A BIOLOGICAL CLASSIFICATION OF ENCAPSULATUS PNEUMONIÆ (FRIEDLÄNDER'S BACILLUS).

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In a previous study (1) of the classification of the *encapsulatus* group of organisms certain technical difficulties were encountered which made a serological differentiation of these bacilli confusing and impracticable. The data presented at that time suggested the existence of two types, but these were ill defined and comprised only a small number of all the strains studied. The difficulties attending the immunological classification were ascribed to the failure to obtain potent sera and to the interference of the capsule of the organisms with agglutination. Numerous other investigators had made unsuccessful attempts at a classification, also, and for the most part they have attributed their failures to one or both of these causes.

A review of the literature reveals how contradictory and confusing the data are on the subject. Kraus (2) and Landsteiner (3) were the first to demonstrate agglutination in the *encapsulatus* group. Both investigators procured sera which agglutinated only in low dilutions. Sicard (4), Defalle (5), Clairmont (6), and Porges (7) obtained antisera inconstantly. Immune sera were procured by Klemperer and Scheier (8) and Bertarelli (9) but the sera were not specific and agglutinated closely related species. Specific sera were obtained by Friel (10), Coulter (11), Small and Julianelle (1), and Ball (12).

Howard (13) protected guinea pigs against Friedländer infection by active immunization, but the serum of these animals conferred no passive protection on normal animals. Landsteiner, Sicard, and Clairmont also were unable to demonstrate protective antibodies in the sera of immunized animals. Perkins (14) found that guinea pigs surviving a spontaneous Friedländer epidemic acquired immunity against the organism isolated during the epidemic. In a later paper (15) he states that "animal inoculation, immunization and agglutination show results which are far too variable to admit of them as means for classification."

Limiting their work to the differentiation of the Friedländer and rhinoscleroma organisms, Erben (16), Ballner and Reibmayr (17), and Galli-Valerio (18, 19)

could determine no serological differences between these organisms. Goldzieher and Neuber (20), on the other hand, obtained fixation reactions with *B. rhinoscleromatis*, using serum from patients suffering from rhinoscleroma and from rabbits immunized to the rhinoscleroma bacillus, but they were not successful when Friedländer's bacillus was used as antigen with the same sera. Babes (21) produced in rabbits an agglutinating serum of low titer against a strain of *B. rhinoscleromatis*. Sdrawosmysloff (22) found no complement-fixing antibodies in the serum of a rhinoscleroma patient.

Schmidt (23), using the serum of a patient convalescent from Friedländer infection of the lung, and the organism isolated from the sputum and from the lung by direct puncture, observed an "amorphous" agglutination. In this instance, no immune properties were demonstrable until after the 1st month of illness, when agglutination, bacteriolysis, and thread reaction were observed. Wolf (24) isolated a strain of Friedländer's bacillus from the urine of a patient whose serum agglutinated equally well the homologous strains and several stock laboratory ones in dilutions of 1:2000 and 1:5000.

Porges attributed the difficulty of immunization with *encapsulatus* strains to the presence of capsules which he thought interfered also with agglutination. Consequently he devised a method by which the capsular material was hydrolyzed by heating suspensions of the bacteria in weak acid. With von Eisler (25) he showed that after destruction of the capsule immune sera could be obtained and that agglutination was facilitated in this way. Streit (26) and Beham (27) took exception to the method because suspensions so treated were apt to clump spontaneously. They effected decapsulation by cultural methods, and obtained agglutination regularly.

Fitzgerald (28) found that immunization of rabbits with *B. rhinoscleromatis* yielded a potent serum which agglutinated four other strains of the organism equally well. These strains were agglutinated only when capsule-free. Small and Julianelle observed that capsule-bearing strains agglutinated only in high concentrations of immune sera, and the flocculation resulting under these conditions gave a compact disk at the bottom of the tube with a clear supernatant fluid. Capsule-free strains on the other hand agglutinated in serum dilutions as high as 1:2500 and gave a finely granular precipitate which was easily broken up. The encapsulated strains, moreover, adsorbed the specific agglutinins from the immune sera, while capsule-free strains did not.

