CHEMO-IMMUNOLOGICAL STUDIES ON CONJUGATED CARBOHYDRATE-PROTEINS

XI. The Specificity of Azoprotein Antigens Containing Glucuronic and Galacturonic Acids

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When rabbits are immunized with artificial antigens containing the azobenzyl glycosides of glucose and glucuronic acid, the antibodies elicited are specific and show no serological crossing (1). Since the configuration of the hexoside radicals in these antigens is identical, it is noteworthy that they give rise in each instance to immune bodies which exhibit no serological cross reactions. Differences in immunological properties must be attributed therefore to differences in the grouping occupying the sixth position in each carbohydrate radical, which in the case of the glucoside is an hydroxyl group (CH_2OH) and in the glucuronide a carboxyl group (COOH).

The uronic acid nucleus of the capsular polysaccharides of certain types of pneumococci appears to be of special importance in determining the serological reactions of the latter substances. In certain instances the uronic acid is glucuronic acid, while in others galacturonic acid is found as an integral part of the polysaccharide molecule. It seemed advisable, therefore, to compare the serological properties of artificially compounded antigens containing glucuronic and galacturonic acids, for in this manner it should be possible to ascertain the relationship between stereoisomerism and the immunological properties of the polar carboxyl group.

Chemical Methods

Tetracetyl p-Nitrobenzyl β-Galactoside.—This glycoside was prepared by shaking 7.7 gm. of acetobromogalactose (2) with 5.8 gm. of silver oxide and 5.8 gm. of *p*-nitrobenzyl alcohol in 80 cc. of ether for 1 hour. After filtering and concentrating the solution *in vacuo*, the oily residue was taken up in hot 50 per cent alcohol.

The glycoside crystallized as the solution was slowly cooled. 3.0 gm. of glycoside were recovered. The substance was recrystallized several times from 50 per cent alcohol. The pure substance melted at 99.5–100.5° (uncorrected).

Rotation.—$[\alpha]_D^{23°} = -35.9°$ in $CHCl_3$ (C = 1 per cent).

Analysis.—$C_{21}H_{25}O_{12}N$. Calculated. C 52.2, H 5.2, $COCH_3$ 35.6.
Found. C 52.6, H 5.4, $COCH_3$ 35.6.

p-Nitrobenzyl β-Galactoside.—17.1 gm. of tetracetyl *p*-nitrobenzyl galactoside were suspended in 100 cc. of methyl alcohol and deacetylated with 1/30 mole of barium methylate according to the method of Isbell (3). After removing the barium by adding the equivalent quantity of N/1 sulfuric acid, the glycoside was recovered from the mother liquors. 9.9 gm. of glycoside were obtained. The product, recrystallized from methyl alcohol, melted at 161–162° (uncorrected).

Rotation.—$[\alpha]_D^{24°} = -32.9°$ in CH_3OH (C = 1.5 per cent).

Analysis.—$C_{13}H_{17}O_8N$. Calculated. C 49.5, H 5.4, N 4.4.
Found. C 50.2, H 5.6, N 4.3.

p-Aminobenzyl β-Galactoside.—2.0 gm. of *p*-nitrobenzyl galactoside were dissolved in 100 cc. of absolute methyl alcohol and reduced catalytically (4). On concentrating the alcoholic solution and dissolving the residual oil in 95 per cent ethyl alcohol, the glycoside crystallized as needles melting at 89–90° (uncorrected).

Rotation.—$[\alpha]_D^{23°} = -50.5°$ in CH_3OH (C = 0.8 per cent).

Analysis.—$C_{13}H_{19}O_6N$. Calculated. C 54.7, H 6.7, N 4.9.
Found. C 54.6, H 6.7, N 4.6.

p-Nitrobenzyl β-Glycoside of Triacetyl Galacturonic Acid Methyl Ester.[1]—18.0 gm. of triacetylbromo galacturonic acid methyl ester (5) were dissolved in 450 cc. of anhydrous ether. 8.4 gm. of *p*-nitrobenzyl alcohol and 6.4 gm. of silver oxide were added. The mixture was shaken for 3 hours at 24°C. until the ethereal solution no longer gave a test for the bromo compound. The glycoside crystallized as the reaction progressed. The mixture was filtered, the filtrate discarded, and the glycoside extracted from the residue of silver salts with chloroform. After evaporating the latter, the glycoside crystallized on dissolving the residue in methyl alcohol. 8.2 gm. of the product were obtained as glistening needles melting at 120–122° (uncorrected).

