CHEMO-IMMUNOLOGICAL STUDIES ON CONJUGATED CARBOHYDRATE-PROTEINS

III. Active and Passive Anaphylaxis with Synthetic Sugar-Proteins

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In the two preceding papers of this series Avery and Goebel (1, 2) have reported the results of chemo-immunological studies on conjugated carbohydrate-proteins.

In the first of these communications Goebel and Avery (1) described the chemical methods by means of which the p-aminophenol glucosides were synthesized from glucose and galactose and the diazotized substances bound to protein. The two newly synthesized sugar-proteins differ from one another chemically only in specific rotation and molecular configuration. In the second paper, Avery and Goebel (2) reported the antigenic properties and serological specificity of the conjugated carbohydrates. They found that the glucosidic radical of the compound antigen endowed the new complex with specific reactivity. This was demonstrated in two ways. First, when the *same* glucoside is attached to two *different* proteins, as serum globulin or egg albumin, the serum prepared by immunization with either one of the antigens is specifically reactive with the other. Second, when the two *different* glucosides,—glucoside and galactoside—are each combined with the *same* protein, the newly formed compounds are serologically distinct. These facts were found to be true even though the individual glucosides are isomers differing only in the spatial configuration of a single carbon atom.

On the other hand the uncombined glucosides alone were found to be non-antigenic. They failed to induce antibody formation in the animal body and caused no visible precipitation when added to immune sera *in vitro*. However, both glucosides possess the capacity of specifically inhibiting the precipitating action of homologous antisugar-protein serum. When galactoside was mixed with serum prepared by immunization with galacto-globulin,[1] the subsequent addition

[1] The terms employed in this paper to represent the sugar-protein compounds are the same as those used by Avery and Goebel (2).

of galacto-albumin to the mixture did not cause precipitation. Furthermore, when the heterologous glucoside was substituted in the same system, no inhibition of the precipitin reaction occurred. These results demonstrate the specificity of the inhibition phenomenon. Avery and Goebel (2) considered that the uncombined glucosides possess the immunological properties of haptens.

The rôle of carbohydrate in anaphylaxis has been a subject of recent experimental investigation.

Tomcsik (3) working with B. lactis aerogenes, and later Tomcsik and Kurotchkin (4) employing B. lactis aerogenes, the pneumobacillus, and a yeast, isolated carbohydrate substances which produced anaphylactic shock in guinea pigs passively sensitized with homologous immune serum. Lancefield (5) also obtained from streptococci carbohydrate material with which anaphylaxis could be induced in guinea pigs passively sensitized with anti-streptococcus serum. Because of the presence of small amounts of nitrogen in the products, none of these authors felt justified in concluding that the carbohydrate alone was responsible for the shock. Avery and Tillett (6) employing the highly purified polysaccharide of the type-specific pneumococci showed that guinea pigs passively sensitized with homologous anti-pneumococcus rabbit serum were thrown into anaphylactic shock by the subsequent injection of the homologous specific carbohydrate. Guinea pigs could not, however, be actively sensitized with the purified polysaccharides alone. Since the materials used in those experiments were protein-free, and in the case of the Type II and Type III substances also nitrogen free, the results conclusively demonstrate the capacity of complex sugars to induce anaphylactic shock in animals passively sensitized with antibacterial sera.

The immunological specificity of pneumococcus polysaccharides has a close analogue in the serological specificity exhibited by gluco-protein and galacto-protein. The immunologic behavior of the synthesized sugar-proteins led to their use in sensitization experiments. The results, reported in this paper, on anaphylaxis with artificially prepared carbohydrate-proteins confirm and extend the serological findings previously reported. The production of both active and passive anaphylaxis was attempted in order to determine the sensitizing properties of the synthetic antigens and to demonstrate the specificity of the reactions.

Gluco-globulin represents phenol β-glucoside-azo-globulin.
Gluco-albumin represents phenol β-glucoside-azo-albumin.
Galacto-globulin represents phenol β-galactoside-azo-globulin.
Galacto-albumin represents phenol β-galactoside-azo-albumin.
The globulin was prepared from horse serum, and the albumin from egg white.

