A SEROLOGICAL STUDY OF THE POLYSACCHARIDES OF MENINGOCOCCUS, B. ANTHRACIS, B. PROTEUS, B. SUBTILIS AND B. MESENTERICUS

By JOSÉ ZOZAYA, M.D.

(From the Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pennsylvania)

(Received for publication, July 24, 1931)

Since the initial studies of Heidelberger, Goebel and Avery (1) on cross-reactions between the specific polysaccharides of Pneumococcus Type II and Friedländer bacillus, the literature has contained little or nothing on reactions of similar nature between the specific polysaccharides of other organisms. However, in the study of bacterial polysaccharides, we have found many interesting cross-reactions that by theory would not have been expected. Among these, the one here reported is of special interest, from both a theoretical as well as a practical point of view. Przemycki (2) obtained a specific carbohydrate substance from the *B. proteus* organism; Furth and Landsteiner (3) also obtained a carbohydrate from *B. proteus* which they found to be specific. Combiesco, Soru and Stamatesco (4) have described a specific carbohydrate obtained from anthrax, and some of the chemical properties, such as rotation, presence of pentoses, hexoses, etc.

The wide difference between the meningococci and the Gram-positive bacilli would not lead one to suspect an immunological relationship between the carbohydrates of the two groups. Our first observation was accidental, and occurred when tests were made to determine the specificity of the anthrax polysaccharide with various immune sera. In the case of the antimeningococcus serum, we observed that an immediate precipitate formed, which increased after incubation. This was repeated with many different samples of antimeningococcic sera. We then proceeded to study the reciprocal test, *i.e.*, meningococcus polysaccharide with anthrax serum and

found that precipitation took place. The titration of the polysaccharide from these organisms, as well as others (*B. mesentericus*, *B. proteus* and *B. subtilis*) was made, using antimeningococcus, antianthrax and antiproteus sera. The results of this test are shown

TABLE I

Titration of Polysaccharide of B. anthrax, B. proteus, B. subtilis and Meningococcus with Antianthrax, Antimeningococcus and Antiproteus Sera

Final dilution of polysaccharide	Antimeningococcus serum (6383) undiluted				Antianthrax serum (8795) undiluted				Antiproteus serum (6202) undiluted			
	1/40,000	1/160,000	1/640,000	1/1,200,000	1/20,000	1/80,000	1/320,000	1/1,200,000	1/2000	1/8000	1/16,000	1/64,000
Meningococcus	4	4	4	4	3	1	1	0	2	2	0	0
Anthrax	4	4	4	3	4	4	4	1	0	0	0	0
Proteus	4	4	4	3	3	1	0	0	4	4	4	3
Mesentericus	4	4	4	4	4	2	0	0	0	0	0	0
Subtilis	4	4	4	3	3	1	0	0	2	1	0	0

Overnight readings.

TABLE II

Titration of Antimeningococcic, Antianthrax and Antiproteus Sera with Different Polysaccharides (1/10,000)

Final dilution of serum	Antimeningococcic serum (8363)						Antianthrax serum (8795)						Antiproteus serum (6206) rabbit					
	1/2	1/4	1/8	1/16	1/32	1/64	1/2	1/4	1/8	1/16	1/32	1/64	1/2	1/4	1/8	1/16	1/32	1/64
Meningococcus	4	4	4	2	0	0	3	2	1	0	0	0	2	1	0	0	0	0
Anthrax	4	4	4	2	0	0	4	4	4	4	2	0	0	0	0	0	0	0
Proteus	4	4	4	2	0	0	3	2	1	0	0	0	4	4	4	4	3	2
Mesentericus	4	4	4	1	0	0	4	2	1	0	0	0	0	0	0	0	0	0
Subtilis	4	4	4	2	0	0	3	2	1	0	0	0	2	1	0	0	0	0

in Table I. We found that, with the antimeningococcic serum, all of the polysaccharides gave more or less the same result, while with antianthrax serum the differences were more marked, showing the anthrax polysaccharide to be more potent and specific in higher dilutions. We then titrated the same sera and a *proteus* antiserum

against the different polysaccharides in order to see if the amount of antibody precipitated by the different carbohydrates varied. The results of this test are given in Table II, according to which the meningococcus, *proteus* and *subtilis* polysaccharides are shown to behave in the same manner as far as the antimeningococcus serum is concerned, while the homologous polysaccharide with the antianthrax serum acts specifically in the higher dilutions of serum, the other four polysaccharides reacting similarly toward each other. The homologous polysaccharide with antiproteus serum is specific in higher dilutions of the former, the meningococcus and *subtilis* acting only at lower dilutions and the anthrax and *mesentericus* negatively. The antimeningococcus serum has the broadest antigenicity, being pre-