It becomes evident from this review of the literature that great confusion exists as to the immunological identity of members of the *encapsulatus* group. The impression is that agglutination not only is difficult, but unreliable and not sharply differential. It seemed not unlikely, however, that the application to this group of the principles governing the immunological relationships of pneumococci and their cell constituents might afford a basis for determining similar relations

among the encapsulated bacilli. This newer concept of the bacterial cell (29–34) involves two separable and distinct constituents—the one a polysaccharide, the soluble specific substance, which endows the cell with type specificity; the other a protein substance, which, regardless of type derivation, exhibits immunologically only the common and undifferentiated characteristics of the species. The dissociation of the polysaccharide from the cell deprives the organism of its type-specific antigenic power. In general, antipneumococcus serum contains both the type-specific and the species-specific antibody; the occurrence of the latter depending upon the extent of the dissociation of the antigenic complex, which may take place both *in vitro* and *in vivo*. The presence of the protein antibody in appreciable amounts may therefore mask and sometimes obliterate the type specificity of an organism. Moreover, under unfavorable conditions, these organisms are known to lose the function of elaborating the soluble specific substance—a condition resulting in loss of type specificity, capsule formation, and accompanied by loss of virulence. These degraded strains lose the property of stimulating the type-specific antibody and as antigen provoke only the common protein response (also Stryker (35), Reimann (36), Amoss (37)). In other words, type specificity is demonstrable only when the antisera are dominantly type-specific and the strains to be agglutinated must be not degraded but encapsulated. Stripping an organism of its capsule therefore will minimize the specific serologic reactivity of the organism. It was with this conception, then, that the problem of the classification of the Friedländer bacilli was subjected to further analysis.

EXPERIMENTAL.

Strains of Microorganisms.—The organisms used in this investigation were all members of the *encapsulatus* group as determined by their individual, cultural, staining, and biochemical characteristics. They had been isolated at different times and places within the past 6 years. Strains E and K were isolated from guinea pigs with pneumonia; Strains H1 and H2 were derived from infections in horses; and the remaining strains, from human sources, the majority of them having been associated with lobar pneumonia. Two strains of *Encapsulatus granulomatis*, and two strains of *B. aerogenes* were included in the agglutination tests as a control of specificity. All the strains had in common the production of mucoid, gummy growth, though there were variations in this respect by the individual strains.

Immunization.—Normal rabbits were bled before immunization was begun and the normal sera were pooled and used for control in the subsequent tests. The animals were immunized by intravenous injections of suspensions of the heat-killed encapsulated organisms. Small doses were injected in the beginning and the amount increased during the course of the injections. Immunization was effected by injecting the organisms on 3 successive days, followed by a rest period of 4 days. Before each course of injections, the titer of the serum of each rabbit was determined; and when the serum showed no further increase in titer, immunization was discontinued. The rabbits were bled 9 or 10 days after the last injection. By this method, an efficient agglutinating serum was obtained in from 4 to 5 weeks.

Agglutination.—Agglutinations were conducted with saline suspensions of living bacilli kept without preservative. The various suspensions were diluted to an equal turbidity before being used for the test. Incubation was carried out for 2 hours at 37°C. in the water bath, and final readings were made after the tubes had been in the ice box overnight. In the more concentrated sera, positive results were usually observable within a few minutes. In agglutinin adsorption tests, heat-killed organisms were used.

Protection.—The protective power of the sera was determined by injecting white mice intraperitoneally with a mixture of 0.2 cc. of the serum and varying dilutions of a broth culture of the organism, both being brought to a volume of 0.5 cc. with sterile broth. The culture used was grown from 4 to 6 hours. The mice were kept under observation for 6 days and those surviving for this period were considered effectively protected.

Precipitin Tests.—In the precipitin tests 0.2 cc. of immune serum diluted to a volume of 0.5 cc. with salt solution was added to an equal volume of increasing dilutions of the soluble specific substance of Friedländer's bacillus. The mixtures were incubated for 2 hours at 37°C. in the water bath. Final readings were made on the following morning after the mixtures had been overnight in the ice box.