Rotation.—$[\alpha]_D^{25°} = -27.8°$ in $CHCl_3$ (C = 1.5 per cent).

Analysis.—$C_{13}H_{11}O_8N(COCH_3)_3(OCH_3)$.
Calculated. C 51.2, H 5.0, OCH_3 6.6.
Found. C 51.2, H 5.2, OCH_3 6.6.

p-Nitrobenzyl β-Glycoside of Galacturonic Acid Methyl Ester.—6.0 gm. of the acetylated glycoside were suspended in 75 cc. of methyl alcohol freshly distilled over sodium. 1.5 cc. of N/1 barium methylate were added at 0° and the mixture

[1] The galacturonic acid used in this research was furnished us by the Department of Agricultural Chemistry of the University of Wisconsin through the courtesy and generous cooperation of Dr. Karl P. Link.

shaken at 0° for 1 hour. A second portion of 1.5 cc. of barium methylate was added and the shaking continued until all of the glycoside had dissolved. The solution stood at 0° overnight. A small amount of yellowish precipitate settled on standing and was removed. The solution was now concentrated *in vacuo* and the residue taken up in hot alcohol, and the latter allowed to cool. 2.8 gm. of the *p*-nitrobenzyl β-glycoside of galacturonic acid methyl ester were recovered. The compound crystallizes as glistening needles melting sharply at 166.5–167.5° (uncorrected).

Rotation.—$[\alpha]_D^{23°} = -53.4°$ in CH_3OH (C = 0.6 per cent).

Analysis.—$C_{13}H_{14}O_8N(OCH_3)$. Calculated. OCH_3 9.0, N 2.45.
Found. OCH_3 8.8, N 2.60.

p-Aminobenzyl β-Glycoside of Galacturonic Acid Methyl Ester.—0.9 gm. of the nitrobenzyl glycoside was dissolved in 50 cc. of methyl alcohol and reduced catalytically with 25 mg. platinum oxide and hydrogen. The reduction was complete in 10 minutes and the theoretical quantity of hydrogen had been utilized. The platinum was filtered off and the filtrate concentrated *in vacuo* and dissolved in a small amount of ethyl alcohol. The glycoside crystallized as well defined long silky white needles. 0.65 gm. was recovered. The compound apparently crystallizes with a half molecule of ethyl alcohol of crystallization. The melting point of the derivative is not sharp. The compound collapses at 76° and melts at 90°. A methoxyl determination shows that the derivative contains ½ mole of alcohol of crystallization. When the derivative is crystallized from a small amount of water, and the substance dried *in vacuo* over calcium chloride at room temperature, the glycoside is obtained as white well defined needles which melt at 108–110° with effervescence.

Rotation.—$[\alpha]_D^{29°} = -75.8°$ in H_2O (C = 1 per cent).

Analysis.—$C_{13}H_{16}O_6(OCH_3)$. Calculated. OCH_3 9.9, N 4.5.
Found. OCH_3 9.6, N 4.2.

p-Aminobenzyl β-Glucoside and β-Glucuronide.—These derivatives were prepared by methods described in the previous study (1).

Immunological Reactions

Methods.—Immunizing antigens were prepared by combining the diazonium derivatives of *p*-aminobenzyl β-glycosides of glucose, galactose, glucuronic, and galacturonic acids with normal horse serum globulin. The galacturonic acid antigen was prepared from the *p*-aminobenzyl galacturonide methyl ester by first hydrolyzing the ester with one equivalent of normal sodium hydroxide. The resulting sodium salt of the acid was diazotized and coupled to serum globulin in the usual way.

The method of immunization of rabbits and the technique of the precipitin and inhibition tests were the same as described in previous studies. Test antigens were prepared by combining the glycosides to the protein of chicken serum in order to avoid protein cross reactions. For the sake of brevity, immunizing antigens

will be referred to in the tables as glucose-globulin, glucuronic acid-globulin etc., whereas the test antigens are referred to as glucose-chick, glucuronic acid-chick, etc.

