IMMUNOLOGICAL RELATIONSHIPS OF CELL CONSTITUENTS OF ENCAPSULATUS PNEUMONIÆ (FRIEDLÄNDER'S BACILLUS).

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A previous publication (1) on the biological classification of Friedländer's bacillus reported the existence of at least three specific types and a heterogeneous group among these bacilli. The groupings, designated as Types A, B, and C, and Group X, are sharply defined and highly specific by a number of immunological reactions. In a later communication (2) it was stated that encapsulated strains are usually virulent, produce soluble specific substance, and as antigen, induce the formation of type-specific antibodies which operate effectively both in test-tube reactions and in animal protection tests. Capsule-free strains, on the other hand, are avirulent, do not produce soluble specific substance, and as antigen stimulate only the undifferentiated species antibody. Type-specific antisera react irregularly with capsule-free strains; and the species-specific antisera, while reacting with capsule-free organisms regardless of type derivations, do not react at all with encapsulated cells.

The accumulated evidence on the serological reactions of Pneumococcus (3) and Friedländer's bacillus (1, 2, 4, 5) discloses that both species are composed of specific types which are referable to the elaboration of soluble specific substance by the organisms. Under certain conditions, the cells degrade into capsule-free bacteria which, among other changes, show lack of virulence and capsule formation, loss of elaboration of specific carbohydrate, and loss of type-specific antigenicity—all of which properties are the opposite of those which characterize their encapsulated antecedents. In virtue of the striking parallelism in the immunological behavior of Pneumococcus and Friedländer's bacillus it seemed of interest to project into the latter group the principles which govern the immunological relationships of the cell constituents of Pneumococcus.

The comparative immunological studies of the soluble specific substance and the nucleoprotein of Pneumococcus (3) reveal that the soluble specific substance, a nitrogen-free carbohydrate, reacts specifically with antipneumococcus serum of the homologous type. In the dissociated, dissolved, state, it does not serve as antigen; but in the form in which it exists in the cell it functions antigenically to produce type-specific antibodies. The nucleoprotein, on the other hand, is protein in nature and induces in the animal an antiserum which contains only the antiprotein or common, species antibody.

The present study is concerned with the immunological relationships of cell constituents of Friedländer's bacillus and the occurrence of these constituents in culture and body fluids of infected animals.

Methods.

The Soluble Specific Substance.—Methods for the fractionation of soluble specific substance of Friedländer's bacillus have been described in papers from this laboratory (5). It was shown at that time that carbohydrate derived from Strain E (Type B) is dextrorotatory, shows an acid equivalent varying from 670 to 716, is nitrogen-free, and on hydrolysis yields about 75 per cent reducing sugars. It reacts only in type-specific sera to a dilution of 1 to 4 million. Purified, nitrogen-free polysaccharides of Types A and B prepared in this laboratory were utilized in the present study through the courtesy of Drs. Heidelberger and Goebel.

The Nucleoprotein.—Several methods were employed for the separation of the protein and none of the methods were entirely satisfactory. The yield was usually small and the solutions underwent denaturation on keeping. The method finally adopted, however, made use of non-encapsulated cells (since no difference could be shown between the protein derived from encapsulated and capsule-free cells, respectively). The growth from the surface of the agar in Blake bottles was washed off in sterile distilled H_2O. To this suspension NaOH was added to an ultimate concentration of .005 N. The suspensions were frozen and thawed successively a dozen times or more and then diluted 3–4 times with distilled H_2O and centrifuged. The resultant supernatant was filtered, so that a sterile cell-free filtrate was obtained. Precipitation was then effected with a minimum amount of N acetic acid and the precipitate was whirled down. The supernatant was discarded and the precipitate was redissolved in a minimum amount of .01 N NaOH. Usually acid precipitation and solution with alkali were repeated and the final product was made up in .85 per cent NaCl. All protein solutions were standardized on the basis of nitrogen content.

Immunological Reactions.—The method of immunization, the reactions of agglutination and precipitation and protection test have been described in an earlier paper (1).

EXPERIMENTAL.

