ANAPHYLAXIS WITH THE TYPE-SPECIFIC CARBOHYDRATES OF PNEUMOCOCCUS.

By OSWALD T. AVERY, M.D., AND WILLIAM S. TILLETT, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 9, 1928.)

Seligmann (1) (1911) was the first to test the capacity of the serum of pneumonia patients to sensitize guinea pigs to Pneumococcus. He inoculated the animals intravenously, with 5 to 6 cc. of serum, and 24 hours later infected them intravenously with suspensions of pneumococci. In no instance was anaphylactic sensitization induced by serum obtained before or after the crisis. Rosenow (2) (1911) found that guinea pigs treated with extracts of pneumococci became hypersensitive to a subsequent injection of these soluble products and that the sensitized animals exhibited a relatively slight yet definite immunity to infection. Clough (3) (1915) by the use of a protein extract of pneumococci produced anaphylactic sensitization in guinea pigs and found that the sensitized animals showed only slight evidence of any increased resistance to infection with virulent pneumococci. Weil and Torrey (4) (1916) injected guinea pigs with inactivated serum obtained from cases of pneumonia during the course of the disease and during convalescence. 4 to 6 days later the animals were tested by the Dale method to determine whether they had acquired an increased hypersensitiveness to autolytic extracts of pneumococci. A positive reaction occurred with the serum of all but two of twenty patients. The sensitizing action when present was demonstrable in serum obtained during the febrile period of the disease and in only two instances did it persist in serum obtained during convalescence. The serum of normal individuals and of patients suffering from diseases other than pneumonia failed to sensitize control animals to the same bacterial extracts. In guinea pigs actively sensitized by repeated intraperitoneal injections of large amounts of heat-killed pneumococci, Zinsser and Mallory (5) (1924) succeeded in obtaining, with alkaline extracts of pneumococci, uterine reactions which they considered comparable in every way to those obtained in anaphylactic sensitization with non-bacterial proteins. They also demonstrated that by passive sensitization, uterine reactions, analogous to those elicited in true protein anaphylaxis, can be obtained in guinea pigs with soluble products of Pneumococcus. However, they found that active sensitization proved difficult, and passive sensitization was never very satisfactory and often impossible.

In guinea pigs previously immunized by killed and living virulent pneumococci, Mackenzie (6) (1924) produced fatal anaphylactic shock by the intravenous in-

jections of amounts of culture filtrate quite innocuous for control animals. While definite anaphylactic response to extracts of the bacterial protein was never elicited in animals similarly immunized, positive results, on the other hand, were obtained by the Dale method when the sensitized uterine muscle was exposed to pneumococcus protein. In this latter type of experiment, however, culture filtrates were found unsuitable because of the narrow margin between the amount necessary to elicit the anaphylactic response, and the quantity primarily toxic for the unsensitized control. In studying the significance of anaphylaxis in pneumococcus immunity Mackenzie observed that although during the course of immunization, the animals developed a high degree of active immunity and that their serum conferred on other guinea pigs passive protection against infection, at no time did the serum show the presence of demonstrable agglutinins for Pneumococcus or precipitins for its soluble derivatives. On the basis of the experimental results, he concludes that there is no significant relationship between anaphylaxis to pneumococcus protein and resistance to infection, since the latter could be demonstrated both in the presence and in the absence of the former.

Previous studies on anaphylaxis with Pneumococcus have dealt with the action of cell solutions and autolytic extracts containing admixtures of bacterial protein and other soluble products, which are often primarily toxic. As a result of the more recent chemo-immunological studies carried out in this laboratory, preparations of the specific carbohydrates of Pneumococcus were available in purified form. These soluble specific substances are free from protein and are devoid of primary toxic properties. In view of their increasing significance in the processes of immunity, it seemed important to determine whether the protein-free polysaccharides isolated from the specific types of Pneumococcus actively participate in the mechanism of bacterial anaphylaxis.

The present paper records the results of attempts by the use of these bacterial carbohydrates, (1) to induce active sensitization in guinea pigs, (2) to produce fatal anaphylactic shock in animals passively sensitized with antibacterial serum of the homologous type, (3) to define the reactions in terms of the chemical and biological specificity of the reagents employed.

EXPERIMENTAL.