Thread Reaction.—In performing these tests, dilutions of the various sera were made in sterile broth. The serum broth tubes were then inoculated with 0.1 cc. of a 4 hour culture of the organisms to be studied and incubation was carried on overnight, for a period of 16 to 18 hours.

Agglutination.

Rabbits were immunized as described to seven of the strains studied. Use was also made of Type II antipneumococcus serum, in view of the recent work of Avery, Heidelberger, and Goebel (38).

The type-specific agglutinations are characteristic and result in a voluminous, compact disk at the bottom of the tube with a clear supernatant fluid. In the lesser dilutions the flocculation occurs immediately and becomes apparent even before incubation.

TABLE I.

Cross-Agglutination Reactions with Strains of Friedländer's Bacillus.

Strain of organism.	Immune sera—final dilution 1:5.								Normal Serum.
	F5	Sc	F2	F6	Pn. II	F10	F11	F12	
F3	++++	++++	−	−	−	−	−	−	−
F5	++++	++++	−	−	−	−	−	−	−
F8	++++	++++	−	−	−	−	−	−	−
F9	++++	++++	−	−	−	−	−	−	−
F15	++++	++++	−	−	−	−	−	−	−
F18	++++	++++	−	−	−	−	−	−	−
F19	++++	++++	−	−	−	−	−	−	−
F20	++++	++++	−	−	−	−	−	−	−
F21	++++	++++	−	−	−	−	−	−	−
F23	++++	++++	−	−	−	−	−	−	−
F25	++++	++++	−	−	−	−	−	−	−
Bu	++++	++++	−	−	−	−	−	−	−
Sc	++++	++++	−	−	−	−	−	−	−
Sm	++++	++++	−	−	−	−	−	−	−
St	++++	++++	−	−	−	−	−	−	−
F2	−	−	++++	++++	++++	−	−	−	−
F6	−	−	++++	++++	++++	−	−	−	−
E	−	−	++++	++++	++++	−	−	−	−
H1	−	−	++++	++++	++++	−	−	−	−
H2	−	−	++++	++++	++++	−	−	−	−
K	−	−	++++	++++	++++	−	−	−	−
F10	−	−	−	−	−	++++	−	−	−
M	−	−	−	−	−	++++	−	−	−
R	−	−	−	−	−	++++	−	−	−
F11	−	−	−	−	−	−	++++	−	−
F12	−	−	−	−	−	−	−	++++	−
F1	−	−	−	−	−	−	−	−	−
F13	−	−	−	−	−	−	−	−	−
F22	−	−	−	−	−	−	−	−	−
F24	−	−	−	−	−	−	−	−	−
G1	−	−	−	−	−	−	−	−	−
G2	−	−	−	−	−	−	−	−	−
A	−	−	−	−	−	−	−	−	−
W	−	−	−	−	−	−	−	−	−

++++ indicates complete, compact agglutination with clear supernatant; − indicates no agglutination.

Strains G1 and G2 are strains of granuloma bacillus, and A and W, *aerogenes*.

None of these strains were agglutinated by Type I or III antipneumococcus sera. The agglutination titer of these sera was as follows: F5, 1:40; Sc, 1:40; F2, 1:80; F6, 1:40; Pn. II, 1:40; F10, 1:40; F11, 1:160; F12, 1:80.

The cross-agglutination reactions are summarized in Table I. It becomes evident from the data that anti-Friedländer sera cause a highly specific agglutination which serves to define distinct type relations. Three types are revealed. Of the thirty strains included in

TABLE II.

Agglutinin Adsorption Reaction.
Result of Agglutination with Type A Serum after Adsorption with Strains of Homologous and Heterologous Types.