I. The Soluble Specific Substance.—(*a*) *Antigenic Properties.*—Repeated observations already published from this laboratory leave no doubt that the chemically purified polysaccharide of Pneumococcus is non-antigenic. Similar studies (6) of Zinsser, Mueller, and their associates also record the lack of antigenicity of "residue antigen" from a number of bacterial species. The "residue antigen" of these investigators is a substance which is extracted from bacteria and which bears a definite relation to the specific character of the bacterial cell.

In the present study observations on the antigenicity of the carbohydrate of Friedländer's bacillus are confined to the immunization of rabbits with the polysaccharide derived from Group X. In this instance a solution of bacterial cells was prepared from an encapsulated strain and it consequently contained dissociated soluble specific substance. Immunization with this product even in the presence of nucleoprotein yielded no specific antibodies, as will be pointed out later. Since the results from various sources indicate that bacterial polysaccharides are not antigenic, the lack of specific antibody response to a solution containing both protein and carbohydrate is evidence of similar conditions in the case of Friedländer's bacillus, also. At the same time it is clear that bacterial dissolution is accompanied by antigenic dissociation.

(*b*) *Serological Properties.*—It has been previously demonstrated (1, 2, 4, 5) that the polysaccharides derived from Friedländer's bacillus react specifically with immune sera of the homologous type. (*Cf.* in this connection Table IV.) In fact, sufficiently conclusive proof has been presented to show that just as has been shown with Pneumococcus, the soluble specific substance confers upon the cell its immunological type specificity.

II. The Nucleoprotein.—(*A*) *Antigenic Properties.*—It is realized that the acetic acid-precipitable material represents more than the nucleoprotein of the bacterial cell, and that it is a mixture of proteins rather than a single antigenic unit. Nevertheless, for the purposes of the present study, this fact offers no difficulty in either the performance or interpretation of the various reactions employed. Rab-

TABLE I.

Agglutination of "S" Strains of Friedländer's Bacillus by Anti-P Sera.

Antigen encapsulated strain	Anti-P sera derived from									Anti-S sera—Type			
	Type A			Type B			Group X			A	B	C	X
	*1:1	1:5	1:10	1:1	1:5	1:10	1:1	1:5	1:10	1:5	1:5	1:5	1:5
Type A	−	−	−	−	−	−	−	−	−	++++	−	−	−
" B	−	−	−	−	−	−	−	−	−	−	++++	−	−
" C	−	−	−	−	−	−	−	−	−	−	−	++++	−
Group X	−	−	−	−	−	−	−	−	−	−	−	−	++++

++++ indicates complete, disc agglutination.

* The figures represent dilution of serum.

TABLE II.

Agglutination of "R" Strains of Friedländer's Bacillus by Anti-P Sera.

Anti-P sera	Protein antigen from	Final dilution of serum							Normal serum 1:5
		1:5	1:10	1:20	1:40	1:80	1:160	1:320	
Type A	Type A	++++	++++	++++	++++	++++	++++	++++	−
	" B	++++	++++	++++	+++	+++	++	++	−
	" C	++++	++++	+++	+++	++	+	+	−
	Group X	++++	++++	++++	++++	++++	++++	++++	−
" B	Type A	++++	++++	++++	++++	++++	+++	++	−
	" B	++++	++++	++++	++++	++++	+++	++	−
	" C	++++	++++	++++	+++	+++	++	++	−
	Group X	++++	++++	++++	++++	++++	++++	++++	−
Group X	Type A	++++	++++	++++	+++	++	+	+	−
	" B	++++	++++	++++	++++	++++	+++	++	−
	" C	++++	++++	+++	+++	++	++	+	−
	Group X	++++	++++	++++	++++	++++	++	+	−

++++ indicates complete agglutination; +++, marked agglutination, ++, moderate agglutination; +, slight agglutination.

bits were immunized to the protein isolated from representative strains of Types A and B and Group X. The immune sera were tested for agglutinins, precipitins, and protective antibodies.

(*a*) *Agglutinins.*—Antiprotein sera do not contain type-specific

agglutinins for encapsulated strains of Friedländer's bacillus. Evidence for this statement is found in Table I where it is seen that antisera prepared by immunization with protein derived from serologically different strains do not react with the encapsulated cell of either homologous or heterologous type.