Specific Precipitin and Inhibition Tests.—The sera of rabbits immunized with azoprotein antigens prepared from the diazonium derivatives of the *p*-aminobenzyl glycosides of glucose, galactose, glucuronic and galacturonic acids were first tested for the presence of homologous

TABLE I

Precipitins in Sera of Rabbits Immunized with Glucose, Galactose, Glucuronic Acid, and Galacturonic Acid Antigens

Antiserum prepared by immunization with	Test antigen used	Final dilution of test antigen		
		1:10,000	1:20,000	1:40,000
Glucose-globulin	Glucose- chick	+++	+++	++±
	Galactose "	±	0	0
	Glucuronic acid-chick	0	0	0
	Galacturonic " "	0	0	0
Galactose-globulin	Glucose- chick	±	0	0
	Galactose "	+++±	+++	++±
	Glucuronic acid-chick	0	0	0
	Galacturonic " "	0	0	0
Glucuronic acid-globulin	Glucose- chick	±	±	0
	Galactose "	0	0	0
	Glucuronic acid-chick	+++	+++	++
	Galacturonic " "	±	±	0
Galacturonic acid-globulin	Glucose- chick	±	0	0
	Galactose "	0	0	0
	Glucuronic acid-chick	±	±	0
	Galacturonic " "	++±	+++	+++

and heterologous precipitins. The results of these tests are summarized in Table I, in which it is seen that each antigen gives rise to antibodies which are distinct and specific. The antisera in each instance show little or no serological crossing. The specificity of these serological reactions is further emphasized by the specific inhibition tests, the results of which are given in Table II. It is evident that the precipitin reactions are inhibited only by the homologous, and not by any of the heterologous glycosides.

In the first paper of this series it was shown that the specificity of conjugated carbohydrate-protein antigens containing glucose and galactose is determined by differences in the spatial configuration of a single asymmetric carbon atom in the carbohydrate radical (6). The

TABLE II

Inhibition of Precipitin Reactions of Glucuronic Acid, Galacturonic Acid, Glucose, and Galactose Antigens in Homologous Antisera

Antiserum prepared by immunization with	0.9% NaCl	M/15 *p*-aminobenzyl glycoside of				Test antigen used (1:5000)	Result
		Glucuronic acid*	Galacturonic acid*	Glucose	Galactose		
0.2 cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*0.5 cc.*	
Glucuronic acid-globulin	0.3					Glucuronic acid-chick	+++
		0.3					0
			0.3				+++
				0.3			+++
					0.3		+++
Galacturonic acid-globulin	0.3					Galacturonic acid-chick	+++
		0.3					+++
			0.3				0
				0.3			+++
					0.3		+++
Glucose-globulin	0.3					Glucose-chick	+++
		0.3					++±
			0.3				++±
				0.3			0
					0.3		+++
Galactose-globulin	0.3					Galactose-chick	++++
		0.3					++++
			0.3				++++
				0.3			++++
					0.3		0

* Used as sodium salt.

stereochemical pattern of the asymmetric carbon atoms of glucose and glucuronic acid is identical, yet antigens prepared from the aminobenzyl glycosides of these two hexoses give rise in rabbits to antibodies which show no serological crossing. Differences in the immunological properties of these two antigens must be attributed therefore to differ-

ences in polarity of the grouping occupying the 6th position in each carbohydrate radical. Thus a new and important factor should be taken into consideration in understanding the specificity of carbohydrates, namely the polarity of groupings within the molecule. The results given in Tables I and II show that the glucuronic and galacturonic acid antigens do not cross react appreciably and that in no instance do heterologous glycosides inhibit the homologous reactions. It may be concluded that antigens containing stereoisomeric uronic

TABLE III

Precipitin Reactions of Glucuronic and Galacturonic Acid Antigens in Antipneumococcus Horse Sera Types I, II, III, and VIII