Strain.	Type.	Serum Type A (F5).						
		Unadsorbed.	Adsorbed by strains of					
			Type A.					Type B.
			F5	F15	Sc	Sm	Bu	E
F3	A	++++	−	−	−	−	−	++++
F5	A	++++	−	−	−	−	−	++++
F8	A	++++	−	−	−	−	−	++++
F9	A	++++	−	−	−	−	−	++++
F15	A	++++	−	−	−	−	−	++++
F18	A	++++	−	−	−	−	−	++++
F19	A	++++	−	−	−	−	−	++++
F20	A	++++	−	−	−	−	−	++++
F21	A	++++	−	−	−	−	−	++++
F23	A	++++	−	−	−	−	−	++++
F25	A	++++	−	−	−	−	−	++++
Sc	A	++++	−	−	−	−	−	++++
Sm	A	++++	−	−	−	−	−	++++
St	A	++++	−	−	−	−	−	++++
Bu	A	++++	−	−	−	−	−	++++
E	B	−	−	−	−	−	−	−

The final dilution of serum in these tests was 1:5.

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

the survey, twenty-four fall into one or another of these types. For the sake of convenience, these groups will be referred to as Type A, which is composed of fifteen strains; Type B, which is composed of six strains; Type C, which comprises three strains. The remaining six cultures, against four of which no sera were prepared, are placed in a

heterogeneous group, Group X. It is not unlikely that, with an extension of this study so as to include a greater number of strains,

TABLE III.

Agglutinin Adsorption Reaction.
Results of Agglutination with Type B Serum after Adsorption with Strains of Homologous and Heterologous Types.

Strain.	Type.	Serum Type B (F2). Unadsorbed.	Adsorbed by strains of: Type B. F2	Type B. E	Type A. Sc	Strain.	Type.	Serum Type B (F6). Unadsorbed.	Adsorbed by strains of: Type B. F2	Type B. F6	Type A. Sc
F2	B	++++	−	−	++++	F2	B	++++	−	−	++++
F6	B	++++	−	−	++++	F6	B	++++	−	−	++++
E	B	++++	−	−	++++	E	B	++++	−	−	++++
K	B	++++	−	−	++++	K	B	++++	−	−	++++
H1	B	++++	−	−	++++	H1	B	++++	−	−	++++
H2	B	++++	−	−	++++	H2	B	++++	−	−	++++
Sc	A	−	−	−	−	Sc	A	−	−	−	−

TABLE IV.

Agglutinin Adsorption Reaction.
Results of Agglutination with Type C Serum after Adsorption with Strains of Homologous and Heterologous Types.

Strain.	Type.	Serum Type C (F10). Unadsorbed.	Adsorbed by strains of: Type C. F10	Type C. M	Type C. R	Type A. Sc
F10	C	++++	−	−	−	++++
M	C	++++	−	−	−	++++
R	C	++++	−	−	−	++++
Sc	A	−	−	−	−	−

additional types may be demonstrated among the heterogeneous strains of Group X.

The B type of the Friedländer bacillus is similar immunologically to Type II Pneumococcus. Avery, Heidelberger, and Goebel have already called attention to this relationship. It will be recalled that they showed that certain strains of Friedländer's bacillus, now designated as Type B, possess specific polysaccharides which, both in chemical and immunological properties, are so similar as to show cross-agglutination and protection, but no reciprocal adsorption of antibodies. It is seen that the six strains of this type react as well with Type II antipneumococcus serum as they do with their homologous sera.

TABLE V.

Protective Action of Anti-Friedländer Serum, Type A, against Strains of Homologous and Heterologous Types.

Amount of culture.	Immune serum Type A.	Strains of Friedländer bacillus.			
		Type A.			Type B.
		F5	F9	Sc	E
cc.	*cc.*				
10^{-3}	0.2	D. 23 hrs.	D. 48 hrs.	D. 24 hrs.	D. 16 hrs.
10^{-4}	0.2	S.	S.	S.	" 40 "
10^{-5}	0.2	"	"	"	" 40 "
10^{-6}	0.2	"	"	"	" 90 "
10^{-5}	0	D. 15 hrs.	D. 24 hrs.	D. 40 hrs.	D. 16 hrs.
10^{-6}	0	" 20 "	" 45 "	" 45 "	" 44 "
10^{-7}	0	" 40 "	" 46 "	" 46 "	" 40 "

S. indicates that the animal survived; D., that the animal died. The numerals represent the number of hours before death occurred.

Agglutinin Adsorption.