It will be recalled that capsule-free (R) strains of Friedländer's bacillus (2) stimulate the formation of the common, undifferentiated

TABLE III.

Agglutination by Anti-P Sera of Suspensions of Friedländer's Bacillus Decapsulated by Porges' Method.

Anti-P serum derived from	Antigen derived from	Dilution of serum				
		1:50	1:100	1:250	2:500	1:1000
Type A	Type A	+++	+++	++++	++++	+++
	" B	++++	++++	++++	++++	+++
	" C	++	+++	+++	++	+
	Group X	+++	+++	++++	++++	+++
	Gran.*	++	++	+	−	−
" B	Type A	++	+++	++++	++++	++++
	" B	++++	++++	++++	+++	++
	" C	++	+++	+++	++	+
	Group X	+++	++++	++++	++++	+++
	Gran.	++	++	+	−	−
Group X	Type A	+	++	++++	++++	++++
	" B	++++	++++	++++	+++	+
	" C	+	+	+++	++	++
	Group X	++	+++	++++	++++	+++
	Gran.	++	++	++	+	−

* This organism is a strain of granuloma bacillus—isolated from an infection of granuloma inguinale.

species antibody; but that they are unable to provoke type-specific antibodies. Accordingly, it seemed pertinent to determine the reaction of antiprotein sera on the non-encapsulated strains. The results of the reactions are given in Table II. It is seen that capsule-free cells derived from any of the serologically different types agglutinate equally well in all the antiprotein sera. The agglutination is

characteristic of the R cells (2) and occurs at a high dilution of serum. Antiprotein sera, in other words, behave in this respect similarly to anti-R sera. It was to be expected, then, that antiprotein sera would agglutinate, also, suspensions of Friedländer's bacilli after decapsulation by Porges' method. Earlier observations (2) pointed out the serological similarity of capsule-free strains obtained by cultural and chemical means. Table III reveals that suspensions of encapsulated cells treated as Porges recommends are agglutinated in antiprotein sera just as are the "R" strains.

TABLE IV.

Precipitation of the Soluble Specific Substance of Friedländer's Bacillus by Anti-P Sera.

Serum	Soluble specific substance of Friedländer's bacillus											
	Type A						Type B					
	2	20	50	100	250	500	2	20	50	100	250	500
Type A (P)	−	−	−	−	−	−	−	−	−	−	−	−
" B "	−	−	−	−	−	−	−	−	−	−	−	−
" C "	−	−	−	−	−	−	−	−	−	−	−	−
Group X "	−	−	−	−	−	−	−	−	−	−	−	−
Type A (S)	−	+	++	++	+	−	−	−	−	−	−	−
" B "	−	−	−	−	−	−	−	++	+++	++++	+++	++
Normal	−	−	−	−	−	−	−	−	−	−	−	−

+++ indicates compact disc precipitation with clear supernatant; +++, marked disc precipitate; ++, thin film-like scale; +, ground glass turbidity.

The figures represent dilution in thousands.

(*b*) *Precipitins.*—It has been shown that type-specific precipitins are induced only by the encapsulated cell. Added confirmation of this fact is derived from the observation that antiprotein sera do not react with the specific polysaccharides of Friedländer's bacillus. This is evident from the results presented in Table IV. It is definite that none of the antiprotein sera are able to cause precipitation of carbohydrate isolated from strains of Type A or B.

That antiprotein sera react with capsule-free strains of Friedländer's bacillus constitutes direct evidence of the presence of the species antibody. That the species antibody is in reality an antiprotein

TABLE V.

Precipitation by Anti-P Sera of the "Nucleoprotein" of Friedländer's Bacillus and Allied Organisms.