EXPERIMENTAL

Guinea pigs weighing 240 to 275 grams were employed. The sensitizing dose, whether serum or sugar-protein, was always injected intraperitoneally. The shocking dose was uniformly injected intravenously into a superficial vein of the hind leg.

For details concerning the chemical procedures involved in the synthesis of the materials, the reader is referred to the article by Goebel and Avery (1).

The serological characteristics of the serum employed and the method of preparation are described by Avery and Goebel (2).

Passive Sensitization

A. Results obtained with sugar-protein compounds

Eight guinea pigs were injected intraperitoneally with the pooled serum of three rabbits immunized with gluco-globulin. Five guinea pigs each received 5 cc. of serum, one received 3 cc., one received 1 cc., and one 0.5 cc. As previously mentioned, serum of this character possesses the capacity to precipitate complex antigens composed of heterologous protein conjugated with the homologous glucoside. The antigluco-globulin sera used in these experiments had an average titre of specific precipitins, as determined by tests made with gluco-egg-albumin, of 1 to 80,000. By reason of the fact that horse globulin alone when used as antigen does not elicit antibodies reactive with egg-albumin, the high precipitin titre of these sera is obviously dependent upon the conjugated glucoside radical.

Twenty-four hours after the administration of gluco-globulin antiserum, each pig received intravenously 1 cc. of gluco-albumin.

From Table I it can be seen that the five pigs sensitized with 5 cc. of serum all died with typical symptoms of anaphylactic shock.

The animals passively sensitized with 3 cc. and 1 cc., respectively, of antigluco-globulin serum had definite and typical symptoms immediately following the injection of 1 cc. of gluco-albumin but recovered. Pig No. 6, which received 0.5 cc. of serum, exhibited only a slight reaction.

A similar experiment was carried out using antigalacto-globulin serum for sensitization and galacto-albumin as the toxigenic antigen.

Sera obtained from three rabbits immunized with galacto-globulin were pooled; the precipitin titre, as determined with galacto-albumin, averaged 1 to 80,000. Eight guinea pigs were injected intraperitoneally as follows: 5 animals received 5 cc. of serum each, one received 3 cc., one received 1 cc., and one, 0.5 cc. The results given in Table II are equally as definite as those shown in Table I. All

TABLE I

Passive Anaphylaxis with Anti-Gluco-Globulin Serum

Reactions induced by the use of the homologous glucoside conjugated with a heterologous protein—Gluco-Albumin

Guinea pig No.	Anti-gluco-globulin serum i.p.	Interval between injection of serum and shock dose	Shocking dose i.v.	Symptoms	Result
	cc.	*hrs.*	*gluco-egg-albumin*		
1	5	24	1 cc.	Typical	†3½ minutes
2	5	24	1 cc.	Typical	†3½ minutes
3	5	24	1 cc.	Typical	†2½ minutes
4	3	24	1 cc.	Marked scratching, bucking, coughing, respiratory distress	Definite symptoms followed by recovery
5	1	24	1 cc.	Violent typical symptoms	Definite symptoms followed by recovery
6	0.5	24	1 cc.	Occasional scratching	No reaction
			galacto-egg-albumin		
7	5	24	1 cc.	None	No reaction
			gluco-egg-albumin		
Same animal 4 hrs. later	—	—	1 cc.	Typical	†4 minutes
			galacto-egg-albumin		
8	5	24	1 cc.	None	No reaction
			gluco-egg-albumin		
Same animal 4 hrs. later	—	—	1 cc.	Typical	†4½ minutes

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

pigs sensitized with homologous immune serum in amounts from 1 to 5 cc. reacted typically and fatally to the intravenous injection of 1 cc. of galacto-albumin; 0.5 cc. of serum was insufficient to sensitize.