Chemical Properties of Pneumococcus Polysaccharides.—The chemical nature of the soluble specific substances derived from each of the three types of Pneumococcus has been described in preceding papers

(7). The essential properties of the particular preparations used in the present experiments are briefly summarized in Table I.

As previously pointed out, all these substances possess the chemical properties of complex sugars; they contain no phosphorus, nor sulfur and they give none of the usual protein color tests. The Type II and Type III substances are nitrogen-free. The Type I substance differs from the other two in containing nitrogen as an apparently essential component; one-half of this nitrogen is present in the amino form. Despite the presence of nitrogen, the substance fails to give

TABLE I.

Soluble Specific Substances of Pneumococcus, Types I, II, III. Carbohydrate-Haptens.

Type	Preparation No.	$[\alpha]_D$	Acid equivalent	N	Reducing sugar on hydrolysis*	Highest dilution giving precipitate with anti-pneumococcus horse serum
				per cent	*per cent*	
I	59	+304	—	5.0†	32.0	1:6,000,000
II	26-A	+72.8	1252	0.0‡	68.2	1:5,000,000
III	53-A	−34	338	0.0‡	70.0	1:5,000,000

* Calculated as glucose.

† Amino N, 2.5 per cent.

‡ Analyzed by the Pregl micro Kjeldahl method, using 50 mg. of substance.

any of the protein color reactions. Quantitative solutions of the substances were made in salt solution and sterilized by heat.

Active Sensitization.—In the present investigation no attempt has been made to induce active anaphylaxis in animals by sensitization with the whole bacterial bodies. The work is confined solely to a study of the anaphylactic response of animals to the type-specific carbohydrate of Pneumococcus.

Guinea pigs of an average weight of 350 gm. were used. They were divided into three groups of six animals each. Of six guinea pigs in Group 1, two received a single initial dose of 50 mg., two 10 mg. and two 5 mg. of the specific carbohydrate derived from Type I pneumococci. The same number of animals in Groups 2 and 3 received similar amounts of the corresponding carbohydrates of organisms of Type II and Type III. 21 days after this initial treatment all the

TABLE II.

Active Anaphylaxis with the Specific Carbohydrates of Pneumococcus.

Type I—Carbohydrate (1).

Guinea pig No.	Sensitizing dose i.p.	Interval	Shocking dose i.v.	Result
	mg.	*days*	*mg.*	
1	5	21	10	No reaction
2	5	21	1	" "
3	10	21	5	" "
4	10	21	1	" "
5	50	21	10	" "
6	50	21	1	" "

Type II—Carbohydrate (2).

Guinea pig No.	Sensitizing dose i.p.	Interval	Shocking dose i.v.	Result
	mg.	*days*	*mg.*	
7	5	21	10	No reaction
8	5	21	1	" "
9	10	21	10	" "
10	10	21	1	" "
11	50	21	10	" "
12	50	21	1	" "

Type III—Carbohydrate (3).

Guinea pig No.	Sensitizing dose i.p.	Interval	Shocking dose i.v.	Result
	mg.	*days*	*mg.*	
13	5	21	10	No reaction
14	5	21	1	" "
15	10	21	10	" "
16	10	21	1	" "
17	50	21	10	" "
18	50	21	1	" "

1 = Preparation 59 (see Table I).
2 = Preparation 26-A (see Table I).
3 = Preparation 53-A (see Table I).
i.p. = intraperitoneal.
i.v. = intravenous.

animals of each group were injected intravenously with the second or shocking dose of from 1 to 10 mg. of the carbohydrate of the homologous type. The results are presented in Table II.