Antigen*	Anti-P serum from	Ultimate dilution of protein						
		200	1000	2000	4000	8000	16,000	32,000
Type A protein	Type A	+++	+++	++	++	+	±	−
	" B	+++	+++	++++	+++	+++	+++	++
	Group X	+++	+++	++	+	−	−	−
		500	2500	5000	10,000	20,000	40,000	80,000
Type B protein	Type A	++++	++++	+++	++	+	±	−
	" B	++	++	+++	+++	+++	+++	+
	Group X	+++	+++	++	+	±	−	−
		200	1000	2000	4000	8000	16,000	32,000
Type C protein	Type A	++	++	++	+	+	±	−
	" B	++	++++	++++	++++	+++	+++	+
	Group X	−	+	++	++	+	−	−
		500	2500	5000	10,000	20,000	40,000	80,000
Group X protein	Type A	++++	++++	+++	++	+	±	−
	" B	++	++	+++	+++	++	++	±
	Group X	++	++	+	+	±	−	−
		500	2500	5000	10,000	20,000	40,000	80,000
B. aerogenes protein	Type A	++	+	+	±	−	−	−
	" B	++	+	+	±	−	−	−
	Group X	++	+	±	−	−	−	−
		300	1500	3000	6000	12,000	24,000	48,000
B. coli protein	Type A	++	+	+	±	−	−	−
	" B	++	++	+	+	±	−	−
	Group X	++	++	+	+	−	−	−
		200	1000	2000	4000	8000	16,000	32,000
Granuloma bacillus protein	Type A	++	++	+	+	−	−	−
	" B	++	++	++	+	+	−	−
	Group X	+	+	±	−	−	−	−

++++ indicates heavy precipitation, supernatant clear; +++, marked precipitation with cloud; ++, marked cloud, no precipitation; +, cloud; ±, faint cloud.

* None of the proteins were precipitated by normal serum.

antibody is evidenced by the reaction of protein precipitation in antiprotein sera. The results of these reactions are presented in Table V. It is seen that sera prepared against protein isolated from three serologically different strains cause the precipitation of protein derived from any of the four different strains. It becomes obvious therefore that nucleoprotein induces the formation of species antibodies which cause agglutination of capsule-free cells and precipitation of protein and that the reactions exhibit none of the type relationships.

In addition, Table V reveals that antiprotein sera of Friedländer's bacillus reacts with protein derived from *B. aerogenes*, *B. coli*, and granuloma bacillus. Stated in another way the protein of Fried-

TABLE VI.

Protection Offered by Anti-P Sera against Infection with Friedländer's Bacillus (Type A).

Type A encapsulated culture	Antiserum derived from				Virulence controls
	Type A		Type B		
	Amount	Result	Amount	Result	
cc.	*cc.*		*cc.*		
.001	.2	D. 20	.2	D. 19	
.0001	.2	" 16	.2	" 15	
.00001	.2	" 39	.2	" 16	D. 24
.000001	.2	" 39	.2	" 43	" 39
.0000001					" 65

D. indicates death, the numerals representing the number of hours before death occurred.

länder's bacillus bears a definite serological relationship to proteins of closely allied species. This lends considerable assistance in the interpretation of the results of former investigators who have observed that anti-Friedländer sera caused agglutination of *B. rhinoscleromatis* (7–10), *B. aerogenes* (11), typhoid (12), and granuloma bacillus (13), etc. The explanation of such cross-agglutinations appears to depend upon the fact that immunization with non-encapsulated strains or prolonged immunization with encapsulated strains stimulates the formation of agglutinins which act not only on R cells

of Friedländer's bacillus, but on R cells of closely related species, as pointed out above.

III. Protection.—Immune sera prepared by immunization with encapsulated Friedländer's bacilli confer upon white mice specific protection against infection by strains of the homologous type (1).

TABLE VII.

Protection Offered by Anti-P Sera against Infection by Friedländer's Bacillus (Type B).

Type B encapsulated culture	Anti-P serum derived from				Virulence controls
	Type A		Type B		
	Amount	Result	Amount	Result	
cc.	*cc.*		*cc.*		
.001	.2	D. 16	.2	D. 15	
.0001	.2	" 16	.2	" 15	
.00001	.2	" 39	.2	" 20	D. 16
.000001	.2	" 39	.2	" 67	" 39
.0000001					" 39

D. indicates death, the numerals representing the number of hours before death occurred.

TABLE VIII.

Precipitation of the Nucleoprotein of Friedländer's Bacillus by Anti-S Sera.