Further evidence of the specificity of the types just mentioned was obtained by agglutinin adsorption tests. The type sera were adsorbed with heat-killed suspensions of both homologous and heterologous type strains. When all the antibodies had been removed for the adsorbing strain, the sera were tested for the presence of agglutinins for the other strains of the homologous type.

The data summarized in Table II show that Serum F5 (Type A)

was adsorbed with five homologous strains, F5, Sc, F15, Sm, and Bu, and in each case the agglutinins were completely adsorbed. Adsorption with a heterologous strain, E, left untouched the agglutinins in this serum. When Serum F2 (Type B) was adsorbed with Strains F2 and E, homologous strains, agglutinins were removed from the serum not only for the adsorbing strains but for all the strains which were agglutinated by the unadsorbed serum. Strain Sc, a heterologous type, however, exerted no influence on the agglutination of the strains of the type serum. Similarly, adsorption of Serum F6 (also Type B) with

TABLE VI.

Protective Action of Anti-Friedländer Serum Type B, against Strains of Homologous and Heterologous Types.

Amount of culture.	Immune serum Type B.	Strains of Friedländer bacillus.			
		Type B.			Type A.
		F6	K	E	F9
cc.	*cc.*				
10^{-2}	0.2	D. 23 hrs.	—	—	—
10^{-3}	0.2	S.	D. 40 hrs.	D. 40 hrs.	D. 19 hrs.
10^{-4}	0.2	"	S.	S.	" 23 "
10^{-5}	0.2	"	"	"	" 92 "
10^{-6}	0.2	"	"	"	" 40 "
10^{-5}	0	D. 44 hrs.	D. 16 hrs.	D. 16 hrs.	D. 24 hrs.
10^{-6}	0	" 48 "	" 18 "	" 44 "	" 40 "
10^{-7}	0	S.	" 21 "	" 44 "	" 44 "

homologous strains removed all agglutinins for members of this group, whereas, adsorption by Sc, a heterologous strain, again had no effect on the agglutinin titer (Table III). Serum F10 (Type C) was no longer agglutinating for its three homologous strains after adsorption had taken place by Strains F10, M, and R. Strain Sc (Type A), however, adsorbed out none of the agglutinins (Table IV). The results obtained by the method of agglutinin adsorption constitute conclusive evidence that the types disclosed by agglutination with anti-Friedländer sera are real types.

Protection Tests.

That Friedländer bacilli are separable into at least three specific types, as demonstrated by the reactions of agglutination and agglutinin adsorption is a fact which gains further demonstration from animal protection tests. It is evident from the data presented in Tables V and VI that the protective action of the type immune sera reveals type specificity in the case of Friedländer bacilli just as sharply as does agglutination. Type A serum (Strain F5) protected mice against homologues of this type to an infective dose of 0.0001 cc. when the cultures themselves killed white mice regularly in a dilution of 0.0000001 cc. within 48 hours. On the other hand it offered no protection against an infection with an organism of a different type. Type B serum (F2) protected against infection by three strains of the homologous type to 0.0001 cc. when the cultures as such were fatal to a dilution of 0.0000001 cc.; yet this serum offered no protection against infection of a different type.

It was not possible to obtain data for the protective power of Type C sera. The three cultures of this type were avirulent for mice, and, despite numerous animal passages, the lethal dose could not be increased. The strains were all capsule-bearing and no "R" colonies could be demonstrated by plating directly from the peritoneal fluid of infected mice.

It is obvious from the results of the protection test that certain anti-Friedländer sera offer definite protection against infection of the organisms and that the protection is type-specific. The protective power of these sera was not as high as had been anticipated however. Avery, Heidelberger, and Goebel obtained a greater degree of protection against Type B infection; but there is no essential discordance between their results and ours, since our animals did not receive as intensive an immunization.

Thread Reaction.

Still further proof of the type specificity of the Friedländer organisms was obtained by means of the thread reaction. This reaction depends upon the character of the growth of an organism in homologous immune serum. Although the mechanism of the reaction may be

TABLE VII.

Thread Reaction.