TABLE II

Passive Anaphylaxis with Anti-Galacto-Globulin Serum

Reactions induced by the use of the homologous galactoside combined with a heterologous protein—Galacto-Albumin

Guinea pig No.	Anti-galacto-globulin serum i.p.	Interval between injection of serum and shock dose	Shocking dose i.v.	Symptoms	Result
	cc.	*hrs.*	*galacto-egg-albumin*		
1	5	24	1 cc.	Typical	†3 minutes
2	5	24	1 cc.	Typical	†8 minutes
3	5	24	1 cc.	Typical	†3½ minutes
4	3	24	1 cc.	Typical	†3 minutes
5	1	24	1 cc.	Typical	†3½ minutes
6	0.5	24	1 cc.	Slight scratching and coughing	Very mild reaction with recovery
			gluco-egg-albumin		
7	5	24	1 cc.	None	No reaction
			galacto-egg-albumin		
Same animal 1 hr. later	—	—	1 cc.	Typical	†3½ minutes
			gluco-egg-albumin		
8	5	24	1 cc.	None	No reaction
			galacto-egg-albumin		
Same animal 1 hr. later	—	—	1 cc.	Typical	†3 minutes

†Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

In Tables I and II it is also shown that the reactions in guinea pigs induced with sugar-proteins and anti-sera are strictly specific.

Animals No. 7 and No. 8 of Table I received antigluco-globulin serum and 24 hours later were injected intravenously with galacto-albumin. No reaction occurred. Four hours later, the introduction of gluco-albumin induced typical fatal shock. Similarly pigs No. 7 and No. 8 of Table II, sensitized with anti-galacto-globulin serum, were unharmed by gluco-albumin; the subsequent administration of the homologous galacto-albumin antigen caused anaphylactic death of these animals. The animals also serve to demonstrate the fact that gluco-albumin and galacto-albumin are not primarily toxic.

B. Results obtained with uncombined glucosides

The specific inhibitory effect exerted by the glucosides on the precipitin reaction of sugar-protein and anti-sera has been described in detail by Avery and Goebel (2) and has been previously commented upon in this paper. It, therefore, seemed of interest to determine whether anaphylactic shock could be elicited by glucosides alone in passively sensitized guinea pigs, and if not, whether the inhibition which these substances have on the precipitin test would also be evident in the anaphylactic reaction.

As shown in Table III, two pigs (Nos. 1 and 2), sensitized 24 hours previously with antigluco-globulin serum, were injected intravenously with 1 cc. of the uncombined homologous glucoside. No reaction occurred. Two hours later 1 cc. of gluco-albumin injected into the same animal caused prompt anaphylactic death.

In guinea pigs Nos. 3, 4, and 5, the introduction of glucoside alone was followed immediately by an injection of gluco-albumin. Except for slight scratching, no response was elicited.

From these results it may be seen that the injection of glucoside into a sensitized animal exerts a definite but transitory protection against the shocking capacity of material which otherwise would be fatal. That the protective action of the glucoside is specific is demonstrated by guinea pigs Nos. 6, 7, and 8. These animals were injected with the heterologous galactoside; when, immediately thereafter they were given gluco-albumin no protection resulted and they died promptly with typical anaphylactic shock.

Table IV presents the results obtained with guinea pigs, which, after having been sensitized with antigalacto-globulin serum, were protected by the galactoside from the toxigenic effect of galacto-albumin.

TABLE III

Passive Anaphylaxis with Anti-Gluco-Globulin Serum

Effect of Uncombined Glucoside

Guinea Pig No.	Anti-gluco-globulin serum i.p.	Interval between injection of serum and anaphylactic test	Injection of glucoside i.v.	Result	Interval between injection of glucoside and sugar-protein	Injection of sugar-protein i.v.	Symptoms	Result
	cc.	*hrs.*	*glucoside*		*hrs.*	*gluco-egg-albumin*		
1	5	24	1 cc.	No reaction	2	1 cc.	Typical	†4 minutes.
2	5	24	1 cc.	No reaction	2	1 cc.	Typical	†4 minutes
3	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Scratches slightly, no other symptoms	No reaction
4	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Scratches slightly, no other symptoms	No reaction
5	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Scratches slightly, no other symptoms	No reaction
			galactoside					
6	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Typical	†7½ minutes
7	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Typical	†3½ minutes
8	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Severe symptoms, falls on side. Apnoea; on feet	Severe shock followed by recovery

