of the pneumococcus carbohydrate with the anti-Friedländer serum differ from those of the opaque disk characteristic of the other reactions in that the precipitate is less heavy, more transparent, and tends to form a gelatinous film.

The "specificity" of the reaction is illustrated in Table IX. Here, four protein-free carbohydrates prepared from four different organ-

TABLE VIII.

Cross-Precipitin Reactions.

Immune sera.	Carbohydrate of Friedländer's bacillus (E).					Carbohydrate of Pneumococcus Type II.				
	1:200,000	1:500,000	1:1,000,000	1:2,000,000	1:4,000,000	1:200,000	1:500,000	1:1,000,000	1:2,000,000	1:4,000,000
Anti-Friedländer E. Rabbit 84.....	++++	+++	++	+	±	++++*	++	+	±	−
Antipneumococcus Type II. Horse 91 A.....	++++	+++	++	+	−	++++	+++	+	±	−

* Thin translucent film-like precipitate.

isms are tested in cross-precipitin reactions with the corresponding antibacterial serum of each strain. The three polysaccharides derived from the three fixed types of Pneumococcus show only the specific type reactions in antipneumococcus serum. The cross-precipitation between the Type II pneumococcus specific substance and the anti-Friedländer serum, and the reverse reactions are again evident.

Precipitin Absorption.—Aliquot portions of diluted anti-Friedländer serum were separately absorbed with the homologous culture and with Pneumococcus Type II. The absorbed antibacterial serum was then tested for precipitins against dilutions of the carbohydrates derived from each of the organisms. The results are given in Table X.

As in the case of agglutinin absorption, the anti-Friedländer serum (E) treated with the homologous bacilli loses its precipitins for both

TABLE IX.

Precipitin Reactions of Soluble Specific Substances of Pneumococcus Types I, II, III, and of Friedländer's Bacillus (Strain E).

Immune sera.	Carbohydrate fraction isolated from‡			
	Pneumococcus.			Friedländer's bacillus (E).
	Type I.	Type II.	Type III.	
Antipneumococcus*Type I.....	++++	−	−	−
" " II.....	−	++++	−	++++
" " III.....	−	−	++++	−
Anti-Friedländer† E.....	−	++	−	++++

− indicates no reaction; ++, delicate film-like scale; ++++, heavy, compact, disk-like precipitate.

* Immune horse serum.

† Immune rabbit serum.

‡ Isolated, purified soluble specific substance in dilution of 1:50,000.

TABLE X.

Precipitin Absorption.

Carbohydrate fraction of		Anti-Friedländer Serum E.			Control.
		Unabsorbed.	Absorbed with Friedländer's bacillus (E).	Absorbed with Pneumococcus II.	Normal rabbit serum.
Friedländer's bacillus (E)	1:10.000	+++	−	++	−
	1:50,000	++++	−	+++	−
	1:100,000	++++	−	+++	−
	1:200,000	++++	−	++++	−
Pneumococcus Type II.....	1:10,000	+	−	−	−
	1:50,000	++	−	−	−
	1:100,000	++±	−	−	−
	1:200,000	+++	−	−	−

the Friedländer and Pneumococcus II specific substances, whereas absorption with Type II pneumococci removes the antibodies for the pneumococcus polysaccharide, but leaves those for the Fried-

länder substance only slightly diminished. The converse of this, in which antipneumococcus serum is absorbed with each of the two cultures, yields similar results, Pneumococcus Type II removing the precipitins for both specific substances and the Friedländer bacillus depleting the serum only of antibodies for its own substance.

Protection.—The facts brought out by the test-tube reactions of agglutination and precipitation find added confirmation in the more final proof of reciprocal protection against infection in the animal body.