Attempts to induce active anaphylactic sensitization with pneumococcus polysaccharides were uniformly negative, as evidenced by the failure of the animals to react to a second injection of the homologous substance (Table II). The complete absence of the signs and symptoms of anaphylaxis in the guinea pigs, treated in the manner described, affords convincing evidence that the type-specific carbohydrate of Pneumococcus, in the dissociated and purified state, is by itself devoid of the property of inducing active sensitization. The criticism may be made, that either the sensitizing or the intoxicating dose or both may not have been adequate to elicit the response; that the interval between the two injections may not have been optimal for the development of sensitization. Unfortunately, the answer to these questions would require more elaborate experiments than the available amounts of the chemically purified substances permit. However, the maximal sensitizing dose of 50 mg. of Type I polysaccharide represents the chemical yield from approximately 5 liters of broth culture, and the amount recoverable from about 500 cc. of culture in the case of the Type III substance. In terms of the effective dosage in protein anaphylaxis, the amounts of bacterial carbohydrate employed both for sensitization and intoxication cover a sufficiently wide range to yield at least some evidence of activity were these substances possessed of sensitizing properties. Moreover, as described later in this paper, the amounts given at the time of the second injection were from 40 to 400 times greater than the minimum dose required to produce fatal anaphylactic shock in animals passively sensitized with antibacterial serum of the homologous type. It also seems unlikely that the failure to elicit the anaphylactic response was due to the lack of an appropriate interval between the two injections since the time chosen, namely 21 days, is usually optimal for the development of maximal sensitivity in the case of true antigens. Therefore, under the experimental conditions, the conclusion seems justified that, in the protein-free form, the immunologically type-specific polysaccharides of Pneumococcus are devoid of the property of inducing anaphylactic sensitization in the animal body.

Passive Sensitization.—The lack of sensitizing action, as tested in the foregoing experiments, adds further evidence in support of the view that the specific carbohydrates of Pneumococcus, when chemically purified, are devoid of antigenic properties. The fact, however, that in the protein-free form they retain unimpaired the function of combining specifically with antibacterial antibodies of the homologous type, led to attempts to ascertain whether these bacterial sugars are capable of provoking anaphylactic shock in animals passively sensitized with specific precipitating serum. Guinea pigs, previously injected with antipneumococcus serum containing a high titer of type-specific precipitins, were later tested to determine whether they had become hypersensitive to the specifically precipitable polysaccharide.

Antibacterial sera of Types I, II and III were produced by the intravenous injection of rabbits with heat-killed suspensions of pneumococci according to the method of Cole and Moore (8). It has long been recognized that it is possible to elicit specific antibodies to Type III in only an occasional rabbit. Tillett (9) has recently shown that this peculiarity in the immune response to Type III is due, in part, to variation in the resistance of individual rabbits and is also dependent upon essential differences in the antigenic complex of organisms of this type. However, by special procedures to be recorded later, the antibacterial rabbit sera employed in these experiments contained a relatively high titer of precipitins for the type-specific polysaccharides. Antipneumococcus horse serum, prepared by the method of Wadsworth, was also used.[1] The immune horse serum of the three specific types contained a higher concentration of carbohydrate-precipitating antibodies than did the corresponding immune rabbit serum. This fact is important in view of the differences in the results obtained with sera of different animal origin.

1. Immune Rabbit Serum.—Guinea pigs weighing 350 to 400 gm. were passively sensitized by a single intraperitoneal injection of antipneumococcus serum. At intervals varying from 1 to 7 days after the administration of the serum, a known amount of a quantitative solu-

[1] The antipneumococcus horse serum (Types I, II and III) employed in this study was furnished through the courtesy of Dr. Augustus B. Wadsworth, Director of Laboratories, New York State Department of Health.

tion of the polysaccharide of the corresponding type was injected into one of the superficial veins of the leg.

TABLE III.

Passive Anaphylaxis to the Specific Carbohydrate of Pneumococcus Type I in Guinea Pigs Sensitized with Type I Antipneumococcus Rabbit Serum.*

Guinea pig No.	Type I antipneumococcus serum (rabbit) i.p.	Time interval	Type I carbohydrate i.v.	Symptoms	Result	Autopsy
	cc.	*hrs.*	*mg.*			
1	1	24	1.0	Typical	†3 min.	Typical
2	1	24	0.05	"	†4 "	"
3	1	24	0.05	"	†5 "	"
4	1	24	0.025	"	†4 "	"
5	1	24	0.005	"	†4 "	"
6	1	24	0.005	"	†3 "	"
7	1	24	0.002	Slight respiratory embarrassment	Recovery	—
8	1	48	0.025	Typical	†5½ min.	Typical
9	1	48	0.025	"	†3½ "	"
10	1	48	0.025	"	†5 "	"
11	1	48	0.010	Scratches, coughs, bucks, dyspnea	Recovery 5 min.	—
12	1	72 *days*	0.025	Typical	†3½ min.	Typical
13	1	7	3.0	"	†3½ "	"
14	1	7	3.0	Scratches, coughs, falls on side	Recovery 6 min.	—
15	1	7	2.0	Slight symptoms	"	—
16	1	7	1.0	No symptoms	No reaction	—

* Preparation 59 (see Table I).
† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

The results of the experiments on passive anaphylaxis are given in Tables III, IV and V. The data presented in Table III show that the protein-free carbohydrate, derived from Pneumococcus Type I,

TABLE IV.