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

TABLE IV

Passive Anaphylaxis with Anti-Galacto-Globulin Serum

Effect of Uncombined Galactoside

Guinea Pig No.	Anti-galacto-globulin serum i.p.	Interval between injection of serum and anaphylactic test	Injection of glucoside i.v.	Result	Interval between injection of glucoside and sugar-protein	Injection of sugar-protein i.v.	Symptoms	Result
	cc.	*hrs.*	*galactoside*		*hrs.*	*galacto-egg-albumin*		
1	5	24	1 cc.	No reaction	2	1 cc.	Marked scratching, coughing, backing, respiratory distress	Definite symptoms followed by recovery
2	5	24	1 cc.	No reaction	2½	1 cc.	Typical	†4 minutes
3	5	24	1 cc.	No reaction	2½	1 cc.	Typical	†3½ minutes
4	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	None	No reaction
5	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	None	No reaction
6	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	None	No reaction
			glucoside					
7	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Typical	†2½ minutes
8	5	24	1 cc.	No reaction	Followed immediately by	1 cc.	Typical	†3 minutes

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

TABLE V

Desensitization Induced in Passively Sensitized Guinea Pigs by Injection of Glucoside and Homologous Sugar-Protein

Guinea Pig No.	Serum used for passive sensitization i.p.	Previous tests. Injections made 24 hrs. after serum i.v.	Result	Interval between previous tests and desensitization tests	Material injected i.v.	Symptoms	Result
				hrs.			
1	5 cc. anti-gluco-globulin serum	1 cc. glucoside followed immediately by 1 cc. gluco-egg-albumin	No reaction	4½ [a]	1 cc. gluco-egg-albumin	None	No reaction
2	5 cc. anti-gluco-globulin serum	1 cc. glucoside followed immediately by 1 cc. gluco-egg-albumin	No reaction	4½	1 cc. gluco-egg-albumin	None	No reaction
3	5 cc. anti-gluco-globulin serum	1 cc. glucoside followed immediately by 1 cc. gluco-egg-albumin	No reaction	4½	1 cc. gluco-egg-albumin	None	No reaction
4	5 cc. anti-galacto-globulin serum	1 cc. galactoside followed immediately by 1 cc. galacto-egg-albumin	No reaction	1	1 cc. galacto-egg-albumin	None	No reaction
5	5 cc. anti-galacto-globulin serum	1 cc. galactoside followed immediately by 1 cc. galacto-egg-albumin	No reaction	2	1 cc. galacto-egg-albumin	None	No reaction
6	5 cc. anti-galacto-globulin serum	1 cc. galactoside followed immediately by 1 cc. galacto-egg-albumin	No reaction	3½	1 cc. galacto-egg-albumin	None	No reaction

i.p. = intraperitoneal.
i.v. = intravenous.

The results are, in every respect, identical with those given in Table III both with regard to the transitory nature of the phenomenon and to its specificity.

The mechanism of the protection afforded by the glucosides is not as yet understood. The fact that the protective effect is no longer demonstrable after two hours indicates that "desensitization,"—if such has occurred—is transitory. Instances of what appears to be true desensitization have been observed in these experiments and are recorded in Table V.

Guinea pigs Nos. 1, 2, and 3, passively sensitized with antigluco-globulin serum, were protected from the shocking effect of 1 cc. of gluco-albumin by a previous injection of homologous glucoside. Four and one-half hours later the same pigs received a second injection of 1 cc. of gluco-albumin. No reaction occurred. The absence of shock following the second injection of whole antigen seems to be dependent upon the first dose of gluco-albumin. That the glucoside alone plays no direct part in the refractory state is demonstrated by its ineffectiveness when injected singly, two hours prior to the shocking dose (Table III). Pigs Nos. 4, 5, and 6 of Table V demonstrate the same principle, the difference being that antigalacto-globulin serum was used for sensitization, and galactoside and galacto-albumin were employed to complete the test.

C. Results obtained with uncombined protein

Avery and Goebel (2) have shown that the serum of rabbits immunized with synthetic sugar-proteins (gluco- or galacto-globulin) possesses two distinct antibodies; 1) the specific precipitin so intimately associated with the carbohydrate radical of the compound; 2) the "common" precipitin, reactive with globulin alone. Passive anaphylaxis experiments were, therefore, carried out, using pure horse globulin as the toxigenic material.

