CARBOHYDRATES ADSORBED ON COLLOIDS AS ANTIGENS

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The phenomenon of adsorption has been used in biology for the purification of enzymes, toxins, etc., and offers an interesting and complicated mechanism, showing certain selectivity.

In the field of bacteriology and immunology many workers have used this phenomenon of adsorption in the study of diverse problems.

Nicolle (1) studying the agglutination reaction, grew different organisms (*B. proteus* and *B. typhosus*) in a filtrate of *B. coli*, and found that these organisms agglutinated with anticoli serum. He obtained similar results when talcum powder was used in the place of the organisms. These foreign particles, when placed in *B. coli* filtrate, were equally agglutinated by anticoli serum. He remarks on the constant presence of the agglutinable substance in the cultures of *B. coli*.

Arkwright (2) studying the agglutination of *B. typhosus* made use of *B. coli* and kieselguhr as indicators in showing the presence of agglutination reaction. When he added *B. typhosus* extract to *B. coli* emulsion, agglutination was observed. He says, "In this case the *B. typhosus* extract appeared to act as a 'protective colloid' and each particle (*B. coli* + *B. typhosus* extract) behaved as though it were composed of the extract. An adsorbed coating of each bacillus with *B. typhosus* colloid being assumed as probable."

Coulter (3) showed that red cells agglutinate at pH 4.75, but when sensitized with serum the agglutination point was shifted to that of globulin, pH 5.3. Northrop and De Kruif (4) found that a mixture of bacteria and egg albumin or bacteria and globulin behaved towards acid, like solutions of the respective proteins. The isoelectric point of the organism shifted to that of the added protein.

Loeb (5), working on cataphoresis with collodion particles showed the effect of electrolytes on them and concluded that they follow closely the same law that McTaggart (6) found for gas bubbles and water, giving the collodion particles a negative charge. Loeb (7) further demonstrated that the influence of different electrolytes was the same on gelatin-coated collodion particles, particles of casein and particles of boiled egg albumin. This fact showing that it is immaterial whether the protein is in the form of denatured egg albumin or a protein-like gelatin, furnishes proof that the solutions of genuine proteins are not diphasic

systems. Further experiments of Loeb (8) on the stability of suspensions of solid particles of proteins and the protective action of colloids showed that collodion particles, gelatin-coated, require the same salt concentration to precipitate them as did the "salting out" of gelatin from aqueous solutions.

Freund (9) studied physicochemical reactions of the agglutination of the tubercle bacillus. In this work he found that collodion particles are acid-fast when stained by the Ziehl-Neelsen method and that they remain acid-fast even if they are coated with protein.

Jones (10), studying the phenomenon of agglutination by precipitins, made use of the collodion particles and bacteria, sensitizing them and agglutinating them with the specific precipitin. He explained the results with heated (serum) antigens on the basis that coagulated proteins in suspension are covered with undenatured antigen and the addition of precipitin causes agglutination of the coagulated protein. He further noted that when particles were mixed with cow serum of crystallized egg albumin and then washed until free antigen no longer remained in the wash solution, they behaved like bacteria sensitized to cow serum and subsequently washed. In a later article (11) Jones showed that the amount of adsorption by the collodion particles, judged by agglutination, is not dependent on the concentration of the sensitized protein beyond a certain maximum, a significant observation, for Hitchcock (12) has shown that collodion membranes when in contact with solutions of crystallized egg albumin and gelatin adsorb the protein on their surfaces. The concentration of the protein, being in relation to the amount adsorbed, is expressed fairly well by the equation proposed by Langmuir (13) for the adsorption of gases by a plain surface. Jones also showed that particles exposed to a number of antigenic substances, crystallized egg albumin, rabbit serum, chicken serum, horse serum and cow serum, in succession, are equally agglutinated by all of the appropriate antisera.

Bedson (14) working with herpes virus, demonstrated that collodion particles adsorbed herpes virus or herpes antibodies. There is a definitely increased avidity for the virus in those particles which have been sensitized with herpes antiserum. The observation will be discussed further on.

Mudd, Lucké, McCutcheon and Strumia (15) have shown that collodion particles treated with precipitogen are phagocytized in a manner that is essentially identical with the phagocytosis of bacteria treated with bacteriotropins. They also found that particles so treated showed characteristic properties of protein, such as cohesiveness, wetting properties and were isoelectric at pH 5.5 to 5.8 and hence believe that precipitation, agglutination, the surface changes and increased phagocytosis are all consequences of a specific chemical combination with, and deposit on the surface of the antigen of antibody protein.

Rhoads (16) has adsorbed the virus of poliomyelitis on aluminum hydroxide particles and while he was unable to induce the disease by injection of the material, produced active immunity in *Macacus rhesus*. Carbohydrates as antigens have been studied, more especially since the isolation of the complex bacterial polysac-

charides. Wells (17) comments on carbohydrates as antigens, stating that there is no apparent theoretical reason why complex carbohydrates should not exhibit antigenic functions, as they may exist in the colloidal state.

Nishimura (18) reports the result of his experiments on the production of specific antibodies, judged by the complement-fixation test with inulin, soluble starch and various dextrins. He concludes that in the production of immune bodies by these three kinds of polysaccharides, protein might play the part of the vehicle as all of them have a small percentage of N, probably in the form of protein. He noted that dextrin produced antibodies more readily when mixed with pig serum.

The use of bacterial polysaccharides for antibody formation has been attempted by many investigators. In the few successful cases the carbohydrate has usually been of doubtful purity. Thus the "residue-antigens" of Zinsser and Parker (19) were impure in some respects, Ross (20) found that rats fed Pneumococcus Type I polysaccharide in doses of 0.5 mg., were protected when tested with virulent pneumococci. Schiemann and Caspar (21) found mice to be more resistant when injected previously with a pneumococci specific substance free of protein, while in rabbits they were unable to induce agglutinins, precipitins or protective antibodies. They account for this failure by the probable insufficient dosage.

Francis and Tillett (22) have found that patients injected intradermally with the different polysaccharides from the pneumococci develop circulating antibodies for these polysaccharides. They suggest that the development of these antibodies for heterologous types of pneumococcus was associated with the previous intradermal injection of the type-specific polysaccharides. They believe it possible that in the process of recovery from infection, a highly reactive state exists in the human organism which responds to stimuli otherwise ineffective. Before one can accept this result, further work must be done.

Our work consists in the study of the antigenic potentialities of various polysaccharides and dextran adsorbed on colloids, especially on collodian particles. While the bacterial polysaccharides have not been free of nitrogen in any case, the significance of its presence in the preparation used for antibody production has always been controlled by injection into animals of the unadsorbed polysaccharide in dilutions of 1/10,000. The dextran was free of nitrogen and ash.

