THE ANTIGENIC COMPLEX OF STREPTOCOCCUS HÆMOLYTICUS.

V. Anaphylaxis with the Type-Specific Substance.

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The anaphylactic reactions caused by the group-reactive nucleoprotein P, and by the species-specific substance, C, obtained from extracts of hemolytic streptococcus have been shown in the preceding paper to parallel their precipitin reactions (1). Thus equivalent amounts of nucleoproteins derived from different types of hemolytic streptococci caused acute anaphylactic death in guinea pigs passively sensitized with anti-P serum prepared with nucleoprotein from any of these types. Moreover, nucleoproteins from related species of bacteria, *Streptococcus viridans* and pneumococcus, also caused anaphylactic death if injected into similarly sensitized guinea pigs in sufficiently large doses. This partial relationship had already been observed in precipitation and absorption experiments (2). The nucleoproteins were also capable of sensitizing guinea pigs in active anaphylactic experiments. The other non-type-specific fraction, the species-specific C substance, which seemed to be non-protein and probably carbohydrate, was also effective in producing acute anaphylactic death in passively sensitized guinea pigs. Only those antibacterial sera which showed a high titer of C antibodies in the precipitin test, however, were under the conditions of the experiments capable of sensitizing guinea pigs passively to shock with the C substance. As in the precipitin reaction, so also in the anaphylactic reaction, the C substance showed no type specificity but gave cross-reactions with sera prepared against all types of hemolytic streptococci. While the chemical data were not sufficient to prove conclusively that this substance was a carbohydrate, still they indicated that this was the case. They were substantiated by the failure of trypsin or of pepsin to affect

either the precipitin titer or the minimal anaphylactic dose of C. These two non-type-specific substances extracted from hemolytic streptococci differed both chemically and in their failure to cause reciprocal precipitin or anaphylactic reactions with their respective antisera.

The present experiments are concerned with the anaphylactic reactions of the type-specific protein, M, and with certain cross-reactions observed with some of the extracts containing M which may be due to a third non-type-specific fraction, Y.

Methods.

The preparation of extracts and of antisera has been described, and the technique of the anaphylactic test is the same as that employed with the non-type-specific fractions (1).

The protein fraction, M, which gave type-specific precipitin reactions, was tested for type-specific anaphylactic reactions as shown in Experiment 1.

Experiment 1.—Guinea pigs, sensitized with antibacterial serum, were tested with intravenous injections of homologous and of heterologous HCl extracts which contained the type-specific M. Surviving guinea pigs were reinjected on the following day with homologous M. The M.A.D. for each extract was determined, and doses were recorded as approximate multiples of the M.A.D. with a definite homologous serum. Control unsensitized guinea pigs were never shocked by these extracts. Table I shows some of the type-specific reactions.

Guinea pigs sensitized with eight different antibacterial sera showed only slight reactions when tested with intravenous injections of 2 to 50 M.A.D. of heterologous M, although the controls, similarly sensitized, died in acute anaphylactic shock when given 1 or 2 M.A.D. of homologous M. The animals surviving heterologous M, either succumbed to injection of homologous M on the following day or suffered a severe but sublethal shock. The increase in amount of homologous M required to produce death following shock with heterologous M was probably due to the presence in these extracts of traces of non-type-specific substances as impurities. Such an experiment was evidence of type-specific anaphylactic shock with the M fraction. A micro Kjeldahl nitrogen determination made on the most active lot

of HCl extract from Strain S43 showed that the M.A.D. for guinea pigs passively sensitized with homologous Serum R322 was 0.2 mg. of protein.

Active sensitization with HCl extracts was attempted; but, although doses and intervals between injections were varied considerably, no positive results were obtained. In view of the difficulty of finding the right conditions for sensitization with P, however, these negative results were not considered conclusive.

Occasional sera were encountered which gave non-type-specific anaphylactic, as well as precipitin, reactions with relatively purified HCl extracts from Strain S43. Since it was obvious that the type-specific M was not completely isolated but that traces of the other reactive fractions were almost certainly present, it was possible that these results were due to reactions of the latter with their respective antibodies which are usually present in antibacterial sera. Experiment 2 was performed to test this hypothesis.