Anti-S Friedländer sera	Friedländer protein derived from							
	Type A		Type B		Type C		Group X	
	*600	6000	1200	12,000	900	9000	500	5000
Type A	++	+	+	++	++	+	++	+
" B	+	−	+	−	±	−	+	−
" C	++	+	++	+	++	+	++	+
Group X	+	−	++	−	+	±	+	−

* These figures represent the dilution of protein.

Immunization with non-encapsulated cells, however, yields no protective antibodies (2). Protective substances, therefore, accompany type-specific antibodies. It was anticipated then that lacking type-specific agglutinins and precipitins, Friedländer antiprotein sera would afford no passive protection against active infection. That this is

actually the case is borne out by the data given in Tables VI and VII. The lack of any protective action by antiprotein sera against infection with virulent strains of homologous and heterologous types is striking.

(*b*) *Serological Properties.*—

1. Precipitation of Protein in Anti-S Sera.—Anti-Friedländer sera resulting from immunization with encapsulated strains are dominantly type-specific. Consequently anti-S sera contain negligible amounts

TABLE IX.

Precipitation by Anti-R Sera of the "Nucleoprotein" of Friedländer's Bacillus.

Antigen	Anti-R serum from	Dilution of protein						
		200	1000	2000	4000	8000	16,000	32,000
Type A protein	Type A	+++	+++	++	++	+	−	−
	" B	+++	++	++	+	−	−	−
	Group X	++	+	+	±	−	−	−
		500	2500	5000	10,000	20,000	40,000	80,000
Type B protein	Type A	+++	++	+	−	−	−	−
	" B	++	++	+	−	−	−	−
	Group X	++	+	+	−	−	−	−
		200	1000	2000	4000	8000	16,000	32,000
Type C protein	Type A	++	+	+	−	−	−	−
	" B	++	+	+	−	−	−	−
	Group X	++	+	+	±	−	−	−
		500	2500	5000	10,000	20,000	40,000	80,000
Group X protein	Type A	+++	++	+	−	−	−	−
	" B	++	++	+	−	−	−	−
	Group X	+++	+++	++	+	−	−	−

of antiprotein as has been pointed out previously (2) by the agglutination of R cells in anti-S sera. In virtue of the presence of antiprotein, such sera may cause precipitation of protein. This fact is illustrated in Table VIII. Type A anti-Friedländer serum definitely contains the species antibody, while the remaining type sera contain traces or none at all.

2. Precipitation of Protein in Anti-R Sera.—Capsule-free strains of Friedländer's bacillus are agglutinated in antiprotein sera (Table

II). Evidence of the reciprocal nature of this reaction was obtained in the precipitation of protein by anti-R sera. It is seen from the results presented in Table IX that anti-R sera cause the precipitation of protein from Friedländer's bacillus of the different types. The conclusion can be drawn, therefore, that the common species antibody reacts with the undifferentiated antigen of Friedländer's bacillus whether the antigen is in the form of non-encapsulated cells or in the form of dissolved protein.

TABLE X.

Precipitation of Friedländer Protein by Anti-P Sera after Adsorption of the Species Antibody by R Strains.

Antigen protein derived from	Anti-P serum (Type A) after adsorption by R strains derived from encapsulated strains of											
	Type A			Type B			Type C			Group X		
	*1:200	1:2000	1:8000	1:500	1:5000	1:10,000	1:200	1:2000	1:8000	1:500	1:5000	1:8000
Type A	—	—	—	—	—	—	—	—	—	—	—	—
" B	—	—	—	—	—	—	—	—	—	—	—	—
" C	—	—	—	—	—	—	—	—	—	—	—	—
Group X	—	—	—	—	—	—	—	—	—	—	—	—

* The figures represent the dilution of protein.

(*c*) *Precipitation of Protein in Antiprotein Sera.*—It has been shown above that protein derived from any of the serological types is precipitated in all antiprotein sera. (*Cf.* Table IV.)

Adsorption of Antibodies in Antiprotein Sera by Non-Encapsulated (R) Strains of Friedländer's Bacillus.