Method

Preparation of the Colloid Carrier.—Merck's C.P. collodion was used. This was precipitated in distilled water, washed several times and then dried between filter papers. The dry collodion was dissolved in warm acetone C.P. The acetone solution was placed in a jar and agitated fairly rapidly by an electric stirring machine. Water was slowly added to the collodion until a white heavy suspension of collo-

dion particles was noticed. The material was further diluted with distilled water until the larger clumps separated out. The acetone was removed by vacuum distillation at 45°C.

The large particles were removed by rapid centrifugation for 1 minute. The rest of the particles were obtained by further centrifugation for 10 minutes. There remained in the liquid a great many particles which were not brought down by centrifugation. These were obtained by adding to the liquid NaCl crystals, and the precipitated particles were centrifuged. All of the centrifuged particles were washed with physiological salt solution at least three times. The washed particles were kept in the ice box until used.

The casein used was prepared from skimmed milk by the method of Van Slyke and Baker. This was suspended in salt solution and used for adsorption in the same way as the collodion particles.

When organisms were used for adsorption they were washed in salt solution and the centrifuged material then used. We always used living organisms. The adsorption on them was carried out by the same method used with other particles.

Adsorption.—Solutions of the polysaccharide in 0.5 to 1 per cent concentration were used. To the centrifuged collodion particles 1 cc. of the polysaccharide solution was added and carefully mixed with a glass rod so as to avoid the formation of large clumps. The container was then placed on the ice for 10 minutes or at other times overnight (we observed no difference). Physiological salt solution was added and the particles washed four times. This is unnecessary when using non-antigenic substances, but was always done. After centrifuging, a small amount of salt solution was added very carefully and the collodion particles made into a homogeneous suspension. This suspension was standardized with a silica turbidity comparator to equal a suspension of *E. typhi* of 5,000,000,000 per cc. A few drops of chloroform were added to the so prepared antigen as preservative. When serum was adsorbed to the particles the same procedure was followed, the serum being diluted 1–10 and mixed with the collodion particles; after refrigeration, it was washed four times, and then the polysaccharide was adsorbed. In case of several polysaccharides, each was adsorbed separately to eliminate the error of dilution which would be present if they were mixed.

Immunization of Animals.—The rabbits were injected intravenously with 0.5, 1.0 and 1.0 cc. on consecutive days, followed by a 5 day rest. A second series of increasing doses were now given, and again a 5 day rest. Three injection series were completed before a trial bleeding. Horses were immunized by giving one injection a week. The doses varied between 25 and 250 cc.

Bacterial Polysaccharides.—The polysaccharides were prepared by various methods, some of which have been described in other publications. It is not necessary to give the chemical assay of each carbohydrate since adequate controls were made showing that the plain polysaccharide was not by itself antigenic. The ash content has no importance and fairly impure preparations may be used, as the adsorption technique in itself serves as a purifier of the substance.

In this group of experiments we have also used bacterial bodies as adsorbents. The results of the experiments will be given under the head of the polysaccharide adsorbed on them. The polysaccharides most used were those with which we have had the most experience, both in preparation and immunological reaction, namely those of certain Gram-positive bacilli, *B. anthracis* and *B. proteus;* the Gram-negative bacillus, *Bacterium morgani*; the Gram-positive cocci, the pneumococci and *Streptococcus viridans* (Bargen), and finally the Gram-negative coccus, the meningococcus.

Making of Tests. (*a*) *Polysaccharide Precipitin.*—The amount of the serum dilution used was usually 0.2 cc. and an equal amount of the polysaccharide solution. Mixed and incubated in the water bath at 37°C. The time of incubation was usually 2 to 4 hours. The test was then read and the tubes were placed in the ice box overnight when a second reading was made. In certain cases the tubes were centrifuged for a few minutes before the final reading was established.

The amount of precipitate and its quality (flocculent or disc formation) were estimated and called 4, 3, 2 or 1 according to the amount of precipitate in proportion to the dilution; 0 indicating no precipitate.

(*b*) *Horse Precipitin Tests.*—Equal amounts of test serum and antigen (horse or bovine serum diluted 1 to 10) were placed in contact in micro test-tubes and the amount of precipitate formed at the union of the two layers was read after standing 1 hour at room temperature as indicated by +++ (heavy and settling), ++ (marked ring), + (distinct ring), ± (faint), 0 (negative).

(*c*) *Agglutinations.*—Equal amounts of the serum dilution and antigen (2,000 million per cc. suspension of killed organisms) were mixed and incubated at 37°C. or 56°C. for various lengths of time from 2 to 18 hours, according to the type of organism used. The readings were made and tabulated as follows: 4 (complete agglutination), 3 (marked), 2 (less marked but definite), 1 (slight) and 0 (negative).

We shall first report the results with collodion particles as the colloid utilized in immunization; then we shall give the results using casein, then alumina and finally bacteria adsorbed with different polysaccharides. All the tests were *in vitro* tests, except the antipneumococcic in which mice were used.

Results of Immunization with Collodion Particles as the Colloid

Anthrax.—The sera of rabbits immunized with collodion particles and other combinations were studied for agglutinins, anti-horse precipitins and precipitins for the specific carbohydrate. The results are shown in Table I. The variations in the different groups immunized with collodion particles adsorbed with polysaccharide and other combinations do not show any marked difference from those in the

group (Rabbits 10, 11 and 12) immunized with the washed precipitate of antianthrax globulin + polysaccharide. Serum was used with the idea that it might increase the amount of polysaccharide adsorbed. The results with Rabbits 13 and 14 are given for the purpose of demonstrating the low antigenicity of the killed anthrax antigen. Rabbits 15 and 16 are only two from a group of ten, none of which showed any antibody response after prolonged treatment with the pure poly-

TABLE I

Agglutination, Horse Precipitin and Polysaccharide Precipitin Tests with the Sera of Rabbits Immunized with Different Combinations of Collodion Particles Adsorbed with Anthrax Polysaccharide

(Tests against same antigen, after three series of three injections each)

Immunized with...		Collodion particles + anthrax SSS			Collodion particles + anthrax globulin + anthrax SSS			Collodion particles + normal horse serum + anthrax SSS			Anthrax globulin + anthrax SSS precipitate			Anthrax organisms		Anthrax SSS	
Rabbit No.....		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Agglutination	1/4	4*	4	2	4	4	4	4	4	4	4	4	4	2	2	0	0
	1/8	4	3	1	4	4	4	4	4	2	4	4	4	1	2	0	0
	1/16	4	2	0	4	4	4	2	3	1	4	2	4	0	1	0	0
	1/32	4	0	0	2	4	2	1	2	0	1	0	3	0	0	0	0
	1/64	2	0	0	0	2	1+	0	0	0	0	0	1	0	0	0	0
Horse precipitin		0**	0	0	+	+	+	±	+	++	++	+++	+++	0	0	0	0
Bovine precipitin		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anthrax SSS 1:1,000		1†	2	1	1	1	1	2	1	1	3	2	2	0	0	0	0

* Read after 15 hours at 37°C. 4 = complete; 3 = marked; 2 = less marked; 1 = slight; 0 = negative.