Experiment 2.—Five guinea pigs, I-1 to I-5, sensitized with Type S23 antibacterial sera received intravenous injections of HCl extract from a strain of heterologous type, as shown in Table II. All these animals died in typical anaphylactic shock. In order to determine whether this cross-reaction was due to traces of the common nucleoprotein P, Guinea Pigs I-6 to I-12 were sensitized with a highly potent anti-P serum and were given intravenous injections of the extract used in the first part of the experiment. Such slight symptoms resulted that P was eliminated as the cause of the cross-reactions in I-1 to I-5. The extract was next tested for the presence of the other known commonly reactive substance, C, by injecting it into guinea pigs sensitized with an antibacterial serum of high potency for C antibody. Since none of these animals showed more than very slight symptoms, the common C substance was also eliminated as the cause of the cross-reactions.

All surviving guinea pigs (I-6 to I–16) were tested again, usually on the following day, with suitable homologous extracts. Typical anaphylactic death resulted, except in the cases of I-6 and I-7 which suffered + + + and + + shock respectively, thus showing that the animals had been effectively sensitized.

Table II shows the cross-reactions of occasional *Type* S23 antibacterial sera with certain HCl extracts from another type. The possible explanation that traces of the common substances, P and C, were responsible for these non-type-specific reactions was eliminated in each instance by testing the extract for the presence of P and of C

TABLE I.

Anti-M Reactions.

Type-Specific Anaphylactic Shock with Antibacterial Sera and HCl Extracts.

Guinea pig	Sensitized with serum*		Shocked by intravenous injections				
			Test No.	Days after sensitization	HCl extract from strain**	Dose M.A.D.‡	Result
	No.	Cc.					
H-1	R323	1.0	1	6	S43 (hom.)	20.0	†4.5 min.
H-2	"	1.0	1	6	S39 (het.)	2.0	±
			2	7	S43 (hom.)	1.0	†4 min.
H-3	R321	0.5	1	1	S43 (hom.)	1.0	†4.5 min.
H-4	"§	0.5	1	1	S39 (het.)	5.0	++
			2	3	" "	10.0	±
			3	4	S43 (hom.)	2.0	†4 min.
H-5	Q866	0.5	1	2	S43 (hom.)	2.0	†4 min.
H-6	"	0.5	1	2	S39 (het.)	2.5	±
			2	3	S43 (hom.)	2.0	++
H-7	Q864	0.5	1	2	S43 (hom.)	2.0	†3 min.
H-8	"	0.5	1	2	S39 (het.)	2.5	±
			2	3	S43 (hom.)	4.0	++
H-9	R322	0.5	1	2	S43 (hom.)	2.0	†4.5 min.
H-10	"	0.5	1	2	S39 (het.)	2.5	±?
			2	3	S43 (hom.)	6.0	+++
H-11	R324	0.5	1	2	S43 (hom.)	2.0	†4 min.
H-12	"	0.5	1	2	S39 (het.)	2.5	±?
			2	3	S43 (hom.)	6.0	†4 min.
H-13	Q613	1.0	1	1	S39 (hom.)	1.0	†7 min.
H-14	"	1.0	1	1	S43 (het.)	50.0	++
			2	3	S39 (hom.)	2.0	†3.5 min.
H-15	Q317	1.0	1	2	S39 (hom.)	1.0	++++
			2	3	" "	2.5	+++
H-16	"	1.0	1	2	S43 (het.)	2.0	±
			2	3	S39 (hom.)	2.5	+++

by injecting it into guinea pigs sensitized with sera of known high potency for P and for C antibodies. It was shown previously in the reciprocal experiment[1] that "purified" C did not shock guinea pigs sensitized with one of the sera which gave cross-reactions in the present experiment (Serum R264 used for I-5). This is further evidence that C is not the cause of the cross-reactions in the present instance. Since this hypothesis proved, therefore, untenable, it was necessary to assume either (1) that still another non-type-specific substance was present in the antigenic complex of the hemolytic streptococcus, or else (2) that the M substance was not strictly type-specific but that it might be nearly enough related to M from a different type to cross occasionally with an antibody which is *chiefly* specific for the latter. Considerable evidence has been accumulated indicating that the second hypothesis is incorrect. A comparison of the M.A.D. of different lots of extracts in terms of animals sensitized with homologous type serum and of others sensitized with heterologous type

[1] See Paper IV of this series, Table VII, G-4 and G-5.

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* Sera R321, R322, R323, and R324 were against Strain S43, *Type* S60.
" Q864 and Q866 " " " S60, " "
" Q613 " Q317 " " " S23, " S23.

** Strain S43 represents *Type* S60.
" S39 " " S23.

‡ M.A.D. of S43 extract in terms of Serum R321.
" " S39 " " " " " R261 or R264.

§ 6 other guinea pigs sensitized with this serum and tested with varying combinations of these 2 antigens reacted in the same way.