For sensitization, one pig received intraperitoneally 1 cc. of serum prepared by immunization with gluco-globulin; a second pig received 1 cc. of serum derived from a rabbit immunized with galacto-globulin. Twenty-four hours later each received 12 mgms. of globulin. Both animals died in typical anaphylactic shock (Table VI). A normal pig receiving the same dose of globulin gave no reaction.

TABLE VI

Passive Anaphylaxis with Anti-Gluco-Globulin and Anti-Galacto-Globulin Sera

Reactions induced by the use of horse globulin

Guinea pig No.	Sensitizing serum i.p.	Time interval	Shocking dose i.v.	Symptoms	Result
		hrs.			
1	1 cc. anti-gluco-globulin serum	24	1 cc. horse-globulin (12 mgms.)	Typical	†3 minutes
2	1 cc. anti-galacto-globulin serum	24	1 cc. horse-globulin	Typical	†2½ minutes
3	Normal control	—	1 cc. horse-globulin	None	No reaction

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

Active Anaphylaxis

For purposes of testing the capacity of the synthetic sugar-proteins to produce active sensitization, 10 guinea pigs were injected intraperitoneally with 5 cc. of gluco-globulin and 10 other animals were similarly inoculated with 5 cc. of galacto-globulin.

In the preparations employed, 5 cc. of sugar-globulin contained 50 mgms. of protein. It is estimated (1) that 15 per cent by weight of this complex represents chemically combined glucoside. Consequently each pig received approximately 7.5 mgms. of the synthesized sugar-protein. The 20 pigs were tested 21 days later for active sensitization. As in the experiments on passive anaphylaxis, the sensitizing dose consisted of material in which the glucoside was joined to a protein heterologous to that used for sensitization. As previously mentioned, this precaution was taken in order to eliminate the possibility of protein-antiprotein reactions entering into the results.

In Table VII, the results of active sensitization obtained by the use of gluco-proteins are given.

Pigs Nos. 1, and 2, previously sensitized with gluco-globulin, were injected intravenously with 1 cc. of gluco-egg-albumin. Each promptly succumbed with typical symptoms. Pigs Nos. 3 and 4 of Table VII demonstrate the specificity of the sensitization; both of the animals when tested with 1 cc. of galacto-albumin showed no reaction. However, when, 3 hours later, 1 cc. of gluco-albumin was introduced, they reacted fatally. Pigs Nos. 5, 6, 7, and 8 were used to determine the influence of homologous uncombined glucoside on the reaction. In these tests the same relations were found to exist as described in the experiments on passive

TABLE VII

Active Anaphylaxis in Guinea Pigs Sensitized with Gluco-Globulin

Guinea Pig No.	Sensitizing dose i.p.	Interval between sensitizing injection and test	1st Injection		Interval between 1st and 2nd injection	2nd Injection		Interval between 2nd and 3rd injection	3rd Injection	
			Material i.v.	Result		Material i.v.	Result		Material i.v.	Result
	gluco-globulin	*days*	*gluco-egg-albumin*	*min.*	*hrs.*		*min.*	*hrs.*		*min.*
1	5 cc.	21	1 cc.	†5	—	—	—	—	—	—
2	5 cc.	21	1 cc.	†3½	—	—	—	—	—	—
			galacto-egg-albumin			*gluco-egg-albumin*				
3	5 cc.	21	1 cc.	No reaction	3	1 cc.	†3	—	—	—
4	5 cc.	21	1 cc.	No reaction	3	1 cc.	Severe symptoms. Recovery	—	—	—
			glucoside							
5	5 cc.	21	1 cc.	No reaction	1½	1 cc.	†3	—	—	—
6	5 cc.	21	1 cc.	No reaction	2	1 cc.	†2½	—	—	—
7	5 cc.	21	1 cc.	No reaction	2	1 cc.	†4½	—	—	—
8	5 cc.	21	1 cc.	No reaction	Followed immediately	1 cc.	No reaction	—	—	—
			galactoside			*galacto-egg-albumin*			*gluco-egg-albumin*	
9	5 cc.	21	1 cc.	No reaction	4	1 cc.	No reaction	½	1 cc.	†4½