Passive Anaphylaxis to the Specific Carbohydrate of Pneumococcus Type II in Guinea Pigs Sensitized with Type II Antipneumococcus Rabbit Serum.*

Guinea pig No.	Type II antipneumococcus serum (rabbit) i.p.	Time interval	Type II carbohydrate i.v.	Symptoms	Result	Autopsy
	cc.	*hrs.*	*mg.*			
1	2	24	1.0	Typical	†2½ min.	Typical
2	2	24	0.5	"	†3 "	"
3	2	24	0.1	"	†4 "	"
4	2	24	0.03	"	†5 "	"
5	2	24	0.025	"	†4½ "	"
6	2	24	0.01	Slight dyspnea	Recovery	—

* Preparation 26-A (see Table I).
† Death of animal.
i.p. = intraperitoneal injection.
i.v. = intravenous injection.

TABLE V.

Passive Anaphylaxis to the Specific Carbohydrate of Pneumococcus Type III in Guinea Pigs Sensitized with Type III Antipneumococcus Rabbit Serum.*

Guinea pig No.	Type III antipneumococcus serum (rabbit) i.p.	Time interval	Type III carbohydrate i.v.	Symptoms	Result	Autopsy findings
	cc.	*hrs.*	*mg.*			
1	3	24	1.0	Cough, bucking, dyspnea severe	Severe reaction, recovery	—
2	3	24	1.0	Typical	†3½ min.	Typical
3	3	48	1.0	"	†4 "	"
4	3	48	0.5	"	†2½ "	"
5	3	72	2.0	"	†3 "	"
6	3	72	1.0	"	†3½ "	"

* Preparation 53-A (see Table I).
† Death of animal.
i.v. = intravenous injection.
i.p. = intraperitoneal injection.

in amounts as small as 0.005 mg., produced rapid and fatal anaphylactic shock in guinea pigs passively sensitized with 1 cc. of immune rabbit serum Type I. The symptoms and autopsy findings were in every respect identical with those characteristic of true protein anaphylaxis. The reaction was readily elicited 24 hours after administration of the sensitizing serum and could still be provoked 3 to 7 days later; as the time interval increased, however, larger amounts of the toxigenic substance were required and the response became progressively less. In the case of the nitrogen-free polysaccharides of Pneumococcus Type II and Type III, typical and fatal anaphylactic

TABLE VI.

Type Specificity of the Anaphylactic Response to Pneumococcus Carbohydrate in Guinea Pigs Sensitized with Antipneumococcus Rabbit Serum.

Guinea pig No.	Antipneumococcus serum (rabbit)		Time interval	Heterologous carbohydrate		Result	20 min. later same animals injected with homologous carbohydrate		Result	Autopsy findings
	Type	*cc.*	*hrs.*	*Type*	*mg.*		*Type*	*mg.*		
1	I	1.0	48	II	0.05	No reaction	I	0.025	†5 min.	Typical
2	II	1.0	24	I	1.0	" "	II	0.03	†3½ "	"
3	II	2.0	72	I	1.0	" "	II	0.025	†5 "	"
4	II	2.0	72	III	1.0	" "	II	0.5	†4 "	"
5	II	1.0	74	I	0.25	" "	II	0.25	†3½ "	"

The sensitizing serum was given intraperitoneally; the carbohydrates were injected intravenously.

† Death of animal.

shock was produced in passively sensitized animals by the injection of 0.025 mg. and 0.5 mg. of the substances respectively.

The bacterial sugars themselves are not primarily toxic. Normal control guinea pigs injected with large amounts have in no instance exhibited any toxic symptoms.