** Read after 1 hour at room temperature. +++ = heavy precipitate with settling; ++ = marked; + = distinct; ± = faint; 0 = negative.

† Polysaccharide precipitin test read after 4 hours.

saccharide. The horse precipitin test is of interest when we notice that only Rabbits 1, 2 and 3 of the first series were negative. Rabbits 13, 14, 15 and 16 were also negative. The polysaccharide precipitin test was weak, (1/4) but definite. Each rabbit had received approximately 0.03 mg. of the polysaccharide adsorbed on collodion particles. The results of the polysaccharide precipitin test are found at the bottom of Table I.

For comparison with the results obtained in rabbits, a horse (No. 1) was immunized with the same antigens using increasing doses, from 25 to 250 cc. Another horse (No. 2) was used as control, receiving equal amounts of a solution of polysaccharide 1/10,000, without collodion particles.

Keeping in mind that anthrax is a disease of horses and that any horse may have had the disease naturally or been artificially immunized, we tried the agglutination test before starting the immunization and found that No. 1 had none, while Horse 2 yielded a 3 agglutination in a 1/10 dilution. The result of the agglutination and polysaccharide precipitin test with the sera of these horses is shown in Table II.

TABLE II

Agglutination and Polysaccharide Precipitin Test with Serum from Horses Immunized with Collodion Particles Adsorbed with Anthrax Polysaccharide

Final dilution	Agglutination					SSS 1–1,000			
Test	1/10	1/20	1/40	1/80	1/160	1/2	1/4	1/8	1/16
Horse 1	4	4	3	2	0	3	3	2	1
Horse 2	3	2	0	0	0	0	0	0	0
Negative control	3	1	0	0	0	0	0	0	0

Read after 4 hours at 37°C.

At this time, we may mention the enormous difficulty encountered in finding a suitable antigen for the anthrax agglutination test. The majority of antigens give positive reactions with normal sera, and tests with freshly prepared antigens that have not been extensively controlled are unreliable.

To establish further the presence of antibodies in Horse 1 and the absence of them in Horse 2, we concentrated the serum with ammonium sulfate (usual method for concentration of antibacterial serum). The results of these agglutination and polysaccharide precipitin tests are shown in Table III.

In the agglutination test we have a very high titer for Horse 1, while in Horse 2, the titer is low. The agglutination titer noted in the latter horse, before we started treatment, was raised by concentration. The polysaccharide precipitin test was difficult to read on account of

the viscosity of the globulin (concentrated twenty-five times) and centrifugation was necessary before a clear reading was obtained.

TABLE III

Agglutination and Polysaccharide Precipitin Tests with the Serum Globulin Concentrated Twenty-Five Times of Horses 1 and 2 Which Had Been Immunized with Collodion Particles Adsorbed with Anthrax Polysaccharide

Test	Agglutination test									Polysaccharide precipitin test (1/10,000)							
Final dilution	1/40	1/80	1/160	1/320	1/640	1/1,280	1/2,500	1/5,000	1/10,000	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
No. 1	4	4	4	4	4	4	4	4	2	4	4	4	4	4	3	2	0
No. 2	3	2	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0
Control	2	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0

Read after 4 hours at 37°C.

TABLE IV

Agglutination (Type I), Horse Precipitin and Polysaccharide Precipitin Tests with the Serum of Rabbits Immunized with Meningococcus Polysaccharide Adsorbed on Collodion Particles

Immunized with		Collodion particles + meningococcus SSS		Collodion particles + antimeningococcus globulin + meningococcus SSS		Collodion particles + normal horse serum + meningococcus SSS			Meningococcus Type I organisms		Meningococcus SSS	
Rabbit No.		1	2	3	4	5	6	7	8	9	10	11
Agglutination	1/8	2*	0	3	2	0	3	1	2	0	0	0
	1/16	3	2	4	3	2	4	2	4	2	0	0
	1/32	4	4	4	4	2	4	0	4	4	0	0
	1/64	4	4	4	4	3	3	0	4	4	0	0
	1/128	4	4	3	2	2	2	0	4	4	0	0
Horse precipitin		0**	0	++	+	+++	+	++	0	0	0	0
Bovine precipitin		0	0	0	0	0	0	0	0	0	0	0
Meningococcus SSS 1:1,000		0†	0	0	0	0	0	0	0	0	0	0

* Read after 15 hours at 56°C.

** Read after 1 hour at room temperature.

† Read after 4 hours at 37°C.

Considering the concentration and the probable non-specific precipitation, a titer of 1/4 is very low and probably is non-specific when

one considers that the control globulin also gives a reading at this dilution.

We believe that these tests show conclusively, the non-antigenicity of our anthrax polysaccharide alone.

Meningococcus.—The polysaccharide used was a mixture of a polysaccharide prepared from a broad strain of meningococcus (Types I and III), and a small porportion of Types II and IV polysaccharides. At this time we believed all of the types to be immunologically similar. Table IV shows the results of the agglutination test with Type I meningococcus antigen. Table V gives the results of agglutination tests with three of the classical strains. A definitely higher aggluti-

TABLE V

Meningococcus Agglutination Test with the Sera of Rabbits Immunized with Collodion Particles Adsorbed with Meningococcus Polysaccharide

Immunized with.....	Collodion particles + meningococcus polysaccharides						Controls					
Rabbit No.....	1			2			Positive serum			Negative serum		
Antigen.....	Type I	Type II	Type III	Type I	Type II	Type III	Type I	Type II	Type III	Type I	Type II	Type III
1/24	4	3	4	4	3	4	4	4	4	0	0	0
1/50	3	2	4	3	2	4	4	4	4	0	0	0
1/100	3	0	4	2	0	2	4	4	4	0	0	0
1/200	0	0	3	0	0	0	4	4	4	0	0	0

Read after 5 hours at 56°C.

nation titer against Types I and III is shown. At the moment we will merely present the results, which show the possibility of group specificity of the type polysaccharide when used as antigens. The polysaccharide precipitins appeared later, after another series of injections.

Horses were placed under treatment and the results of the tests will be given under the title of multiple antigens.