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

TABLE VIII

Active Anaphylaxis in Guinea Pigs Sensitized with Galacto-Globulin

Guinea Pig No.	Sensitizing dose i.p.	Interval between sensitizing injection and test	1st Injection		Interval between 1st and 2nd injection	2nd Injection		Interval between 2nd and 3rd injection	3rd Injection	
			Material i.v.	Result		Material i.v.	Result		Material i.v.	Result
	galacto-globulin	*days*	*galacto-egg-albumin*	*min.*	*hrs.*		*min.*	*hrs.*		*min.*
1	5 cc.	21	1 cc.	†3½	—	—	—	—	—	—
2	5 cc.	21	1 cc.	†4	—	—	—	—	—	—
			gluco-egg-albumin			*galacto-egg-albumin*				
3	5 cc.	21	1 cc.	No reaction	2½	1 cc.	†4	—	—	—
4	5 cc.	21	1 cc.	No reaction	2	1 cc.	†3			
			galactoside							
5	5 cc.	21	1 cc.	No reaction	1½	1 cc.	†4½			
6	5 cc.	21	1 cc.	No reaction	3	1 cc.	†3			
7	5 cc.	21	1 cc.	No reaction	3	1 cc.	†2			
8	5 cc.	21	1 cc.	No reaction	Followed immediately by	1 cc.	Very slight symptoms. Recovery			
			glucoside			*gluco-egg-albumin*			*galacto-egg-albumin*	
9	5 cc.	21	1 cc.	No reaction	4	1 cc.	No reaction	1	1 cc.	†5
10	5 cc.	21	1 cc.	No reaction	4	1 cc.	No reaction	1	1 cc.	†3½

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

anaphylaxis. When glucoside was injected immediately before gluco-albumin, complete inhibition of anaphylaxis resulted. However, when glucoside was injected one and one-half to two hours before the shocking dose (Pigs Nos. 5, 6, and 7), no protection occurred. Pig No. 9 of Table VII is further evidence of the specificity of active sensitization; in this animal attempts to inhibit shock with heterologous galactoside and to desensitize with galacto-albumin were ineffectual since the subsequent injection of gluco-albumin produced characteristic death.

TABLE IX

Active Anaphylaxis in Guinea Pigs Sensitized with Gluco-Globulin and Galacto-Globulin

Reactions induced by the use of horse globulin—the protein common to both antigens

Guinea pig No.	Sensitizing serum i.p.	Time interval	Shocking dose i.v.	Symptoms	Result
		days			
1	5 cc. gluco-globulin	21	1 cc. horse-globulin (18 mgms.)	Typical	†3½ minutes
2	5 cc. gluco-globulin	21	0.5 cc. horse-globulin (9 mgms.)	Typical	†14 minutes
3	5 cc. galacto-globulin	21	1 cc. horse-globulin (18 mgms.)	Typical	†3 minutes
4	5 cc. galacto-globulin	21	0.5 cc. horse-globulin (9 mgms.)	Typical	†7½ minutes
5	Normal control	—	1 cc. horse-globulin (18 mgms.)	None	No reaction

† Death of animal.
i.p. = intraperitoneal.
i.v. = intravenous.

In Table VIII, a similar group of experiments was carried out employing galacto-globulin for sensitization instead of gluco-globulin. Galacto-albumin was the toxigenic agent; galactoside was injected for inhibition tests. Results comparable in every respect to those recorded in Table VII were obtained. Consequently a detailed description need not be given.

Table IX presents the results obtained in guinea pigs actively sensitized with gluco-globulin (animals Nos. 1 and 2) or with galacto-globulin (Nos. 3 and 4) and subsequently injected with horse globulin.

All the animals gave typical reactions. Active sensitivity in these pigs was in all probability induced by the uncombined globulin present in the sensitizing material.