The specificity of the reaction is shown in Table VI. Guinea pigs sensitized with the immune serum of one type are wholly unaffected by the intravenous injection of the carbohydrate of a heterologous type. These same animals, however, succumb promptly with all the characteristic symptoms of anaphylaxis, when subsequently injected

with a fraction of a milligram of the polysaccharide corresponding in type to that of the immune serum with which they were originally sensitized.

The results of the intravenous injection of minute quantities of these specific polysaccharides into serum-sensitized guinea pigs, establish the fact that passive anaphylaxis can be produced by the

TABLE VII.

Absence of Anaphylactic Response to the Specific Carbohydrate of Pneumococcus Type I in Guinea Pigs Sensitized with Type I Antipneumococcus Horse Serum.

Guinea pig No.	Type I antipneumococcus serum (horse)	Time interval	Type I carbohydrate	Symptoms	Result
	cc.	*hrs.*	*mg.*		
1	4	24	2.5	Slight coughing, no other symptoms	Survival
2	1	24	2.5	None	"
3	4	24	1.0	Occasional cough, no other symptoms	"
4	1	24	1.0	None	"
5	4	24	0.5	Slight abdominal breathing	"
6	1	24	0.05	None	"
7	1	24	0.025	"	"
8	1	24	0.025	"	"
9	4	48	1.0	"	"
10	4	48	0.01	"	"
11	4	72	2.0	"	"
12	4	72	1.0	"	"
13	4	72	0.01	"	"

Guinea Pigs 1 to 6 received immune horse serum Lot 345 (Albany).
Guinea Pigs 7 to 12 received immune horse serum Lot 7 P_6 (New York).
Sensitizing serum was injected intraperitoneally.
Carbohydrate was injected intravenously.

protein-free carbohydrates of Pneumococcus, and that the reactions are type-specific. Moreover, the carbohydrates of Types II and III have been chemically purified to the point where they no longer contain nitrogen. The Type I substance, even when subjected to equally vigorous methods of purification, always retains a residue of nitrogen which appears to be an essential component of the carbo-

hydrate molecule, since it cannot be split off without loss of immunological specificity. Despite the presence of nitrogen this substance gives none of the protein color tests. The experimental evidence, therefore, is that the protein-free carbohydrates—the type-specific haptens—of Pneumococcus produce anaphylactic shock in animals passively sensitized with the precipitating serum of rabbits immunized with the bacterial cells from which these substances are derived.

TABLE VIII.

Absence of Anaphylactic Response to the Specific Carbohydrate of Pneumococcus Type II in Guinea Pigs Sensitized with Type II Antipneumococcus Horse Serum.

Guinea pig No.	Type II anti-pneumococcus serum (horse)	Time interval	Type II carbohydrate	Symptoms	Result
	cc.	*hrs.*	*mg.*		
1	1	24	1.0	None	Survival
2	1	24	0.25	"	"
3	5	48	2.0	"	"
4	5	48	1.0	"	"
5	5	72	2.0	"	"
6	5	72	1.0	"	"

Guinea Pigs 1 to 4 received intraperitoneally immune serum of Horse 92.
Guinea Pigs 5 and 6 received intraperitoneally immune serum of Horse 107.
Carbohydrate injected intravenously.

2. Immune Horse Serum.—The successful experiments on passive anaphylaxis, recorded above, were all carried out on guinea pigs sensitized with immune rabbit serum. On the other hand, attempts to render guinea pigs passively anaphylactic by the use of anti-pneumococcus horse serum were uniformly unsuccessful (Tables VII to IX).

The absence of the anaphylactic response to pneumococcus carbohydrate in guinea pigs passively sensitized with immune horse serum, when contrasted with the invariably fatal reaction induced in animals sensitized with immune rabbit serum, seemed at first inexplicable; it was all the more surprising in view of the fact that the horse serum

in vitro precipitated the type-specific carbohydrate in higher dilution than did the corresponding serum of the immune rabbits.

This difference in the behavior of two sera, each of different animal origin but both containing precipitating antibodies of the same type, may possibly be explained, in part at least, by the fact, first noted by Zinsser and Parker (10), that antipneumococcus horse serum contains a substance which interferes with the fixation of complement. The same phenomenon has been observed in this laboratory by Lancefield (11) and by Vollmond (12), who found that in a system containing the purified, type-specific carbohydrate (hapten) of Pneumococcus

TABLE IX.