Bacterium morgani.—The results of the tests on immunized rabbits shown in Table VI are self-explanatory. The agglutinins are very high for this organism. We have no explanation for the negative

horse precipitin test except that the serum may not have been adsorbed on the collodion particle in Rabbits 3, 4, 5 and 6. Again we noted the negative polysaccharide test. On further immunization we obtained a strong reaction. Noticing the agglutinins increase rapidly we tested their specificity by absorbing with the homologous organisms; and in Table VII the results are shown. The absorption was complete after two trials, showing the specificity of the anticarbohydrate antibodies. This experiment was tried with the other antisera and

TABLE VI

Agglutination, Horse Precipitin and Polysaccharide Precipitin Tests on Serum of Rabbits Immunized with Morgani Polysaccharide Adsorbed to Collodion Particles

Immunized with.....		Collodion particles + *morgani* SSS		Collodion particles + anti-morgani serum + *morgani* SSS		Collodion particles + normal horse serum + *morgani* SSS		*S. morgani* organisms		*Morgani* SSS	
Rabbit No.....		1	2	3	4	5	6	7	8	9	10
Agglutination	1/16	4*	2	4	4	4	4	4	4	0	0
	1/32	4	1	4	4	4	4	4	4	0	0
	1/64	4	0	4	4	4	4	4	4	0	0
	1/128	2	0	4	4	3	4	4	4	0	0
	1/500	0	0	4	4	0	4	4	4	0	0
	1/1,000	0	0	4	0	0	0	4	4	0	0
Horse precipitin		0**	0	0	0	0	0	0	0	0	0
Morgani SSS 1:1,000		0†	0	0	0	0	0	0	0	0	0

* Read after 2 hours at 56°C.

** Read after 1 hour at room temperature.

† Read after 4 hours at 37°C.

the results were similar. We give our results of the test with *Bacterium morgani* on account of the very high agglutination titer.

B. proteus.—The immunization of animals with this polysaccharide has been the least satisfactory in the production of agglutinins, when collodion particles were used as the colloid carrier. The results are shown in Table VIII. The negative horse precipitin test in Rabbits 3 and 4 is due to the use of rabbit antiserum. In the other cases in which we used specific antisera, horse serum was employed. The polysaccharide precipitin test was definite in these series of animals.

Streptococcus viridans (*Bargen*).—Table IX shows the results of

agglutination, polysaccharide precipitin and horse precipitin tests. It is of interest to note the variability in the response of animals to the treatment. We account for the higher antibody content in

TABLE VII

Absorption Tests with Bact. morgani Antigen in Sera Immunized with Bact. morgani Polysaccharide Adsorbed on Collodion Particles and Other Combinations

Immunized with.....	Collodion particles + *morgani* SSS						Collodion particles + anti-morgani serum + *morgani* SSS						Collodion particles + normal horse serum + *morgani* SSS					
Rabbit No.....	7			8			9			10			11			12		
Serum.....	Orig.	1st abs.	2nd abs.	Orig.	1st abs.	2nd abs.	Orig.	1st abs.	2nd abs.	Orig.	1st abs.	2nd abs.	Orig.	1st abs.	2nd abs.	Orig.	1st abs.	2nd abs.
1/4	4	2	0	4	0	—	4	1	0	4	2	0	4	4	0	4	4	0
1/8	4	1	0	4	0	—	4	0	0	4	1	0	4	2	0	4	3	0
1/16	4	0	0	4	0	—	4	0	0	4	1	0	4	1	0	4	0	0
1/32	4	0	0	3	0	—	4	0	0	4	0	0	4	0	0	4	0	0
1/64	4	0	0	2	0	—	4	0	0	4	0	0	4	0	0	4	0	0
1/128	2	0	0	1	0	—	4	0	0	4	0	0	4	0	0	4	0	0
1/256	2	0	0	0	0	—	2	0	0	2	0	0	3	0	0	4	0	0

Read after 2 hours at 56°C.

TABLE VIII

Agglutination, Horse Precipitin and Polysaccharide Precipitin Tests with Serum of Rabbits Immunized with Proteus Polysaccharide Adsorbed on Collodion Particles

Immunized with.....		Collodion particles + *proteus* SSS		Collodion particles + anti-proteus serum + *proteus* SSS		Collodion particles + normal horse serum + *proteus* SSS		*Proteus* organisms		*Proteus* SSS	
Rabbit No.....		1	2	3	4	5	6	7	8	9	10
Agglutination	1/4	4*	4	4	4	3	3	4	4	0	0
	1/8	3	3	2	3	2	2	4	4	0	0
	1/16	2	0	1	0	1	0	4	4	0	0
	1/32	0	0	0	0	0	0	4	4	0	0
Horse precipitin		0**	0	0	0	++	+	0	0	0	0
Bovine precipitin		0	0	0	0	0	0	0	0	0	0
Proteus SSS 1:1,000		2†	3	2	3	2	1	4	4	0	0

* Read after 4 hours at 56°C. ** Read after 1 hour at room temperature.

† Read after 2 hours at 37°C. and overnight in ice box.

Rabbits 3, 4 and 5 on the basis that more polysaccharide had been adsorbed, for the amount of globulin adsorbed cannot in itself have been responsible. The protein alone does not account for the increased reaction, as Rabbits 7, 8 and 9 received collodion particles adsorbed with normal horse serum first.

TABLE IX

Agglutination, Horse Precipitin and Polysaccharide Precipitin Tests with the Serum of Rabbits Immunized with Strep. viridans (Bargen) Polysaccharide Adsorbed on Collodion Particles

Immunized with.....		Collodion particles + Bargen SSS		Collodion particles + Bargen globulin + Bargen SSS			Collodion particles + normal horse serum + Bargen SSS			Bargen organism		Bargen SSS	
Rabbit No.....		1	2	3	4	5	6	7	8	9	10	11	12
Agglutination	1/4	2*	4	4	4	4	3	2	0	4	4	0	0
	1/8	1	3	4	4	4	2	0	0	4	4	0	0
	1/16	0	1	4	4	3	1	0	0	4	4	0	0
	1/32	0	1	4	4	2	0	0	0	4	4	0	0
	1/64	0	0	4	2	0	0	0	0	4	4	0	0
Horse precipitin		0**	0	+++	++	+++	++	++	+++	0	0	0	0
Bovine precipitin		0	0	0	0	0	0	0	0	0	0	0	0
Bargen SSS 1:1,000		1†	3	2	3	3	2	1	0	4	4	0	0

* Read after 2 hours at 56°C.
** Read after 1 hour at room temperature.
† Read after 2 hours at 37°C.