In all tables the following symbols are employed:

−	indicates	no shock.
±?	"	trace of shock.
±	"	slight shock.
+	"	mild shock.
++	"	moderate shock.
+++	"	moderately severe shock.
++++	"	severe shock.
+++++	"	very severe shock.
†	"	animal died.
hom.	=	homologous.
het.	=	heterologous.
M.A.D.	=	minimal anaphylactic dose.

TABLE II.

Anti-Y Reactions.

Non-type-Specific Anaphylactic Shock with Certain Antibacterial Sera and HCl Extracts.

Guinea pig	Sensitized with serum No.*	Shocked by intravenous injections of HCl extract from Strain S43, *Type* S60			
		Days after sensitization	M.A.D. in terms of serum against homologous *Type* S60	M.A.D. in terms of serum against heterologous *Type* S23	Result
Antibacterial sera giving cross-reactions with HCl extracts from a strain of another type					
I-1	Q308	2	2	2	†4 min.
I-2	"	4	20	1.3	†4 "
I-3	R261	6	2	2	†4.5 "
I-4	"	1	2	2	†3 "
I-5	R264	2	2	2	†6 "
Pure Anti-P sera					
I-6	R500	6	2.5	2.5	+
I-7	"	3	10	10	±?
I-8	"	1	10	10	+
I-9	"	1	10	10	+
I-10	"	1	2	2	+
I-11	R594	1	2	2	±?
I-12	"	3	10	10	−
Antibacterial serum containing C antibody					
I-13	R446	1	1.3	1	+
I-14	"	2	15	1	+?
I-15	"	1	1	1−	±
I-16	"	2	?**	3	±

* Guinea Pigs I-1, -2, -3, -5, -6, -11, -12 were sensitized with 1 cc. of serum; all others with 0.5 cc.

R500 was an anti-P serum against P from Strain S39, *Type* S23.

R594 " " " " " " " " S43, " S60.

All others were antibacterial sera:

R446 was against Strain S3, *Type* S3

Q308 " " " S23, " S23.

R261 " " " S39, " "

R264 " " " " " "

** HCl extract from Strain S39, *Type* S23, given to this animal.

sera shows wide variation. For example, with the extract used to test Guinea Pig I-1, the M.A.D. in terms of *Type* S23 and *Type* S60 sera was the same, while with an extract from the same strain prepared at another time (used for I-2) the M.A.D. in terms of heterologous *Type* S23 serum was fifteen times the M.A.D. in terms of homologous *Type* S60 serum. This difference in degree of heterologous activity of different lots of extract seems significant in indicating that M itself is not responsible for the cross-reaction, but rather that some non-type-specific substance, also extracted at times by HCl, is the cause of these atypical reactions.

The following experiment shows the effect of tryptic digestion on the anaphylactic reactions of this so called Y substance as well as its effect on the type-specific M.

Experiment 3.—A concentrated HCl extract from Strain S43 was digested with 0.5 per cent trypsin for 50 minutes with the technique previously described.[2] Controls with heated trypsin were included. Guinea Pig O-1, sensitized with serum prepared against Strain S43, served as control and died in acute anaphylactic shock on injection of 1 M.A.D. of the undigested extract (containing heated trypsin). Guinea Pig O-2, on the contrary, sensitized in the same way, was unaffected by the same dose of digested extract, although it was proved sensitive by injection on the next day of the same amount of untreated extract which resulted in typical anaphylactic death.

The same solution, known to give cross-reactions characteristic of the non-type-specific Y, was tested in Guinea Pigs O-3 and O-4, sensitized with serum prepared against a heterologous type strain. The control, O-3, died of anaphylactic shock when given 1 M.A.D. of the undigested extract, while O-4 suffered no shock from the same amount of digested extract. On the following day, however, O-4 was killed by 1 M.A.D. of untreated extract, thus proving that it was sensitive. Table III shows these results.

The results of this experiment were clear. Tryptic digestion destroyed the type-specific M contained in S43 HCl extract, as shown by the failure of the digested material to shock O-2, which had been sensitized with the homologous serum. The animal was sensitive, for it was killed by subsequent injection with untreated extract. The control animal, O-1, which received the undigested (heated trypsin) extract, died in 3 minutes. Similar results were obtained when the

[2] Experiment 3 described in Paper II of this series.

digested and the undigested S43 HCl extracts were tested in guinea pigs sensitized with heterologous Serum R264: the digested extract did not affect O-4 (although subsequent injection with untreated extract caused acute anaphylactic death), while the undigested control extract was still effective in shocking Guinea Pig O-3. Like all

TABLE III.