Antipneumococcus horse serum Type	Test antigen used	Final dilution of test antigen				
		1:10,000	1:50,000	1:250,000	1:500,000	1:1,000,000
I	Galacturonic acid-chick	+++	++±	+±	+	±
	Glucuronic " "	±	±	0	0	0
II	Galacturonic " "	±	0	0	0	0
	Glucuronic " "	+++	+++	+±	±	0
III	Galacturonic " "	++±	+±	+	±	0
	Glucuronic " "	++++	+++	++	+	±
VIII	Galacturonic " "	++	+	0	0	0
	Glucuronic " "	++±	++±	++	±	±
Normal horse serum	Galacturonic " "	0				
	Glucuronic " "	0				

acids display a specificity as sharply defined as that exhibited by simple hexoside antigens. It should be emphasized, furthermore, that the immune response of rabbits is directed toward the carbohydrate radical as a whole, and not toward any individual grouping in particular.

Precipitin Reactions of Glucuronic and Galacturonic Acid Antigens in Antipneumococcus Horse Sera Types I, II, III, and VIII.—In previous work it has been demonstrated that the capsular polysaccharides of Types III and VIII pneumococci are constituted from molecules of

glucose and glucuronic acid (7). Azoprotein antigens containing glucuronic acid react in high dilutions with antipneumococcus horse sera Types II, III, and VIII, whereas the corresponding glucose antigen shows little or no serological activity (1). Since it is now known that the capsular polysaccharide of Type I Pneumococcus contains galacturonic acid (8), one might expect the artificial galacturonic acid antigen to precipitate in antipneumococcus horse serum Type I. That this is the case is seen from the results given in Table III. The galacturonic acid antigen precipitates, however, in Types III and VIII serum as well. This reaction might be explained by assuming that Types III and VIII pneumococcus contain a galacturonic acid constituent which gives rise to antibodies reactive with the

TABLE IV

Precipitin Reactions of Glucuronic and Galacturonic Acid Antigens in Antipneumococcus Horse Serum Type III before and after Absorption with the Homologous Capsular Polysaccharide

Antipneumococcus horse serum Type III	Test antigen used	Final dilution of test antigen		
		1:5000	1:20,000	1:80,000
Unabsorbed	Glucuronic acid-chick	++++	+++	++±
	Galacturonic " "	++	++	+±
Absorbed with SSS III	Glucuronic " "	0	0	0
	Galacturonic " "	0	0	0

artificial antigen. When antiserum is first absorbed with homologous capsular polysaccharide, however, the absorbed serum fails to react with the galacturonic acid antigen, as shown in Table IV. It is obvious, therefore, that the reaction of the galacturonic acid antigen in antipneumococcus horse serum Type III (and probably in Type VIII as well) is one which takes place between the antigen and the type specific carbohydrate immune body.

Since the results of the present study demonstrate that antigens containing isomeric uronic acids give rise in rabbits to specific antibodies, it is difficult to understand the reaction of galacturonic acid antigen with immune bodies elicited by immunization of horses with organisms containing glucuronic acid in the encapsulating poly-

saccharide. It is believed, however, that the precipitation of galacturonic acid antigen in antipneumococcus horse sera Types III and VIII is of a non-specific nature. Evidence for this hypothesis is provided by the results of the specific inhibition tests given in Table V. The reaction of glucuronic acid antigen in Types II, III, and VIII antipneumococcus horse sera is inhibited only by the glucuronide, whereas

TABLE V

Inhibition of Precipitin Reactions of Glucuronic and Galacturonic Acid Antigens in Antipneumococcus Horse Sera Types I, II, III, and VIII

Antipneumococcus serum Type	0.9% NaCl	M/15 *p*-aminobenzyl glycoside of		Test antigens (1:10,000		Result
		Glucuronic acid	Galacturonic acid	Glucuronic acid-chick	Galacturonic acid-chick	
0.2 cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	
I	0.3				0.5	+++
		0.3			0.5	+++
			0.3		0.5	0
II	0.3			0.5		+++
		0.3		0.5		0
			0.3	0.5		+++
III	0.3			0.5		+++±
		0.3		0.5		0
			0.3	0.5		+++±
	0.3				0.5	++±
		0.3			0.5	0
			0.3		0.5	0
VIII	0.3			0.5		+++
		0.3		0.5		0
			0.3	0.5		+++
	0.3				0.5	++
		0.3			0.5	0
			0.3		0.5	0

the precipitation of galacturonic acid antigen is inhibited by either uronide. On the other hand, the galacturonic acid antigen, which contains the uronic acid found in the Type I polysaccharide, precipitates in Type I antipneumococcus serum even in the presence of the glucuronide. The precipitation is of course inhibited by homologous galacturonide.