TABLE X

Agglutination Test with Serum of Horse Immunized with Collodion Particles Adsorbed with Streptococcus (Bargen) Polysaccharide

Serum dilution.....	1/2	1/4	1/8	1/16	1/32	1/64
Horse 50.....	4	4	4	2	1	0
Normal horse serum.....	0	0	0	0	0	0

Read after 2 hours at 56°C.

In view of these responses, we immunized a horse with collodion particles and Bargen polysaccharide. This animal received 855 cc. of suspension, equivalent in polysaccharide to about 1.9 mg. Tables X and XI show the agglutination and polysaccharide precipitin tests with the serum of this horse.

B. dysenteriae (Shiga and Hiss).—The polysaccharides from these two organisms were selected, first, because we had done considerable work with the Shiga variety, and second to determine whether the specific antibody found in antidysentery (Shiga) serum is an anti-carbohydrate antibody, or an antitoxin. These rabbits received 17 cc. of antigen which is equivalent to about 0.05 mg. of its respective polysaccharides.

TABLE XI

Polysaccharide Precipitin Test with the Serum of a Horse Immunized with Collodion Particles Adsorbed with Streptococcus (Bargen) Polysaccharide

Serum dilution	1/2	1/4	1/8	1/16	1/32	1/64
Horse 50	4	4	4	1	0	0
Normal horse serum	0	0	0	0	0	0

Read after 2 hours at 37°C. and overnight in ice box.

TABLE XII

Agglutination Tests with Serum of Rabbits Immunized with Collodion Particles Adsorbed with B. dysenteriae (Shiga) and B. dysenteriae (Hiss) Polysaccharides

Organism	*B. dysenteriae* (Hiss)						*B. dysenteriae* (Shiga)					
Serum dilution	1/50	1/100	1/200	1/400	1/800	1/1,600	1/2	1/4	1/8	1/16	1/32	1/64
(Shiga) No. 68	0	0	0	0	0	0	4	4	4	3	2	1
(Hiss) No. 70	4	4	4	4	4	3	0	0	0	0	0	0
Normal rabbit serum	0	0	0	0	0	0	0	0	0	0	0	0

Read after 2 hours in water bath at 56°C.

Table XII shows the agglutination test of the two test rabbits. It is of interest to note the specificity of each serum. In the preparation of agglutinating antiserum we always get cross-reactions with Shiga and Hiss types. We cannot account for the difference in titer of the two types of sera, except by supposing that one (Hiss) might be more easily adsorbed. Tests in mice are under way to see if the toxin prepared from the organisms is neutralized by this antiserum.

Pneumococci.—Consistent failure has been the result in our hands of immunization experiments with these polysaccharides. This in-

cludes Type I which seems to contain normally about 5 per cent nitrogen, though this is not in the form of protein.

We have carried out immunization experiments in horses and rabbits with the object of producing immune serum, and have used mice for experiments on active immunity. Our first attempt was to immunize mice with Pneumococcus Types I, II and III polysaccharides adsorbed on collodion particles. A control series was injected with

TABLE XIII

Virulence Test on Mice Injected with 0.5 Cc. Intravenously and Intraperitoneally of a Suspension of Collodion Particles Adsorbed with Pneumococcus Polysaccharide Types I, II and III Separately

Type of organism...	Pneumococcus Type I				Pneumococcus Type II				Pneumococcus Type III			
Route of injection....	Intravenous		Intraperitoneal		Intravenous		Intraperitoneal		Intravenous		Intraperitoneal	
cc.												
10^{-2}	18	18	—	—	—	—	—	—	—	—	—	—
10^{-3}	18	S	18	24	18	24	18	18	—	—	—	—
10^{-4}	S	S	24	S	18	18	18	72	18	18	24	48
10^{-5}	S	S	24	S	24	36	24	S	24	36	S	S
10^{-6}	S	S	S	S	S	S	S	S	24	72	S	S
10^{-7}	24	48	24	96	24	36	72	S			24	48
Control.....	Virulence		Polysaccharide		Virulence		Polysaccharide		Virulence		Polysaccharide	
cc.												
10^{-9}	24	24	72	—	24	36	S	—	36	S	48	48
10^{-8}	22	22	36	36	24	24	36	—	36	36	24	48
10^{-7}	18	36	36	—	24	36	24	—	24	36	48	—

S = survival; numerals indicate the number of hours elapsing before death.
— = not done.

polysaccharide alone. One injection was given to two series of mice, in one intravenously and in the other intraperitoneally. 7 days later these mice were tested for immunity against various amounts of live, virulent cultures of pneumococcus. The result of our first test is given in Table XIII. Encouraging results were observed, and further work is being done with the object of working with other colloids as well as of determining the optimum time factor and dosages.

We then proceeded with the immunization of horses with collodion particles adsorbed with Types I, II and III polysaccharides respectively. (The Type III polysaccharide used was kindly supplied to us

TABLE XIV

Homologous Polysaccharide Precipitin Test with the Sera of Horses Immunized with Collodion Particles Adsorbed with Types I, II and III Pneumococcus Polysaccharides

Dilution of serum..	1/2	1/4	1/8	1/16	1/32
Type I					
Horse 49	3	2	0	0	0
Type II					
Horse 52	3	3	2	1	0
Type III					
Horse 87	3	3	2	2	0

Read after 3 hours at 37°C. and overnight in ice box.

TABLE XV

Protection Test in Mice with Serum of Horses Immunized with Collodion Particles Adsorbed with Pneumococcus Polysaccharides

Type.....	Pneumococcus I		Pneumococcus II		Pneumococcus III	
Horse No.....	49		52		87	
cc.						
10^{-8}	S	S	S	S	36	36
10^{-7}	S	S	S	S	36	36
10^{-6}	72	S	S	S	24	36
10^{-5}	48	S	S	S	24	36
10^{-4}	48	48	S	S	24	24
10^{-3}	48	72	24	24	24	24
Virulence						
10^{-9}	48	S	24	S	96	S
10^{-8}	48	48	24	24	24	24
10^{-7}	24	24	24	24	24	24

S = survival. Numerals indicate the number of hours elapsing before death.

by Dr. M. Heidelberger.) Horse 49 was immunized against Type I, having received during 2 months 855 cc. of the antigen, approximately 2 mg. in terms of polysaccharide. Horse 52 was immunized against Type II, receiving during 4 weeks 425 cc. of antigen, or about 0.9

mg. in terms of polysaccharide. And finally, Horse 87, immunized against Type III, received in 4 weeks 425 cc. of antigen or 0.9 mg. of polysaccharide. 1 day after the last injection, the horses were trial bled, and agglutination, polysaccharide precipitin and protection tests were made. Tables XIV and XV show the results of these tests. We did not observe any agglutination with any of the types. At this time Type III antiserum does not show any protection although it clearly gives a precipitin test at a dilution of 1/16 of serum. The immunization of the horses is being continued.