Tryptic Digestion of HCl Extracts Containing M and Y; Effect on Anaphylactic Shock.

Guinea pig	Sensitized with serum No.*	Shocked by intravenous injections of 1 to 1.5 M.A.D.					
		Test No.	Days after sensitization	HCl extract		Result	Probable chief cause of reaction
				from strain:	treated with:		
To show tryptic digestion of M							
O-1	R322	1	1	S43 (hom.)	Inactive trypsin	†3 min.	M
O-2	"	1	1	" "	Active trypsin	—	—
		2	2	" "	—	†4 min.	M
To show tryptic digestion of Y							
O-3	R264	1	1	S43 (het.)	Inactive trypsin	†3.5 min.	Y
O-4	"	1	1	" "	Active trypsin	—	—
		2	2	" "	—	†14 min.	Y

* Guinea pigs sensitized with 0.5 cc. of serum.

Serum R322 was an antibacterial serum from a rabbit immunized with Strain S43, *Type* S60; Serum R264 was an antibacterial serum from a rabbit immunized with Strain S39, *Type* S23

the other anaphylactic tests, this corroborated the previous experiments, the results of which were tested only by the precipitin reaction, and was further evidence that these two fractions were proteins. It did not, however, serve to show that this extract really contained two substances. The following absorption experiment differentiated them in such a way as to remove the doubt as to the existence of Y.

Experiment 4.—Three guinea pigs, sensitized with untreated serum from Rabbit R261, were used as controls on the activity of the extracts: J-1 died when tested with HCl extract from a heterologous strain, while J-2 and J-3 also died when tested with HCl extract from the homologous strain. Three other guinea pigs, J-4 to J-6, were sensitized with the same serum which had been absorbed by the technique previously described (2) with a strain (S24) known to be heterol-

TABLE IV.

Antibacterial Serum, R261, Rendered Type-Specific in Its Anaphylactic Reactions by Absorption with Heterologous Bacteria, Strain S24.

Guinea pig	Sensitized with Serum R261*		Shocked by intravenous injections				
	Cc.	Preliminary treatment of serum	Test No.	Days after sensitization	HCl extract from strain	Dose M.A.D. **	Result
J-1	0.5	None (control)	1	1	S43 (het.)	2	†3 min.
J-2	0.5	" " ‡	1	1	S39 (hom.)	1–?	†60 "
J-3	0.5	" " §	1	1	" "	1	†5 "
J-4	0.9	Absorbed	1	1	S39 (hom.)	1–?	+++
J-5	0.9	"	1	1	S43 (het.)	2	–
			2	2	S39 (hom.)	2–	†3.5 min.
J-6	0.45	"	1	1	S43 (het.)	10	–
			2	2	S39 (hom.)	2+	†5.5 hrs.

* Serum R261 was from a rabbit immunized with Strain S39, *Type* S23.
** All M.A.D. in terms of Serum R261.
‡ J-2 controls J-4 and J-5.
§ J-3 " J-6.

ogous to all the other strains used in this experiment. J-4, tested with the homologous HCl extract, suffered severe shock but recovered since too small a test dose was used; J-5 and J-6 were first tested with the same heterologous extract which had killed J-1, but they were unaffected by it. The next day, however, they succumbed to homologous HCl extract, thus showing that they had been sensitized. All necessary controls were negative. Table IV summarizes the results.

The serum used in this experiment sensitized animals to shock with heterologous HCl extracts, but after it was absorbed with entirely

heterologous bacteria, it induced only type-specific anaphylactic reactions. Guinea Pigs J-5 and J-6, sensitized with absorbed serum, withstood respectively 2 and 10 M.A.D. of heterologous HCl extract. Subsequent injection on the following day with the homologous extract resulted in acute anaphylactic death in the case of J-5 and in delayed death in the case of J-6, which, however, had been sensitized with only half as much absorbed serum as the other animals. The absorption method, therefore, eliminated the confusing cross-reactions and brought out type-specific anaphylactic as well as precipitin reactions, thus showing that M and Y must be different substances.

Tables V to VIII, inclusive, show how much was accomplished towards demonstrating type-specific anaphylactic reactions in this serum and in others which crossed with heterologous HCl extracts, by the method of desensitizing guinea pigs with extracts of various kinds and subsequently testing with the homologous extract containing M. This *in vivo* saturation of antibodies was comparable, in part, to the *in vitro* absorption described above. The results are somewhat complicated in this instance, however, by the necessity of using extracts which, although they contained one reactive substance in excess, contained in addition traces of others as impurities. Table V shows desensitization with one of these extracts.