The six strains of Friedländer's bacilli tested for virulence in mice have each caused a fatal infection in doses as small as 1 ten-millionth cc. The protective power of immune sera against Friedländer's bacilli and pneumococci was tested by injecting white mice intraperitoneally with increasing doses of virulent organisms together with a fixed amount of type serum. Animals surviving 10 days were considered effectively protected. All animals except the virulence controls received 0.2 cc. of immune serum.

The serum of a rabbit immunized with the Friedländer bacillus (Strain E) was first tested for its power to protect mice against infection with virulent cultures of homologous and heterologous types of the Friedländer bacillus.

The protocol given in Table XI shows that 0.2 cc. of the anti-Friedländer serum Type E protected animals against 0.1 cc. of a virulent culture of the homologous strain which without immune serum caused death of the mice in doses of one-millionth cc. On the other hand, the same serum afforded no protection in mice against a virulent heterologous Strain Sc, which, by the reaction of agglutination, was shown to be of another type.

Since the E strain of Friedländer's bacillus was found to be agglutinated and its soluble specific substance to be precipitated by antipneumococcus serum Type II, it was of interest to determine whether protection against infection with a biologically different organism was possible by the use of antipneumococcus serum Type II. The results of this experiment are given in Table XII.

It is evident from Table XII that mice inoculated with 0.2 cc. of antipneumococcus serum Type II were effectively protected against at least a thousand lethal doses of the virulent E strain of Friedländer's bacillus. As in the agglutinin and precipitin tests the

"specific" nature of this protective reaction is shown by the fact that antipneumococcus serum Type I afforded no protection whatever against infection (Table XIII).

TABLE XI.

Protective Action of Anti-Friedländer Serum (E) against Friedländer's Bacillus of Homologous and Heterologous Types.

Anti-Friedländer Serum E.	Friedländer's bacillus.			
	Strain E.		Strain Sc.	
	Amount of culture.	Result.	Amount of culture.	Result.
cc.	*cc.*		*cc.*	
0.2	0.1	S.	0.1	D. 17
0.2	0.01	"	0.01	" 18
0.2	0.001	"	0.001	" 22
0.2	0.0001	"	0.0001	" 22
0	0.00001	D. 40	0.00001	" 30
0	0.000001	" 18	0.000001	" 23

In this and the following tables S. indicates survived; D., death, the numerals representing the hours before death of the animal occurred.

TABLE XII.

Protective Action of Antipneumococcus Serum Type II against Friedländer's Bacillus (E).

Friedländer's bacillus (E).	Antipneumococcus Serum II.		Virulence controls.
	Amount.	Result.	
cc.	*cc.*		
0.001	0.2	S.	
0.0001	0.2	"	
0.00001	0.2	"	
0.000001	0.2	"	
0.0000001	0.2	"	
0.00001	0		D. 51
0.000001	0		" 41
0.0000001	0		" 44

The anti-Friedländer serum prepared by immunization with Strain E protected mice against a hundred thousand lethal doses of another strain (K), which, by agglutination, was classified as belonging to the same type as E. Similarly antipneumococcus serum

Type II afforded protection against the K strain just as it did against the E strain; a fact which emphasizes again the immunological similarity of Friedländer's bacillus of this type to Type II pneumococcus. The same protocol (Table XIII) shows that antipneumococcus serum Type I is wholly without protective action against this type of Friedländer's bacillus. Moreover, Table XIV brings out the fact that protective power against Type II pneumococcus infection is not possessed by the anti-Friedländer serum produced by Strain Sc, which both by agglutination and protection has been shown to belong to a type different from the effective Type E strains.

TABLE XIII.

Protective Action of Antipneumococcus Serum against Friedländer's Bacillus (K).

Friedländer's bacillus (K) (Type E).	Antipneumococcus sera.		Anti-Friedländer Serum E.	Virulence controls.
	Type I. 0.2 cc.	Type II. 0.2 cc.	Rabbit 84. 0.2 cc.	
0.1		D. 42	D. 18	
0.01		" 18	S.	
0.001	D. 18	S.	"	
0.0001	" 18	"	"	
0.00001	" 42	"	"	D. 21
0.000001	" 18			" 42
0.0000001				" 19

TABLE XIV.