Because agglutination of R cells occurs in antiprotein sera, and precipitation of protein is obtained in anti-R sera, experiments were conducted to gain information concerning the identity of the antibody involved in both reactions. Each antiprotein serum was adsorbed with heat-killed suspensions of R cells derived from encapsulated strains of Types A, B, and C, and Group X. Adsorption was continued until all the agglutinins were removed for the adsorbing

strain. It was found that removal of agglutinins by one strain adsorbed the agglutinins for the remaining R strains also. Moreover, the loss of agglutinins by adsorption was accompanied by a loss of precipitins. A typical protocol is presented in Table X. It is clear that adsorption of a serum resulting from immunization with protein derived from a strain of Type A, for example, with any of the four "R" strains deprives the serum of precipitins for protein derived from any of the types. It appears therefore that the undifferentiated species antibody is the same antibody whether it occurs in anti-R or antiprotein sera.

TABLE XI.

Occurrence of Soluble Specific Substance in Culture Filtrate. Friedländer Bacilli.

Strain	Dilution of filtrates															
	After 4 hrs.				After 8 hrs.				After 12 hrs.				After 24 hrs.			
	1:1	1:5	1:20	1:50	1:1	1:5	1:20	1:50	1:1	1:5	1:20	1:50	1:1	1:5	1:20	1:50
Type A	+	−	−	−	+	+	−	−	+++	++	+	−	++++	++++	++	+
" B	−	−	−	−	±	−	−	−	+	+	−	−	++++	+++	+	−
" C	+	−	−	−	++	+	−	−	++	++	+	−	++++	++++	+	−
Group X	±	−	−	−	++	+	−	−	++	+	−	−	++++	++	+	−

Occurrence of Soluble Specific Substance in Culture Filtrates of Friedländer's Bacillus.

It has been shown by Dochez and Avery (14) that the soluble specific substance of Pneumococcus is demonstrable in culture filtrates, and that the progressive increase of the carbohydrate bears a striking relation to the growth curve of the culture. Their results show clearly that the soluble specific substance is a product of metabolic activity rather than a product of cell disintegration.

In similar fashion, the polysaccharide of Friedländer's bacillus is demonstrable in actively growing cultures. Cell-free filtrates obtained at different intervals during the growth are precipitated specifically by anti-Friedländer sera. The data submitted in Table XI show that in some instances specific carbohydrate is present in filtrates as early as 4 hours after growth has been initiated. The

amount of the carbohydrate increases rapidly so that after 24 hours' growth a definite reaction may be obtained in culture filtrate in dilution of 1:20, and in one case (Type A) even in a dilution of 1:50.

Occurrence of Protein in Culture Filtrates of Friedländer's Bacillus.

The type specificity of anti-Friedländer sera depends in part upon the integrity, and the absence of R cells in the culture used for immunization. It becomes of importance, therefore, to determine the rapidity of the disintegration of Friedländer's bacilli. Dissociation may be estimated by the presence of the common or protein antigen. Accordingly an analysis of the protein content of culture filtrates of Friedländer's bacillus was made by the usual protein pre-

TABLE XII.

Occurrence of Protein in Culture Filtrates of Friedländer Bacilli.

Strain	Dilution of filtrates											
	At 12 hrs.		At 24 hrs.		At 72 hrs.		5 days		9 days			
	1:1	1:10	1:1	1:10	1:1	1:10	1:1	1:10	1:1	1:5	1:10	1:20
Type A	−	−	−	−	−	−	−	−	+	±	−	−
" B	−	−	−	−	−	−	−	−	++	+±	+	−
" C	−	−	−	−	−	−	−	−	++	+	±	−
Group X	−	−	−	−	−	−	−	−	++	+	±	−

cipitation test. As brought out in Table XII, no protein was demonstrated in culture filtrates after 5 days' growth. On the 9th day, precipitation of protein was obtained in all the filtrates studied. In contrast to the carbohydrate which is elaborated during the period of active growth, the protein is demonstrable after this period and when cell disintegration takes place.

This fact becomes of great significance in immunization with suspensions of Friedländer's bacillus. Despite the fact that both disintegration and R cells are absent in the cultures used for immunization, anti-Friedländer sera may contain variable amounts of protein antibody. This is evidence that the body defenses not only engender type-specific antibodies, but also include a mechanism which causes a cleavage or disintegration of the specific antigen.