DISCUSSION

The experiments reported in this paper demonstrate the capacity of artificially prepared sugar-proteins to produce both active and passive anaphylaxis. The tests were devised and carried out in such a manner as to emphasize the significance of the carbohydrate radical. The fact that guinea pigs, passively sensitized with antigluco-globulin serum, or actively sensitized with gluco-globulin, can be subsequently shocked with gluco-albumin, demonstrates that the antigen-antibody specificity in these instances is directly dependent upon the carbohydrate fraction of the antigenic compounds.

The introduction of the sugar radical into the protein molecule endows the new complex with a sharply defined specific antigenicity. This fact is brought out by experiments in which galactoside was substituted for glucoside in the preparation of sugar-proteins used for sensitization. The same specific relations hold in the production of anaphylaxis with galacto-proteins as that described for gluco-proteins. Attempts to incite anaphylactic shock with heterologous material were ineffectual. The results of the anaphylactic experiments conform to the results anticipated by the serological findings of Avery and Goebel (2).

Landsteiner (7), employing complex antigens, has reported experiments on anaphylaxis of a similar character to those presented in this report. He found that guinea pigs sensitized with one azoprotein could be shocked by the injection of a second compound containing the same azo-groups attached to a different protein.

In addition to the new specificity which the carbohydrate radical confers upon the conjugated proteins, the uncombined glucosides by themselves also exert a definite influence on the reactivity of sensitized animals. When sensitized pigs are injected with the homologous glucoside immediately before the introduction of the toxigenic sugar-protein, they are completely protected from shock. However, the protection afforded by the glucoside alone is apparently only transitory; for, when the interval between introduction of glucoside and

shocking agent was as long as two hours, the injection of homologous sugar-protein produced prompt and typical anaphylactic death. That the temporary protection just mentioned is specific, was demonstrated by the experiments in which uncombined carbohydrate of heterologous type was shown to exert no such protective action.

The transitory, specific protection afforded by the glucosides alone is not yet understood. Landsteiner (7) found in experiments on anaphylaxis with azoproteins that the azo-component, when injected one hour before the conjugated azoprotein, inhibited shock. He considered that a state of anti-anaphylaxis had been induced. In the tests with glucosides and sugar-proteins, sufficient evidence has not been obtained to interpret the mechanism other than to say that the inhibitory effect of the glucosides disappears in at least two hours.

Active and passive anaphylaxis has also been elicited with uncombined globulin. Whether animals were passively sensitized with anti-gluco-globulin or antigalacto-globulin serum, the toxigenic action of globulin was equally effective. Since the sera employed contained anti-globulin antibodies, these results were to be expected. Guinea pigs actively sensitized with either gluco-globulin or galacto-globulin, were found to be equally sensitive to uncombined globulin. Since the material used to produce active sensitization contained free globulin, the subsequent intoxication with horse globulin is obviously based on a simple protein- anti-protein reaction.

CONCLUSIONS

1. Guinea pigs passively sensitized with the serum of rabbits immunized with an artificially prepared sugar-protein (gluco-globulin) exhibit typical anaphylactic shock when subsequently inoculated with gluco-albumin; the serum of rabbits immunized with a second synthetic sugar-protein (galacto-globulin) similarly sensitizes guinea pigs to galacto-albumin. The reactions, in each instance, are specific and depend for their specificity on the carbohydrate component, and not on the protein fraction of the synthesized sugar-protein.

2. Guinea pigs actively sensitized with gluco-globulin or galacto-globulin are similarly subject to anaphylactic shock, when injected, after 21 days, with sugar-proteins containing carbohydrate identical with that present in the sensitizing antigen, regardless of the kind of protein with which it is combined.

3. The unconjugated glucosides, although themselves not capable of inducing shock, inhibit the anaphylactic reaction when injected immediately prior to the introduction of the toxigenic sugar-protein. The protective action of the glucosides disappears within two hours after injection. In order to elicit the phenomenon, the carbohydrate must be the same as that combined in the sugar-protein complex.

4. Anaphylactic shock may be induced by uncombined globulin in guinea pigs passively sensitized with either antigluco-globulin serum or antigalacto-globulin serum; globulin is similarly effective in animals actively sensitized with gluco-globulin or galacto-globulin. The reactions elicited by globulin alone are dependent upon the common protein present in the antigens, and exhibit only species specificity.

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