Protective Action of Anti-Friedländer Sera against Pneumococcus Type II.

Pneumococcus Type II culture.	Virulence controls.	Anti-Friedländer serum.		Antipneumococcus serum Type II (Horse 91 A). 0.2 cc.
		Rabbit 84 D immunized with Strain E. 0.2 cc.	Rabbit 77 D immunized with Strain Sc. 0.2 cc.	
cc.				
0.2		D. 46	D. 20	D. 42
0.1		" 46	" 20	" 72
0.01		S.	" 20	S.
0.001		"	" 20	"
0.0001		"	" 26	"
0.00001	D. 36			
0.000001	" 46			
0.0000001	" 46			

The further data presented in Tables XIV, XV, and XVI demonstrate the comparable protective power of antipneumococcus Type II and the anti-Friedländer E sera against infection with virulent Type

TABLE XV.

Protective Action of Anti-Friedländer Serum E against Pneumococcus Type II.

Pneumococcus Type II culture.	Immune sera.				Virulence controls.
	Anti-Friedländer E (Rabbit 84).		Antipneumococcus II (Horse 91 A).		
	Amount.	Result.	Amount.	Result.	
cc.	*cc.*		*cc.*		
0.2	0.2	D. 19	0.2	D. 24	
0.1	0.2	S.	0.2	" 48	
0.01	0.2	"	0.2	S.	
0.001	0.2	"	0.2	"	
0.00001	0		0		D. 20
0.000001	0		0		" 20
0.0000001	0		0		" 36

0.0000001 cc. of this culture = 450 colonies.

TABLE XVI.

Comparative Protective Value of Anti-Friedländer and Antipneumococcus Serum against Pneumococcus Type II.

Culture Pneumococcus Type II.	Immune sera.				Virulence control.
	Anti-Friedländer E (Rabbit 84).		Antipneumococcus Type II (Horse 91 A).		
	Amount.	Result.	Amount.	Result.	
cc.	*cc.*		*cc.*		
0.2	0.2	S.	0.2	D. 44	
0.1	0.2	"	0.2	" 20	
0.01	0.2	"	0.2	" 44	
0.001	0.2	"	0.2	S.	
0.0001	0.2	"	0.2	"	
0.00001	0		0		D. 26
0.000001	0		0		" 26
0.0000001	0		0		" 44

II pneumococci. Indeed the protective potency of the anti-Friedländer serum in pneumococcus infection in two of the three experiments is greater than, and in the third test, equal to that exhibited

by the Pneumococcus Type II immune serum itself. This is all the more striking since in the former case immune rabbit serum was used and in the latter the serum of a horse which had received more intensive immunization.

The peculiar "specificity" of the reciprocal protection of antisera of two kinds of bacteria so widely different in other biological characters is confirmatory of the immunological relationships of these organisms brought out by the cross-reactions of agglutination and precipitation.

DISCUSSION.

While comparison of the chemical properties of the two soluble specific substances isolated from Pneumococcus Type II and Friedländer's bacillus (Strain E) reveals many points of resemblance, differences are also found which are too great to be ignored. That the substances in reality are identical and that the observed differences depend only upon impurities present may possibly be the case, but the evidence so far obtained is entirely opposed to this assumption. It has been pointed out in the preceding paper (1) and in the papers dealing with the soluble specific substances of pneumococci (2, 5) that widely differing methods of preparation, calculated to remove different kinds of accompanying inert matter, have yielded strictly comparable products. This fact cannot be taken to indicate that the specific substances as at present isolated are pure chemical compounds, but it at least makes reasonable the assumption that in each instance a large proportion of the adventitious impurities has been eliminated. The view that the specific substance isolated from Pneumococcus Type II and that recovered from the Friedländer bacillus (Strain E) are not identical is further supported by certain of the serological findings, especially those which show that the absorption of agglutinins and precipitins is not reciprocal with the two organisms. If the fact that bacteria possess mutual absorptive capacity be accepted as the criterion of their antigenic identity then the failure of the organisms in question to exhibit this property may be taken as further evidence of the lack of identity of the substances involved.