TABLE VI

Precipitin Reactions of p-Aminobenzene Sulfonic and Carboxylic Acid Antigens in Antipneumococcus Horse Sera Types I, II, III, and VIII

Antipneumococcus horse serum Type	Test antigen used	Final dilution of test antigen						
		1:1000	1:2500	1:5000	1:10,000	1:20,000	1:40,000	1:80,000
I	Carboxylic acid-chick*	+++	++	±	0	0	0	0
	Sulfonic " "	+++	++±	++	+±	0	0	0
II	Carboxylic " "	0	0	0	0	0	0	0
	Sulfonic " "	+	+±	0	0	0	0	0
III	Carboxylic " "	+++±	+++±	+++±	+++±	++±	+±	+
	Sulfonic " "	+++	++++	+++±	++±	++	±	0
VIII	Carboxylic " "	+++±	+++±	++±	+±	±	0	0
	Sulfonic " "	+++	++++	+++	++±	++	+	±
Normal horse serum	Carboxylic " "	0	0	0	0	0	0	0
	Sulfonic " "	0	0	0	0	0	0	0

* Test antigens prepared by combining diazotized *p*-aminobenzene sulfonic and carboxylic acids with chicken serum.

Evidence has been presented indicating that the active acidic groups of bacterial carbohydrates and the free amino groups of antibody are involved in the specific precipitation of homologous immune protein (9). Similarly, the precipitation of glucuronic and galacturonic acid

TABLE VII

*Inhibition of Precipitin Reactions of Acid Azoprotein Antigens in Antipneumococcus Horse Serum Type III**

Test antigen used (1:5000)	0.9% NaCl	0.2 molar inhibiting substance				Result
		p-Amino-benzyl glucuronide	*p*-Amino-benzyl galactu-ronide	*p*-Amino-benzene sulfonic acid	*p*-Amino-benzene carboxylic acid	
0.5 cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	
Glucuronic acid-chick	0.3					++++
		0.3				0
			0.3			+++
				0.3		++++
					0.3	++++
Galacturonic acid-chick	0.3					+++
		0.3				0
			0.3			0
				0.3		±
					0.3	0
Sulfonic acid-chick	0.3					+++±
		0.3				0
			0.3			0
				0.3		0
					0.3	0
Carboxylic acid-chick	0.3					+++
		0.3				0
			0.3			0
				0.3		±
					0.3	0

* 0.2 cc. of antipneumococcus horse serum Type III used in each test. All inhibiting substances used as neutral sodium salts.

antigens in antipneumococcus horse sera might be attributed to a reaction between the acidic groups of the antigen and the basic groups of the antibody protein molecule. Artificial azoprotein antigens containing organic acid radicals, quite unrelated in chemical constitu-

tion to the uronic acids, might likewise be expected to precipitate in antipneumococcus horse sera.

Antigens prepared from *p*-aminobenzene carboxylic and sulfonic acids, though not reactive in normal horse serum, precipitate in antipneumococcus sera, as seen from results of precipitin tests given in Table VI. The precipitation of these antigens in antipneumococcus serum Type III is inhibited indiscriminately by the sodium salt of any one of the uncombined acidic derivatives, as the results of the specific inhibition tests in Table VII show. The reaction of glucuronic acid antigen in the immune horse serum is inhibited, however, only by the glucuronide and not by any of the heterologous acid derivatives. The precipitation of glucuronic acid antigen in Type III antipneumococcus

TABLE VIII

Precipitin Reactions of p-Aminobenzene Sulfonic and Carboxylic Acid Antigens in Antipneumococcus Horse Serum Type III before and after Absorption with the Homologous Capsular Polysaccharide

Antipneumococcus horse serum Type III	Test antigen used	Final dilution of test antigen	
		1:5000	1:10,000
Unabsorbed	*p*-Aminocarboxylic acid-chick	++++±	+++
	p-Aminosulfonic " "	++++±	+++
Absorbed with SSS III	*p*-Aminocarboxylic " "	0	0
	p-Aminosulfonic " "	0	0

horse serum may be regarded therefore as approaching more closely the homologous reaction. The reaction of all these acid antigens in antipneumococcus horse serum Type III (and probably in antisera of other types as well) represents a precipitation of antigen and immune protein, for precipitation does not occur in normal horse serum, nor in immune serum from which the type specific antibody has been removed by absorption. The results of these tests are given in Table VIII.