Thread Formation during Growth of Friedländer's Bacillus in Presence of Immune Sera of Homologous and Heterologous Types.

Anti-Friedländer serum.		Strain Sc, Type A. Dilution of serum.						Strain E, Type B. Dilution of serum.						Strain M, Type C. Dilution of serum.						Broth control.
		1:5	1:10	1:50	1:100	1:500	1:1000	1:5	1:10	1:50	1:100	1:500	1:1000	1:5	1:10	1:50	1:100	1:500	1:1000	
Type A	F5	++++	++	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Type B	F2	−	−	−	−	−	−	++++	++++	++	±	−	−	−	−	−	−	−	−	−
	F6	−	−	−	−	−	−	++++	++	+	−	−	−	−	−	−	−	−	−	−
Type C	F10	−	−	−	−	−	−	−	−	−	−	−	−	++++	+++	+	−	−	−	−
Group X	F11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	F12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Normal serum.		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

++++ indicates growth was compact and completely sedimented with clear supernatant; +++ indicates marked, but not complete sedimentation; ++, clumping, more easily broken up; +, partial clumping; ±, slight clumping; −, no clumping.

the same as that involved in agglutination, it is usually more delicate than agglutination, and frequently positive results are obtained at a dilution of serum beyond the range of agglutination.

The findings are presented in Table VII. It is seen that a strain gives the thread reaction only when it is grown in immune serum of the homologous type. The reaction extends over the same range of dilutions as agglutination. It was expected that the thread reaction would be detectable in greater dilutions, but the growth of the organism is so profuse as probably to obscure the clumped growth that might occur at the higher dilutions.

Precipitin Reaction.

It has been stated earlier in this paper that a part of the difficulty in demonstrating types by serological reactions in the *encapsulatus* group has been explained on the basis that capsules inhibit agglutination and interfere with agglutinin formation in the body. The methods which were devised to strip the organism of its capsule, at the same time removed from the organism its specificity.

The first workers considered the capsule to be a nucleoprotein (39, 7, 28, 11). The chemical nature of the substance was studied by Rettger (40), who considered it a glycoprotein, possibly a pseudomucin. Toenniessen (41) isolated from a strain of Friedländer's bacillus a polysaccharide which yielded on hydrolysis an osazone, probably of galactose. Kramár (42) isolated a polysaccharide which on inversion yielded galactose. None of these investigators, however, recognized the relationship between the chemical nature and biological specificity of the capsular material.

Recently Mueller, Smith, and Litarczek (43) derived from a Friedländer bacillus a carbohydrate-containing material which reacted to a very high dilution of homologous immune serum. Heidelberger, Goebel, and Avery (44, 38) obtained a nitrogen-free polysaccharide from Strain E (Type B) which reacted specifically with immune serum of the homologous type in dilutions of 1:4 millions. Blake (45) was the first, and as far as we know the only one, to show the appearance of the soluble specific substance of Friedländer's bacillus in the blood and urine during an active infection. Although he could demonstrate no agglutinins in the serum of a patient, he found increasing amounts of the soluble specific substance of Friedländer's bacillus in both blood and urine. This is an interesting example of similarity in Friedländer and Pneumococcus pneumonia, since it is possible in both infections (46) to demonstrate the presence of the soluble specific substance of the causative organism in the blood and urine of patients suffering from severe infection.

Since the relation of capsular material and specificity has been recognized it seemed desirable to determine the action of immune sera of the different types on the isolated specific substance of Friedländer bacilli. Nitrogen-free polysaccharides of Strain Sc (Type A) and Strain E (Type B) were obtained[1] and cross-precipitin reactions were carried out in the usual manner. The amount of immune serum was kept constant at 0.2 cc. while the polysaccharide was used in increasing dilutions.

TABLE VIII.

Precipitation of the Soluble Specific Substance of Friedländer Bacilli by Immune Sera of Homologous and Heterologous Types.