However, granted a chemical difference between the two specific substances, it becomes necessary to account for their marked im-

munological similarity. In the absence of further evidence as to the structural relations of the two substances, which can only be obtained when large amounts of material become available, it seems reasonable to assume that both contain in a portion of the complex molecule the same or a closely similar configuration of atoms. This essential similarity in molecular grouping would then determine the immunological similarity of the two substances.

In the case of Pneumococcus it has been shown that the polysaccharides by themselves are not antigenic, and it is believed that they become antigenic only when attached to some other substance, possibly the protein of the cell. The type-specific character of the antigenic response, however, is dependent almost entirely upon the nature of the polysaccharide and not upon the substance to which it is attached. Therefore, since the specific carbohydrate substance of the Friedländer bacillus (Strain E) and that of Type II pneumococcus possess in common similar chemical properties, the antigenic response to each may also be similar even though the proteins or other substances with which they are combined be quite dissimilar. A discussion of the actual number of antigens and antibodies present must be deferred until more facts are available (*cf.*, however, in this connection, Landsteiner and van der Scheer (6)).

A striking and probably analogous example of common antigenic properties in substances of remote biological origin is furnished by the phenomenon of heterogenetic specificity originally described by Forssman (7). This investigator showed that following the injection of animal tissues of unrelated species common hemolytic antibodies for sheep corpuscles appear. Landsteiner (8) and Taniguchi (9) have shown that such heterogenetic antigens consist of two component parts, one a protein, the other probably a lipoid substance. Landsteiner and Simms (10) have found that the lipoid constituent, although itself practically devoid of antigenic properties, acquires true antigenicity when combined with protein, and that the antibodies thus induced react with the isolated lipoid fraction.

The fact that two biologically unrelated organisms, Pneumococcus Type II and Friedländer's bacillus (Strain E), possess certain similar serological and antigenic properties suggests that examples of heterogenetic specificity likewise occur among bacteria. In the case

of bacteria, however, the specific substance involved instead of being a lipoid appears to be a polysaccharide. From the results reported in the present papers it further appears probable that when the analogous specific polysaccharides of otherwise totally unrelated microorganisms correspond sufficiently in chemical constitution an immunological correspondence also results. This type of immunological correspondence in no way invalidates the systematic classification of bacteria based upon the more usual and general methods of species determination. It is of greater immediate significance in connection with the study of problems dealing with bacteria as disease-producing agents than in the study of bacteria in their genetic relationships.

SUMMARY.

The chemical and immunological properties of the soluble specific substances of a strain of Friedländer's bacillus and Pneumococcus Type II are described and correlated, and the serological and antigenic similarity of these biologically unrelated organisms is discussed as an example of heterogenetic specificity among bacteria.

BIBLIOGRAPHY.

1. Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, xlii, 701.
2. Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, xlii, 727.
3. Personal communication.
4. Krumwiede, C., Cooper, G., and Provost, D. J., *J. Immunol.*, 1925, x, 55.
5. Avery, O. T., and Heidelberger, M., *J. Exp. Med.*, 1923, xxxviii, 81. Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, xl, 301.
6. Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1925, xlii, 123.
7. Forssman, J., *Biochem. Z.*, 1911, xxxvii, 78.
8. Landsteiner, K., *Biochem. Z.*, 1921, cxix, 298.
9. Taniguchi, T., *J. Path. and Bact.*, 1921, xxiv, 253, 254.
10. Landsteiner, K., and Simms, H. S., *J. Exp. Med.*, 1923, xxxviii, 127