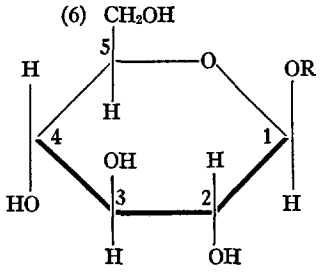
The azoprotein antigens containing the galacturonide, or the aromatic acid radicals, possess but one property in common, namely acidic groups of divergent nature. It does not appear illogical to assume, therefore, that the reaction of these antigens in antipneumococcus horse sera represents a neutralization of the charge of basic groups

of the antibody protein by the acid groups of the hapten, followed by precipitation.

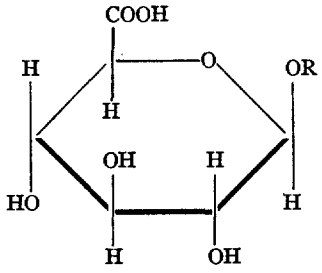
In conclusion it should be pointed out that none of the acid azoprotein antigens precipitate in antipneumococcus rabbit sera. The reason for this is not understood. It is apparent, however, that certain fundamental differences exist between the antibodies of the horse and the rabbit (10). The great affinity of acid antigens for the antibodies of immune horse sera and their failure to precipitate in the corresponding rabbit antisera represent another striking difference between the immune protein of these two animal species.

DISCUSSION

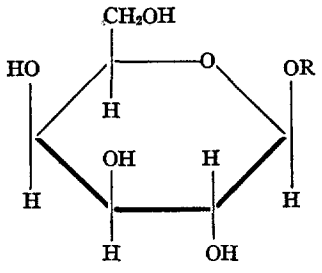
In view of our present knowledge concerning the constitution of hexoses (11) and hexose uronic acids (12), it may be stated with fair certainty that the *p*-aminobenzyl β-glycosides of glucose, galactose, glucuronic acid, and galacturonic acid are pyranoside derivatives. The configuration of these substances can therefore be represented by the following formulae in which R signifies the *p*-aminobenzyl group—$CH_2C_6H_4NH_2$.



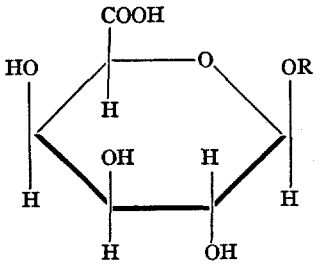
p-Aminobenzyl β-glucoside



p-Aminobenzyl β-glucuronide



p-Aminobenzyl β-galactoside



p-Aminobenzyl β-galacturonide

As indicated earlier in this communication, both the glucoside and glucuronide have an identical configuration and differ only in the grouping occupying the 6th position; this relationship is true also of the galactoside and galacturonide. The glucoside and glucuronide differ structurally from the galactoside and galacturonide, however, in the spatial configuration of the H and OH groups occupying the 4th carbon atom. In the first communication of this series (6) it was shown that in antigens containing simple hexosides a rotation of the H and OH groups through an angle of 180° on carbon atom 4 of the glycoside radical sufficed to confer a distinct and individual specificity upon each. That this is true of the corresponding uronides is now evident from the results of the foregoing serological analysis. This fact is important, for it undoubtedly finds application in understanding the specificity of the naturally occurring bacterial polysaccharides, which in certain instances contain glucuronic acid, and in others galacturonic acid. In a previous study on the specificity of artificial antigens containing glucose and glucuronic acid (1) it was found that a change in polarity of the terminal grouping of a simple carbohydrate suffices to confer a distinct specificity upon each. This fact is further substantiated by the results of the present communication in which it is seen that antigens containing galactose and galacturonic acid give rise likewise to antibodies which are specific and show no serological crossing.