Occurrence of Soluble Specific Substance in Friedländer Infections.

The soluble specific substance of Pneumococcus has been demonstrated in the serum and urine of patients during pneumonia by Dochez and Avery (14). Blake (15) has shown that this is also a fact in pneumonia due to Friedländer's bacillus. This is the only reference of its kind concerning Friedländer infection which has come to our attention. In the present study experiments were performed to detect specific carbohydrates in rabbits infected by intraperitoneal injections of Friedländer's bacilli. The urine and blood of the infected rabbits were collected and tested for soluble specific substance

TABLE XIII.

Occurrence of Soluble Specific Substance in the Blood and Urine of Animals Infected with Friedländer's Bacilli.

Type of infection	Body fluid	Dilution of fluid		
		1:1	1:5	1:10
Type B	Serum	++	+	−
	Urine	+	−	−
" C	Serum	+++	++	+
	Urine	++	+	−

The precipitin reaction was obtained only in homologous immune serum.

by the usual precipitin technique. It is seen from Table XIII that the specific polysaccharide of Friedländer bacilli is present in both urine and serum of rabbits, and is demonstrable within 18 hours after infection.

DISCUSSION.

The soluble specific substance of Friedländer's bacillus endows the cell with type specificity, and is separable from the bacterial cell as a pure, nitrogen-free polysaccharide. When dissociated from the cell, it does not function as antigen, but in the form in which it exists in the cell it stimulates the formation of antibodies which cause type-specific agglutination of encapsulated cells, precipitate the carbohydrate derived from organisms of the homologous type, and afford

passive protection in white mice against infection by bacilli of the same type. As reacting substance, it is precipitated only in antisera resulting from immunization with encapsulated strains from which it is derived.

The nucleoprotein is separable from dissolved Friedländer's bacilli by precipitation with acetic acid in the cold. This constituent differs in nature and in serological behavior from the soluble specific substance. It is protein in nature and is a common, undifferentiated constituent of all types. It is antigenic and provokes in the animal the common protein or species antibody. The species antibody does not react with encapsulated bacilli of any type nor with the soluble specific substance of either homologous or heterologous types, and does not protect against infection with Friedländer's bacillus. Anti-protein sera, however, cause agglutination of capsule-free cells derived from any of the serological types by either cultural or chemical methods; and they react also with protein from all types. Moreover, the protein antibody is of a sufficiently general nature to react with protein from allied bacteria. In this fact resides the explanation for the confusing cross-agglutination reactions obtained with related organisms by former workers. That the protein antibody is of more or less common occurrence among other species of bacteria gains evidence from the results of numerous investigators. The studies from this laboratory show this with Pneumococcus, and the work of Lancefield (16), Hitchcock (17), and Tunnicliff (18) discloses a distinct serological relationship between various species of the Gram-positive cocci. The contributions of Dopter (19) and Eberson (20) depict similar relationships among the Gram-negative cocci, and Smith and TenBroeck (21) and Felix (22) offer comparable data for members of the typhoid-colon group.

Antisera prepared by immunization with protein or with a degraded non-encapsulated R culture contain antibodies which are identical in their immunological reactions.

The presence of soluble specific substance in filtrates of growing cultures suggests that it is a product of growth activity of the cell. The presence of protein in filtrates of old cultures only, indicates that it is a product of cell autolysis and disintegration.

CONCLUSIONS.

1. The soluble specific substance of Friedländer's bacillus is non-antigenic when dissociated from the cell. It is different for each type and it is highly reactive in the corresponding anti-S serum.

2. The nucleoprotein is antigenic, induces the species or protein antibody which reacts with capsule-free cells and protein derived from all types. Antiprotein sera do not react with either the encapsulated cell or the polysaccharide derived from it, and they offer no protection against infection.

3. Anti-R and antiprotein sera are identical in their behavior.

4. The carbohydrate of Friedländer's bacillus is demonstrable in filtrates of actively growing cultures and in the blood and urine of infected animals.

5. The protein is demonstrable in filtrates of only old, disintegrating cultures.

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