Anti-Friedländer serum.		Type A soluble specific substance.								Type B soluble specific substance.								Salt control.
		20*	50	100	250	500	1000	2000	4000	20	50	100	250	500	1000	2000	4000	
Type A	F5	++	++	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−
	Sc	++	++	++	+	±	−	−	−	−	−	−	−	−	−	−	−	−
Type B	F2	−	−	−	−	−	−	−	−	+	++	++	++	+	+	±	−	−
	F6	−	−	−	−	−	−	−	−	+	++	++	+	+	±	−	−	−
	E	−	−	−	−	−	−	−	−	+++	+++	+++	++	+	±	−	−	−
Type C	F10	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group X	F11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	F12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Normal serum.		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

++++ indicates heavy compact, disk precipitate; +++, marked disk precipitate; ++, thin, film-like scale; +, ground glass turbidity; ±, slight turbidity; −, no reaction:

* These figures represent dilutions in thousands.

The results of the precipitation tests are presented in Table VIII, and they show that the polysaccharide of each type reacts only with immune sera of the corresponding type and that precipitation occurs at a high dilution of the soluble specific substance. Further evidence to be reported in a subsequent publication will show that the organism

[1] The polysaccharides were obtained through the courtesy of Dr. Heidelberger and Dr. Goebel.

without its capsule is devoid of type specificity. It becomes obvious, therefore, that by removal of the capsule, specificity is lost.

DISCUSSION.

Although numerous attempts have been made by various investigators to classify the Friedländer group of bacilli on the basis of serological reactions, confusion rather than system has resulted. The newer conception of the immunological relationships of the capsular and somatic substances of the bacterial cell has furnished us, in part at least, with an explanation of the earlier failures. The principles involved are those brought out by the immunochemical studies on the type specificity of Pneumococcus, which have been carried on in this laboratory. The work has revealed a cellular mechanism in which two distinct and separable substances are concerned in the antigenic and serological behavior of the cell. One of these, the capsular or ectoplasmic layer of the cell, is the soluble specific substance which is now identified as a polysaccharide and is chemically different for each of the fixed types of Pneumococcus. The differential specificity of the bacterial types is dependent solely upon the presence of this substance.

That the carbohydrate fraction of the Friedländer bacillus is the substance which determines specificity among organisms of the *encapsulatus* group has been demonstrated in the present study, and in other papers from this laboratory. Removal of the capsule either by hydrolysis or by cultural methods results in a loss of specificity of the organism.

The second constituent of the Pneumococcus cell is the somatic protein substance which, regardless of type derivation, exhibits immunologically only the common and undifferentiated characteristics of the species. Immunization by a number of workers with a capsule-free Friedländer bacillus has yielded a serum which reacted with capsule-free strains of any type of these organisms.

Antigenically, the polysaccharide of the bacterial cell is inert. However, in the form in which it exists in the cell, this carbohydrate complex is the dominant and effective antigen yielding on immunization the corresponding type-specific antibody. It is now recognized that cell dissolution, whether occurring spontaneously in the body or arti-

ficially *in vitro*, is accompanied by more or less antigenic dissociation. The result of this process is reflected in the immune serum by the increasing amount of non-specific antibody with diminution or complete loss of type-specific response.

In previous studies it was observed that prolonged immunization induced a serum of high titer for the Friedländer organism, but that the serum agglutinated as well a number of organisms which were unable to remove agglutinins of the specific type by adsorption of the serum. Other workers also produced high titered sera at the expense of specificity.

The lack of recognition of two distinct antibodies in a single immune serum, each specifically reactive with only one of the cell constituents, and conversely the failure to distinguish the type-specific from the species-specific antigen of the cell, explain some of the difficulties encountered in the earlier efforts at classification of the *encapsulatus* group.

In a later paper the immunological relationships of these two constituents the protein and carbohydrate, and of encapsulated and capsule-free strains of the Friedländer bacillus, will be described.

SUMMARY.

A biological classification has been made of thirty strains of Friedländer's bacillus. This study reveals that there exist among these strains three sharply defined and specific types and one heterogeneous group. The three types are Type A, fifteen strains; Type B, six strains; Type C, three strains; and Group X, six strains. The agglutination, agglutinin adsorption, protection, thread, and precipitin reactions have been employed in the working out of this classification, and the types have been proved highly specific by means of each serological test.

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