The result obtained with Type III polysaccharide immunization is not surprising when one considers the difficulty experienced in pro-

TABLE XVI

Agglutination and Polysaccharide Precipitin Test with Sera of Rabbits Immunized with Collodion Particles Adsorbed with Anthrax, Meningococcus and Bargen Polysaccharides, at the Same Time

Antigen.....	Anthrax			Meningococcus Type I			*Strep. viridans* (Bargen)			
Rabbit No.....	1*	2	3	1**	2	3	—*	1	2	3
1/20	4	4	4	3	4	4	1/2	4	2	4
1/40	3	1	2	1	2	4	1/4	2	0	3
1/80	1	0	0	0	0	4	1/8	1	0	3
1/160	0	0	0	0	0	2	1/16	0	0	1
SSS 1–1,000	0†	0	±	1	1	3	—	3	2	2

* Read after 2 hours at 56°C. ** Read after 15 hours at 56°C.
† Read after 2 hours at 36°C. and overnight in ice box.

ducing protective serum even when the whole Type III pneumococcus organism is used for immunization. In the treatment of horses, more than 6 months is usually necessary before any definite protection is shown in their serum.

Multiple Polysaccharides Adsorbed on Collodion Particles

Having successfully used as antigens, collodion particles adsorbed with single polysaccharides, we now adsorbed different polysaccharides on the same particles. Table XVI shows the results of agglutination and polysaccharide precipitin tests on the sera of rabbits immunized with collodion particles adsorbed with three distinct polysaccharides.

The variations observed in the different rabbits are probably due to individual variation in the response of the animal to the antigen.

Table XVII shows the results of agglutination tests with serum from Horse 73 which received 460 cc. of collodion particles adsorbed with

TABLE XVII

Agglutination with Anthrax, Meningococcus Type I, Pneumococcus Type I and Streptococcus (Bargen) with Serum of Horse 73 Immunized with Collodion Particles Adsorbed with Polysaccharides of Same Organisms

Final dilution	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Anthrax	4*	4	4	4	3	1	0
Meningococcus Type I	4**	4	4	3	1	0	0
Pneumococcus Type I	0†	0	0	0	0	0	0
Streptococcus (Bargen)	4*	4	3	0	0	0	0

* Read after 2 hours at 56°C.

** Read after 15 hours at 56°C.

† Read after 2 hours at 37°C.

TABLE XVIII

Protection Test in Mice with Serum of Horses Immunized with (72) Casein and (73) Collodion Particles Adsorbed with Anthrax, Meningococcus, Streptococcus (Bargen) and Pneumococcus Type I Polysaccharides

Organism	Type I pneumococcus			
Horse No.	72		73	
cc.				
10^{-8}	S	S	S	S
10^{-7}	S	S	S	40
10^{-6}	S	S	S	40
10^{-5}	S	S	40	40
10^{-4}	S	S	24	40
Virulence				
10^{-9}	72	S	–	–
10^{-8}	40	40	–	–
10^{-7}	24	40	–	–

S = survival after 96 hours. Numbers indicate hours elapsing before death.

anthrax, meningococcus, streptococcus (Bargen) and Pneumococcus Type I polysaccharides. The agglutination test for pneumococcus was negative, so mouse protection tests were made with virulent organisms, the result of the test being shown in Table XVIII.

Results of Immunization with Aluminum Hydroxide as Colloid.—We adsorbed alumina with different polysaccharides, first one at a time, then two and three. The results of the multiple polysaccharide adsorption given in Table XIX suffice to show the success obtained.

TABLE XIX

Agglutination Test with Anthrax, Meningococcus Type I and Streptococcus (Bargen) of Serum of Rabbit 42, Immunized with Aluminum Hydroxide Adsorbed with Polysaccharides of the Same Organisms

Final dilution	1/4	1/8	1/16	1/32	1/64	1/128	SSS 1~1,000
Anthrax	4*	4	4	4	4	2	3
Meningococcus Type I	4**	4	3	1	0	0	2
Streptococcus (Bargen)	4*	2	1	0	0	0	2

* Read after 2 hours at 56°C. ** Read after 15 hours at 56°C.

TABLE XX

Homologous Agglutination Test with Sera of Rabbits Immunized with Casein Adsorbed with Morgani, Bargen and Anthrax Polysaccharides, Separately

Antigen	Casein + *morgani* SSS			Casein + Bargen SSS			Casein + anthrax SSS		
Rabbit No.	64	65	–	69	70	71	66	67	68
1/16	4	4	–	4	2	1	2	4	2
1/32	4	4	–	4	1	0	1	4	1
1/64	4	4	–	3	0	0	0	4	0
1/128	3	4	–	1	0	0	0	4	0
1/256	3	4	–	0	0	0	0	2	0
Milk agglutinins	+	+	–	+	+	+	+	+	+

Read after 2 hours at 56°C.

We have successfully immunized a horse with alumina adsorbed with meningococcus polysaccharide and another with alumina adsorbed with anthrax, streptococcus (Bargen), meningococcus and Pneumococcus Type I polysaccharide.

Results of Immunization with Casein as Colloid.—The use of the protein, casein, as the colloid carrier is of importance as showing that a protein carrier can effectively adsorb polysaccharides, the combina-

TABLE XXI

Agglutination with Sera of Rabbits Immunized with Casein Particles and Bacterium morgani, B. anthracis and Streptococcus (Bargen) Polysaccharides Together

Immunized with.....	Casein + *Bacterium morgani*, *B. anthrax* and Streptococcus (Bargen) SSS								
Rabbit No...	60			61			62		
Antigen.....	*Morgani*	Bargen	Anthrax	*Morgani*	Bargen	Anthrax	*Morgani*	Bargen	Anthrax
1/4	4	0	4	4	2	4	4	3	4
1/8	4	0	4	4	1	4	4	2	4
1/16	3	0	3	4	0	2	4	1	4
1/32	2	0	2	4	0	1	4	0	2
1/64	1	0	1	4	0	0	4	0	1
1/128	0	0	0	4	0	0	4	0	0
Milk agglutination	4			4			4		

Positive and negative controls as in previous table. Read after 2 hours at 56°C.