That antigens containing glucuronic and galacturonic acids, each containing a polar carboxyl group, give rise in rabbits to specific antibodies, emphasizes anew the fact that the spatial relationship of the lesser polar hydroxyl (OH) groups suffices to determine the serological specificity of carbohydrates. It should be emphasized, however, that in the rabbit the specificity of the antibody to which these artificial carbohydrate-protein antigens give rise is determined in all instances by the glycoside radical as a whole and not by any particular grouping within the carbohydrate molecule. It appears at first difficult to understand the precipitation of simple uronic acid antigens in antipneumococcus horse sera and their failure to precipitate in the corresponding immune rabbit sera. It must be remembered, however, that in these instances we are dealing with the sera of different animal species, the

immune protein of which may differ considerably in chemical properties. Furthermore we know as yet but little of the structure of the pneumococcus polysaccharides in which certain of the hydroxyl groups are known to be in chemical combination, whereas others may be masked by steric hindrance effects not obtaining in the simple monosaccharides. It appears, however, that the antibodies to which the polysaccharides give rise, in horses at least, are directed toward the molecule as a whole, and toward the uronic acid nucleus as well.

Antigens containing benzene carboxylic and sulfonic acid radicals, quite unrelated in chemical constitution to the pneumococcus polysaccharides, precipitate vigorously in antipneumococcus horse sera Types I, III, and VIII. These reactions are in each instance inhibited non-specifically by the glycosides of uronic acids and by the sodium salts of the uncombined benzene sulfonic and carboxylic acids themselves. It is evident therefore that a portion of the anticarbohydrate globulin of pneumococcus horse serum is capable of reacting with antigens containing acid groups of widely divergent nature. The chemical properties of the immune globulin must differ from those of the globulin of normal horse serum, for precipitation of these acid antigens does not occur in normal serum. The reactive groups of the immune protein with which these azoproteins combine, are believed to be basic groups which may unite not only with the acidic groups of the homologous polysaccharide, but which can combine non-specifically with the acidic groups of the azoproteins in question. This concept is supported by the results of experiments now being carried out, in which it has been found that partial acetylation of the amino groups of Type III pneumococcus antibody deprives the latter of the ability to precipitate these same acid-containing azoproteins.

In conclusion, it should be said that the highly selective immunological specificity of bacterial polysaccharides can be understood only when a more complete picture of their chemical constitution has been gained.

SUMMARY

1. Azoprotein antigens containing glucuronic and galacturonic acids give rise in rabbits to specific antibodies. The immune sera show no serological crossing with antigens containing glucose or galactose.

2. The galacturonic acid antigen reacts in antipneumococcus horse serum Type I in high dilutions.

3. Azoprotein antigens containing galacturonic acid, benzene sulfonic and carboxylic acids precipitate in antipneumococcus horse sera of various types but not in normal horse serum. The mechanism underlying these cross reactions is discussed.

BIBLIOGRAPHY

1. Goebel, W. F., *J. Exp. Med.*, 1936, **64**, 29.
2. Fischer, E., and Armstrong, E. F., *Ber. chem. Ges.*, 1902, **35**, 833.
3. Isbell, H. S., *Bureau Standards J. Research*, 1930, **5**, 1179.
4. Vorhees, V., and Adams, R., *J. Am. Chem. Soc.*, 1922, **40**, 1397.
5. Morell, S., and Link, K. P., *J. Biol. Chem.*, 1935, **110**, 719.
6. Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1929, **50**, 533.
7. Goebel, W. F., *J. Biol. Chem.*, 1935, **110**, 391.
8. Heidelberger, M., Kendall, F. E., and Scherp, H., *J. Exp. Med.*, 1936, **64**, 559.
9. Chow, B. F., and Goebel, W. F., *J. Exp. Med.*, 1935, **62**, 179.
10. Horsfall, F. L., Jr., and Goodner, K., *J. Exp. Med.*, 1935, **62**, 485.
11. Haworth, W. N., The constitution of sugars, London, Edward Arnold & Co., 1929.
12. Hotchkiss, R. D., and Goebel, W. F., *J. Biol. Chem.*, 1936, **115**, 285.