Absence of Anaphylactic Response to the Specific Carbohydrate of Pneumococcus Type III in Guinea Pigs Sensitized with Type III Antipneumococcus Horse Serum.

Guinea pig No.	Type III anti-pneumococcus serum (horse)*	Time interval	Type III carbohydrate	Symptoms	Result
	cc.	*hrs.*	*mg.*		
1	5	24	2.0	None	Survival
2	5	24	1.0	"	"
3	5	48	2.0	"	"
4	5	48	1.0	"	"
5	5	72	2.0	"	"
6	5	72	1.0	"	"

* Immune horse serum (Albany, Lot 68).

complement fixation takes place in the presence of homologous immune rabbit serum but does not occur when immune horse serum is used.

When the nucleoprotein of the cell, instead of the carbohydrate hapten, was used in the test, both Lancefield and Vollmond found that complement fixation occurred with the immune sera of both species.

The fact that the anaphylactic response to the specific hapten does not occur in guinea pigs passively sensitized with antipneumococcus horse serum may be referable to the presence in the serum of the same substance which inhibits the fixation of complement with

pneumococcus polysaccharide. There is a certain amount of evidence in favor of the view that complement actively participates in the mechanism of anaphylaxis. The chief facts supporting this view may be briefly summarized as follows: (1) the diminution of complement during anaphylactic shock (13); (2) the prevention of anaphylaxis by hypertonic salt solution which is also known to inhibit the fixation of complement *in vitro* (14); (3) the absence of anaphylactic response to protein in guinea-pigs after depletion of their complement by the injection of sensitized sheep cells (15); (4) the action of complement in the formation of anaphylotoxins (16, 17). It may be possible, therefore, that the substance in antipneumococcus horse serum which is known to interfere with the fixation of complement in the test-tube, may also inhibit the action of complement in the animal body and thus prevent the anaphylactic reaction when the type-specific hapten (carbohydrate) of Pneumococcus is injected into guinea pigs sensitized with immune horse serum.

DISCUSSION.

Tomcsik (18) isolated from *B. lactis aerogenes* a soluble, specific substance consisting largely of carbohydrate which failed to give the protein reactions and which, despite repeated attempts at further purification, contained 0.9 per cent of nitrogen. In passively sensitized guinea pigs, the injection of 0.02 mg. of this substance induced fatal anaphylactic shock, while doses of 1 mg. failed to cause any reaction in unsensitized control animals. By the Schultz-Dale method, positive uterine reactions were obtained when minute amounts of the substance were added to the reaction bath, and distinct contractions were observed in dilutions as high as 1:20,000,000. Tomcsik and Kurotchkin (19) in a subsequent paper have reported similar results with the specific carbohydrate material isolated from *B. lactis aerogenes*, the pneumobacillus and a yeast. Lancefield (20) has recently isolated from streptococci carbohydrate material which produces anaphylactic shock in guinea pigs passively sensitized with antistreptococcus serum. Because of the presence of small amounts of nitrogen in the products they used, none of these authors felt justified in concluding that the bacterial carbohydrate alone produced the anaphylactic shock.

The bacterial carbohydrates used in the present study represent the most highly purified preparations isolated in this laboratory by Heidelberger and Goebel from type-specific strains of Pneumococcus. The Type II and Type III substances are nitrogen-free, and the Type I substance, despite the presence of apparently essential nitrogen (5 per cent), is protein-free, as far as can be determined by chemical analysis. These bacterial derivatives belong to the unique group of immunologically specific substances which Landsteiner (21) called haptens. When split off from the complex antigens of which they form a part, these substances are no longer antigenic but still retain the property of binding the type-specific antibodies produced by the whole antigen. As previously pointed out, the carbohydrate haptens of Pneumococcus are immunologically identical with the bacterial products originally referred to as the soluble specific substance.

Considering the carbohydrate nature and the non-antigenic properties of pneumococcus haptens it is not surprising that they fail to induce active anaphylactic sensitization. In passive anaphylaxis, the results obtained with the carbohydrates of Pneumococcus demonstrate that protein-free and even non-nitrogenous substances of the nature of bacterial polysaccharides are capable of inducing anaphylactic shock in guinea pigs sensitized with antibacterial serum of the homologous type.