TABLE XXII

Agglutination and Polysaccharide Precipitin Tests with Anthrax, Meningococcus Types I, II, III, IV and V, Pneumococcus Type I and Streptococcus (Bargen) with the Serum of Horse 72, Immunized with Casein Adsorbed with Polysaccharide from the Same Organisms

Test	Agglutination						Polysaccharide precipitin test (1/10,000)		
Final dilution.....	1/4	1/8	1/16	1/32	1/64	1/128	1/2	1/4	1/8
Anthrax.....	4	4	4	4	2	1	4*	2	0
Pneumococcus Type I.....	0	0	0	0	0	0	3	2	1
Streptococcus (Bargen).....	4	4	2	1	0	0	4	1	0
Meningococcus Type I*.....	4	4	4	4	4	4	4	2	0
Meningococcus Type II.....	4	4	4	4	3	2	—	—	—
Meningococcus Type III.....	4	4	4	3	1	0	—	—	—
Meningococcus Type IV.....	4	3	1	0	0	0	—	—	—
Meningococcus Type V.....	4	4	4	1	0	0	—	—	—

* Precipitin tests read after 2 hours at 37°C. and overnight in the ice box.

** Tests for agglutination with meningococcus read after 15 hours at 56°C., all the others after 2 hours at 37°C.

tion being antigenic. Avery and Goebel have made use of proteins, combining them chemically with polysaccharides, but we used casein

adsorbed with various polysaccharides. Table XX shows the results of agglutination tests with sera of animals immunized with casein adsorbed with anthrax, *morgani* and Bargen polysaccharides respectively, and Table XXI shows the results of the agglutination test with the serum of animals immunized with casein adsorbed with the same three polysaccharides at the same time.

Horse 72 was immunized with casein adsorbed with meningococcus, Pneumococcus Type I, streptococcus (Bargen) and anthrax polysaccharides. The results of the agglutination test with the serum of this

TABLE XXIII

Agglutination and Polysaccharide Precipitin Tests with Sera of Rabbits Immunized with Streptococcus (Bargen) Adsorbed with Different Polysaccharides

Immunized with..	Bargen organisms + *proteus* SSS				Bargen organisms + *morgani* SSS				Bargen organisms + anthrax SSS			
Rabbit No.....	78		79		80		81		82		83	
Antigen.....	*Proteus*	Bargen	*Proteus*	Bargen	*Morgani*	Bargen	*Morgani*	Bargen	Anthrax	Bargen	Anthrax	Bargen
1/4	4	4	2	4	4	4	4	4	4	4	4	4
1/8	4	4	1	4	4	4	4	4	4	4	2	4
1/16	4	4	0	4	4	4	4	4	2	4	0	4
1/32	4	4	0	4	4	4	4	4	0	4	0	4
1/64	3	4	0	4	4	4	4	4	0	4	0	4
1/128	0	4	0	4	4	4	4	4	0	4	0	4
SSS 1–1,000	2	4	2	3	0	4	1	4	0	4	0	4

Read after 2 hours at 56°C.

horse is shown in Table XXII. The pneumococcus did not show any agglutination. The mouse protection test against virulent Type I pneumococcus is shown in Table XVIII. The polyvalency against meningococcus is of interest.

Results of Immunization with Bacterial Cells as Colloids.—Before attempting immunization experiments with adsorbed polysaccharides on bacteria, we made a few preliminary experiments *in vitro*. We selected *Streptococcus viridans* (Bargen) as the bacterium. It was grown in Douglas media for 24 hours, centrifuged and washed once with saline. To the centrifuged cells the polysaccharide was added and thoroughly mixed. We allowed this to stand in the ice box for $\frac{1}{2}$

hour, after which it was washed four times with saline. The last washing was free from detectable amounts of polysaccharide. These organisms were suspended in saline in a concentration of about 2,000 billion, and agglutination tests were made with serum homologous to the adsorbed polysaccharide with positive results. This no doubt was the phenomenon observed by Nicolle, Arkwright and Jones in their studies on agglutination. Having successfully repeated this with many different polysaccharides, we began the immunization of

TABLE XXIV

Agglutination and Polysaccharide Precipitin Tests with Sera of Rabbits Immunized with Bargen Organisms Adsorbed with Two Different Polysaccharides on the Same Cell

Immunized with..	Bargen organisms + anthrax and *proteus* SSS			Bargen organisms + *morgani* and anthrax SSS			Bargen organisms + Pneumococcus Type I and *morgani* SSS		
Rabbit No.....	77			75			84		
Antigen.....	Anthrax	*Proteus*	Bargen	Anthrax	*Morgani*	Bargen	Pneumococcus Type I	*Morgani*	Bargen
1/4	4	4	4	4	4	4	0	4	4
1/8	4	4	4	4	4	4	0	4	4
1/16	4	2	4	4	4	4	0	4	4
1/32	2	0	4	4	4	4	0	4	4
1/64	0	0	4	2	4	4	0	4	4
SSS 1–1,000	0	3	4	0	2	0	0	2	4

Read after 2 hours at 56°C.

animals. The results of our tests with different polysaccharides are shown in Table XXIII.

We then adsorbed more than one polysaccharide to the bacterial cell and immunized animals. The results of our tests are shown in Table XXIV. It is of interest to note that the specific antigen of the cell remained the most prominent of all both in agglutinins and precipitins. The streptococci adsorbed with Pneumococcus Type I polysaccharide agglutinated *in vitro*, but did not produce agglutinins against the pneumococci when used as antigen. Unfortunately this serum was mislaid and we were unable to make protection tests. Comparing these results with our previous experiments with pneumo-

coccus, we can expect some protective antibodies to have been produced.

Results of Immunization with Dextran Adsorbed on Collodion Particles.—Dextran is the synthetic polysaccharide of *Leuconostoc mesenterioides* from saccharose. This sugar is of importance because it probably resembles in its chemical structure the more complex bacterial polysaccharides. Its molecular composition is being studied by Hibbert of McGill University, who believes it to be a gluco-glucoside of some sugar. Further work is necessary to definitely establish its composition.

TABLE XXV

Agglutination Test with Collodion Particles Adsorbed with Dextran as Antigen of Antidextran Serum R 12

Serum dilution	1/2	1/4	1/8	1/16	1/32	1/64
R 12	4	4	4	3	2	2
Normal rabbit serum	0	0	0	0	0	0

Read after 5 hours in water bath at 37°C.

TABLE XXVI

Titration of Antidextran Serum against Dextran Polysaccharide (1–5,000)

Serum dilution	1/2	1/4	1/8	1/16	1/32
R 12	4	4	4	2	1
Normal rabbit serum	0	0	0	0	0

Read after 5 hours in water bath at 37°C. and vault overnight.

The material used was kindly supplied by Prof. Hibbert, and was very pure, nitrogen- and ash-free. Rabbits were immunized with collodion particles adsorbed with a solution of dextran (1 per cent). The immunization was continued for 4 weeks when the animals were bled. An equal number of rabbits (6) were injected with a solution of dextran 0.05 per cent in 5 cc. doses, the treatment continuing for 4 weeks longer than in the work thus far described. They were then bled and tested. Serum R 12 was selected as a representative sample for our tests.