TABLE III

Agglutination Tests with Anthrax, Meningococcus and Proteus Antigens with Antimeningococcus, Antianthrax and Antiproteus Sera

Final dilution of serum	Antimeningococcus serum (8363)						Antianthrax serum (8795)						Antiproteus serum (6206)					
	1/20	1/40	1/80	1/160	1/320	1/640	1/160	1/320	1/640	1/1200	1/2500	1/5000	1/10	1/40	1/80	1/160	1/640	1/2500
Meningococcus..	4	4	4	4	4	3	3	3	2	2	0	0	4	4	3	1	0	0
Anthrax.....	4	4	4	3	2	2	4	4	4	4	3	2	0	0	0	0	0	0
Proteus.....	4	4	3	1	0	0	0	0	0	0	0	0	4	4	4	4	4	2

cipitated by all the heterologous polysaccharides used. With the anthrax serum, we found specificity of the homologous carbohydrate in the higher dilutions of the carbohydrate, while the other four acted similarly. Finally with the antiproteus serum we found specificity of its homologous polysaccharide in high dilutions and some similarity to the *subtilis* and meningococcus.

Agglutination tests were made with meningococcus, anthrax and *proteus* against antimeningococcus, antianthrax and antiproteus sera. The results are shown in Table III. In this test we confirmed the observations obtained in a previous test with the polysaccharide, the meningococcus remaining a broad antigen for the three sera, while anthrax and *proteus* were more limited. It is of interest to note the polyvalency of the sera as well as of the antigens, for this may have

some bearing on the agglutination in typhus cases with *proteus* antigen, since in some typhus sera we have obtained agglutination with the meningococcus antigen. We will report these studies in a future paper.

In order to secure more information, we made absorption tests with antimeningococcus and antianthrax sera and the various polysaccharides, performing an agglutination test with the homologous organism of the antiserum as well as a precipitation test with the homologous polysaccharide, to determine whether antibody was absorbed by the specific carbohydrate. For this experiment, we had to use concentrated sera prepared according to Felton's (5) water precipitate method, in order to test the dilution obtained in the absorption process and still possess carbohydrate precipitable substance. The original serum showed precipitin in dilution 1/8, while in the concentrated preparation it gave a titer of 1/64.

Method

To 2 cc. of concentrated serum, were added 2 cc. of the polysaccharide 1/1000, incubated for 2 hours at 37°C., and placed in the ice box overnight. The mixture was centrifuged and from the supernatant liquid, 2 cc. were taken and absorbed again with the polysaccharide. This procedure was repeated four times. In each sample the precipitin and agglutination tests were done with the homologous polysaccharide and antigen.

The results of absorption of antimeningococcus serum are shown in Table IV. Here we noticed that the carbohydrate antibody was equally well absorbed by all of the polysaccharides used, while the agglutination titer of the serum was unaffected. In Table V we show the results of absorption of antianthrax serum. We note here that while the heterologous polysaccharides gave a precipitate with this serum, only the homologous polysaccharide absorbed the anthrax specific antibody. Here we have at least two anticarbohydrate antibodies, one specific, easily absorbed by its specific carbohydrate and another probably group specific as those found in antipneumococcus serum or even more broadly non-specific. Yet the agglutination titer of the serum is not changed by any of the absorbing polysaccharides, not even when the specific polysaccharide absorbs its homologous antibody.

The absorption tests with antiproteus serum only will be mentioned because the amount of immune serum was limited and no concentration was obtained, although the meningococcus and *subtilis* poly-

TABLE IV

Precipitin Tests with Meningococcus Polysaccharide 1/10,000, after Absorption of Antimeningococcic Serum with Meningococcus, Anthrax, Proteus, Subtilis and Mesentericus Polysaccharide 1/1000, as Well as the Agglutination Titer of the Same Serum

Final dilution		1/4	1/8	1/16	1/32	1/64	1/128	Agglutination titer
Absorbed with meningococcus polysaccharide 1/1000	1st	1	0	0	0	0	0	3:1/500
	2nd	—	0	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Absorbed with anthrax polysaccharide 1/1000	1st	1	0	0	0	0	0	3:1/500
	2nd	—	0	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Absorbed with *subtilis* polysaccharide 1/1000	1st	2	1	0	0	0	0	3:1/500
	2nd	—	1	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Absorbed with *mesentericus* polysaccharide 1/1000	1st	2	1	0	0	0	0	3:1/500
	2nd	—	1	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Absorbed with *proteus* polysaccharide 1/1000	1st	1	0	0	0	0	0	3:1/500
	2nd	—	0	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Control serum with salt solution	1st	4	4	4	4	4	2	3:1/500
	4th	—	—	—	4	4	2	

saccharides did not absorb the specific precipitable substance for the *proteus* polysaccharide showing the same non-specific phenomenon as was found in the absorption experiments with antianthrax serum. The *proteus* polysaccharide is specific.