Previous studies have shown that the reactions of precipitation and complement fixation with these substances occur in immune rabbit serum and are type-specific. The present work demonstrates that the anaphylactic reaction also occurs when minute amounts of the same carbohydrate haptens are injected into animals passively sensitized with immune rabbit serum. However, an interesting difference in the occurrence of these three immune reactions has been found to exist when antipneumococcus *horse* serum is used instead of *rabbit* serum. Although these substances are equally precipitable by the immune sera of both species, they combine in the reaction of complement fixation in the test-tube and induce passive anaphylaxis in the animal body when immune rabbit serum is used, and they fail to react when immune horse serum is employed. It is not possible at present to state whether the same inhibiting substance in antipneumococcus horse serum which interferes with the fixation of complement in a

system containing the specific carbohydrate also prevents the occurrence of anaphylactic shock by interfering with the action of complement in the animal body. The phenomenon seems peculiar to the carbohydrate haptens since it does not occur when the protein fraction of the cell is used with the same immune horse serum. The elucidation of this phenomenon requires further study. The present paper deals only with the facts as they relate to the reactions induced by the non-protein carbohydrates. The facts appear sufficiently established to justify the conclusion that anaphylactic shock can be induced by the injection of minute amounts of the type-specific carbohydrates of Pneumococcus in guinea pigs passively sensitized with homologous antipneumococcus rabbit serum.

CONCLUSIONS.

1. The type-specific carbohydrates (haptens) of Pneumococcus Types I, II and III, when isolated in protein-free form, are devoid of the property of inducing active anaphylactic sensitization in guinea pigs.

2. The bacterial carbohydrates of Pneumococcus, of which the Type II and Type III substances are nitrogen-free, produce rapid and fatal anaphylactic shock in guinea pigs passively sensitized with the precipitating serum of rabbits immunized with pneumococci of the homologous type; the reactions induced are type-specific.

3. In contrast to the positive results with *immune rabbit serum*, there is a complete absence of anaphylactic response to pneumococcus carbohydrate in guinea pigs passively sensitized with *antipneumococcus horse serum.*

BIBLIOGRAPHY.

1. Seligmann, E., *Z. Immunitätsforsch., Orig.*, 1911, ix, 78.
2. Rosenow, E. C., *J. Infect. Dis.*, 1911, ix, 190.
3. Clough, P. W., *Bull. Johns Hopkins Hosp.*, 1915, xxvi, 37.
4. Weil, R., and Torrey, J. C., *J. Exp. Med.*, 1916, xxiii, 1.
5. Zinsser, H., and Mallory, T. B., *J. Immunol.*, 1924, ix, 75.
6. Mackenzie, G. M., *J. Exp. Med.*, 1925, xli, 53.
7. Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, xlii, 727.

8. Cole, R., and Moore, H. F., *J. Exp. Med.*, 1917, xxvi, 537.
9. Tillett, W. S., *J. Exp. Med.*, 1927, xlv, 713, 1093.
10. Zinsser, H., and Parker, J. T., *J. Immunol.*, 1923, ii, 151.
11. Lancefield, R. C., unpublished data.
12. Vollmond, E., unpublished data.
13. Sleeswijk, J. G., *Z. Immunitätsforsch., Orig.*, 1909, ii, 133.
14. Friedberger, E., and Hartoch, O., *Z. Immunitätsforsch., Orig.*, 1909, iii, 581.
15. Loeffler, F. C., *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 129.
16. Friedemann, U., *Z. Immunitätsforsch., Orig.*, 1909, ii, 591.
17. Friedberger, E., *Z. Immunitätsforsch., Orig.*, 1909, iii, 692; 1909–10, iv, 636.
18. Tomcsik, K., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 812.
19. Tomcsik, K., and Kurotchkin, T. K., *J. Exp. Med.*, 1928, xlvii, 379.
20. Lancefield, R. C., *J. Exp. Med.*, 1928, xlvii, 843.
21. Landsteiner, K., *Proc. Roy. Acad. Sc. Amsterdam*, 1921 (Feb.), 1166; *Biochem. Z.*, 1921, cxix, 294.