In order to show the presence of antibodies in the serum we used collodion particles adsorbed with dextran as antigen in the first agglutination test made. A stable suspension of collodion particles is important in each test in order that the final readings are not confused. The results are shown in Table XXV.

We then adsorbed Serum R 12 to collodion particles, washed them 4 times and tried the agglutination test on different dilutions of dextran from 1/1,000 to 1/4,000. All these tests were negative. This phenomenon will be commented on in the discussion.

TABLE XXVII

Titration of Dextran Polysaccharide against Antidextran Serum R 12

Polysaccharide dilution	1/2,000	1/8,000	1/32,000	1/128,000	1/256,000	1/512,000	1/1,240,000
Serum R 12	4	4	4	4	4	3	1
Normal rabbit serum	0	0	0	0	0	0	0

Read after 5 hours in water bath at 37°C. and vault overnight.

TABLE XXVIII

Polysaccharide Precipitin Test with Serum R 12 Absorbed with Dextran 1/1,000, Using the Homologous Polysaccharide in the Test 1/5,000

	1/4	1/8	1/16	1/32	1/64
Serum R 12	4	4	4	3	1
R 12 absorbed	0	0	0	0	0
Normal rabbit serum	0	0	0	0	0

Read after 5 hours in water bath at 37°C. and vault overnight.

Table XXVI shows the results of the polysaccharide precipitin test in various dilutions of the serum. The potency is high for an anti-carbohydrate antibody. Table XXVII shows the results of the polysaccharide precipitin test in various dilutions of the polysaccharide with Serum R 12.

To show the specificity of this antibody towards its antigen we adsorbed some of the serum with dextran diluted 1/1,000, centrifuged the precipitate and made precipitin tests with the supernatant liquid. The results of this test are shown in Table XXVIII. They prove

conclusively that the precipitate formed by the immune serum and the dextran polysaccharide is specific. Methylated dextran as antigen does not give cross-reactions with this serum.

In a separate article we are reporting the relation of the active group or groups of dextran to some bacterial polysaccharides.

We attempted to immunize animals with levan, a polymerization product of an anhydro-fructo-furanose, produced synthetically from sucrose by *B. mesentericus* or its enzyme, but with negative results so far. Either the active group of levan is attached to the colloid or the antibodies produced were not to be detected by our methods of investigation.

DISCUSSION

Bacteria, being living organisms, have protoplasm, and this protoplasm always contains protein. It has been inferred that antigens must be of protein nature, and that the protein must have a certain complexity. The generalization now seems warranted that this need not be the case, but that some substance of a colloid nature is responsible for antigenicity (*vide* Willstätter's theory of enzyme action (23)). The colloid may be in certain cases a protein, but other forms of colloids which can adsorb the specific principle can suffice instead. The physical attachment makes the combination an antigen. In some cases a carbohydrate, in other cases a lipoid and in yet others a specific nitrogenous (protein) radical give to the antigens their specificity. In the case of pure proteins, we no doubt have a special radical in the whole molecule which is the active group and gives the antibody its specificity. Cross-reactions with antibodies may be explained by chemical similarity in the structure of the active group, an idea suggested by the work of Heidelberger and Avery (24) with the *B. friedlaenderi* and Pneumococcus Type II polysaccharide as well as by the work of Avery and Goebel (25) with gluco- and galacto-albumin and globulin, as also by the work of Obermeyer and Pick, (26) who studied the changes in specificity brought about by subjecting proteins to various chemical reactions, such as that caused by iodine, or nitric acid, the chemical changes thus induced giving rise to new antigens with altered serological properties.

We have shown in this work that dextran, a polysaccharide free of

nitrogen, can become antigenic when adsorbed on collodion particles. The experiments with the antiserum thus obtained demonstrate that carbohydrate antibodies are specific. They can be of high titer, as judged by the precipitin test and by the agglutination test. From the theoretical side it is important to note our failure to obtain positive agglutination reactions with various dilutions of the polysaccharide and collodion particles adsorbed with immune serum. Our belief is that the adsorption of the immune serum to the particle comes about by means of the groups which have specific attraction to the polysaccharide, the hydrophilic groups being left free in this case. The polysaccharide, in solution with water, has the hydrophilic groups orientated towards the water phase. If this is so, one could not expect attraction towards the serum adsorbed on the particle. In the case of the particle adsorbed with the polysaccharide, one may suppose on the evidence of specific antigenicity that orientation occurs in such a way so as to leave the active groups free for a meeting with the specific group of the serum. The serum being attached to the water phase by the hydrophilic group leaves the specific group free to be attached to the adsorbed polysaccharide, causing agglutination.

In another communication, suggestive evidence is reported of the existence of various active groups in one bacterial polysaccharide.

Of theoretical interest are the calculations of the small amounts of polysaccharide needed to produce antibodies, especially in horses, in which 0.5 to 1.0 mg. was sufficient to give a fair titer to the serum. How much of the response in the animal is caused by the polysaccharide-colloid combination and how much is due to the exceedingly small amount of the polysaccharide alone, remains to be definitely determined. We are unable to tell the duration of the immunity produced by the polysaccharide or the degree of hyperimmunization which can be obtained. These points are being studied. There is no doubt that antibodies are detectable a short time after the injection and we have also observed the disappearance of the precipitin antibody on prolonged immunization. We have not been able to explain the latter phenomenon.

The extent of the practical application of our work, experience will decide. The advantages of an antiserum free from protein antibodies would seem plain. It is conceivable that one may prepare specific

bacterial polysaccharides and other antigenic haptenes for use in active immunization against infectious diseases in which the polysaccharides of the organisms play a part. The multiple nature of the haptogens in one particle has many attractive possibilities. The adsorption method may help the chemist to determine active groups in substances which are difficult to analyze by the methods now at hand.

SUMMARY

Evidence is here given that polysaccharides can be rendered antigenic by haptogenic adsorption upon a colloid carrier. The polysaccharides studied were those of *B. anthracis*, the meningococcus, *Streptococcus viridans* (Bargen), *B. proteus*, *S. morgani*, *B. dysenteriae*, both the Shiga and Hiss types, and the pneumococci. With the polysaccharide of Type III pneumococci, we have been unable in 6 weeks to produce any detectable protective antibodies, but we were able to produce anticarbohydrate antibodies. All the bacterial carbohydrates were non-antigenic alone when used in the doses indicated, though containing some nitrogen. Dextran, which was free from nitrogen was also rendered antigenic by the adsorption method.

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