DISCUSSION

The interesting findings of Heidelberger, Goebel and Avery on the immunological and chemical similarity between the polysaccharides obtained from a strain of *Bacillus friedlaenderi* and from Pneumo-

TABLE V

Precipitin Tests with Anthrax Polysaccharide (1/10,000) after Absorption of Anti-anthrax Serum with Meningococcus, Anthrax, Proteus, Mesentericus and Subtilis, as Well as the Agglutination Titer of the Same Serum

Final dilution of serum		1/4	1/8	1/16	1/32	1/64	1/128	Agglutination titer
Absorbed with meningococcus polysaccharide 1/1000	1st	4	4	4	4	3	2	3:1/5000
	2nd	—	4	4	4	3	3	
	3rd	—	—	4	4	2	2	
	4th	—	—	—	4	2	1	
Absorbed with anthrax polysaccharide 1/1000	1st	2	2	1	1	0	0	3:1/5000
	2nd	—	2	0	0	0	0	
	3rd	—	—	0	0	0	0	
	4th	—	—	—	0	0	0	
Absorbed with *subtilis* polysaccharide 1/1000	1st	4	4	4	4	4	3	3:1/5000
	2nd	—	4	4	4	4	3	
	3rd	—	—	4	4	4	2	
	4th	—	—	—	4	4	2	
Absorbed with *mesentericus* polysaccharide 1/1000	1st	4	4	4	4	4	3	3:1/5000
	2nd	—	4	4	4	4	3	
	3rd	—	—	4	4	4	2	
	4th	—	—	—	4	4	2	
Absorbed with *proteus* polysaccharide 1/1000	1st	4	4	4	4	4	2	3:1/5000
	2nd	—	4	4	4	4	3	
	3rd	—	—	4	4	4	3	
	4th	—	—	—	4	4	2	
Control serum with salt solution	1st	4	4	4	4	4	4	3:1/5000
	4th	—	—	—	4	4	2	

coccus Type II, are somewhat similar to our findings with these other organisms. The possibility of a similar chemical composition will be left for future study. Again the possibility arises of having in the

polysaccharides of these organisms a similar or common substance to all of them, as was found in the pneumococcus by Tillett, Goebel and Avery (6).

From previous work and from our present findings, we are aware of the complexity of bacterial antigens and of the necessity for a chemical structural study of the different polysaccharides. It is of interest to remember that while *B. anthracis* and *B. mesentericus* are so different in their biological behavior, Cowles (7) has found a bacteriophage common to both, suggesting a related chemical structure; a finding corroborated by our polysaccharide studies.

The work of Heidelberger, Goebel and Avery with *B. friedlaenderi* and Pneumococcus Type II has been used in detecting an immunological method making it possible to predict chemical similarities in the carbohydrate as Landsteiner (8) has suggested for the erythrocytes in the different species of animals.

CONCLUSIONS

1. The meningococcus polysaccharide reacts with a broad precipitable carbohydrate antibody in common with those of *B. anthracis*, *B. subtilis*, *B. proteus* and *B. mesentericus*.

2. The anthrax and *proteus* polysaccharides are specific in the higher dilutions of serum.

3. Antianthrax serum contains two different polysaccharide precipitable antibodies, one specific and the other non-specific.

4. Agglutinins have no relation to the carbohydrate precipitable substance, specific or non-specific.

5. An immunological method is given for the study of the probable chemical relation or similarity of polysaccharides of different bacteria similar to that given by Heidelberger, Goebel and Avery from a strain of Friedländer bacillus and Pneumococcus Type II.

BIBLIOGRAPHY

1. Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 701.
2. Przemycki, F., *Compt. rend. Soc. biol.*, 1925, **95**, 744.
3. Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.
4. Combiesco, D., Soru, E., and Stamatesco, S., *Arch. roum. path. exp. et micr.*, 1929, **2**, 291.

5. Felton, E. D., *J. Infect. Dis.*, 1925, **37,** 199, 309; 1928, **42,** 248, 256.
6. Tillett, W. S., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1930, **52,** 895.
7. Cowles, P. B., *J. Bact.*, 1931, **21,** 161.
8. Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1925, **42,** 139.