Experiment 5.—Guinea Pigs K-1, K-2, and K-3 were sensitized with antibacterial serum, R261, the same serum used in absorption experiment, No. 4. On the following day at hourly intervals, five subcutaneous injections each of 10 mg. of NaOH extract (chiefly P) from heterologous Strain S3 were given to K-2 and K-3. 1 hour after the last injection, 20 mg. of this heterologous extract, given to K-2 intravenously, caused no symptoms, although 10 mg., given to the sensitized but otherwise untreated control K-1, caused typical anaphylactic death. At the same time, 20 mg. of the homologous NaOH extract (containing M as well as P) caused immediate death when given intravenously to the treated K-3.

This simple experiment showed that a sensitized guinea pig (K-2) was desensitized by subcutaneous injections of P so that it no longer reacted to twice the amount of P required to kill a similarly sensitized animal (K-1) which had not received these subcutaneous injections. Guinea Pig K-3, desensitized in the same way as K-2, was, however, killed by 20 mg. of homologous NaOH extract. Since this always contained the type-specific M, in addition to P, the experiment showed

that desensitization to P was accomplished by subcutaneous injections of P without desensitizing the animal to homologous M.

The effect of such desensitization with NaOH extracts on subsequent shock with HCl extracts was tested in Experiment 6.

TABLE V.

Antibacterial Serum, R261, Rendered Type-Specific in Its Anaphylactic Reactions. Effect of Desensitizing with Heterologous NaOH Extract on Subsequent Shock with Heterologous and with Homologous NaOH Extract.

Guinea pigs sensitized with 0.5 cc. of serum, R261, from a rabbit immunized with Strain S39, *Type* S23.

Guinea pig	Desensitization of guinea pig prior to intravenous injections	Shocked by intravenous injections 1 day after sensitization				
		NaOH extract from strain	Dose	Result	Probable chief cause of reaction	Remarks
			mg.			
K-1	None (control)	S3 (het.)*	10**	†11 min.	P	
K-2	5 subcutaneous injections totaling 50 mg. (5 M.A.D.) of NaOH extract from heterologous strain, S3, 1 day after sensitization	" "	20	±?‡	—	Desensitized to P
K-3	Same as K-2	S39 (hom.)	20	†4 min.	M	Not desensitized to hom. M

* Strain S3 represents *Type* S3.

** 1 M.A.D.

‡ A subsequent test on this animal with homologous M (HCl extract), following a test immaterial to this experiment, resulted in severe shock.

Experiment 6.—Guinea Pigs L-1 to L-4 were sensitized with another Type S23 serum which induced cross-anaphylactic reactions with HCl extracts from a heterologous type. L-1 and L-2 were controls on the activity of the extracts: L-1 succumbed to an intravenous injection of heterologous HCl extract, and L-2 to homologous HCl extract. 2 days after sensitization at hourly intervals L-3 and L-4 were given subcutaneous and intraperitoneal injections of NaOH ex-

tracts from a heterologous strain increasing in dosage from 5 mg. to 16.5 mg. until a total of 50 mg. had been administered. The following day the heterologous HCl

TABLE VI.

Antibacterial Serum, R264, Rendered Type-Specific in Its Anaphylactic Reactions. Effect of Desensitizing with NaOH Extract, from a Strain Heterologous to the Serum and to All Test Extracts, on Subsequent Shock with Heterologous and with Homologous HCl Extracts.

Guinea pigs sensitized with 0.5 cc. of serum, R264, from a rabbit immunized with Strain S39, *Type* S23.

Guinea pig	Desensitization of guinea pig prior to intravenous injections	Shocked by intravenous injections							
		Test No.	Days after sensitization	HCl extract from strain*	Dose M.A.D. in terms of serum No.** R 264	Dose M.A.D. in terms of serum No.** R 321	Result	Probable chief cause of reaction	Remarks
L-1	None (control)	1	2	S43 (het.)	2	2	†6 min.	Y	
L-2	" "	1	1	S39 (hom.)	1	0	†5 "	M	
L-3	5 subcutaneous and intraperitoneal injections, totaling 50 mg. (5 M.A.D.) of NaOH extract from heterologous Strain S3, 2 days after sensitization	1	3	S43 (het.)	2	2	—	—‡	L-3 and L-4 show a certain amount of desensitization to Y but not to hom. M
L-4	Same as L-3	1	3	" "	4	4	+++	Y	
		2	4	S39 (hom.)	5	0	†3.5 min.	M	

* Strain S3 represents *Type* S3.
" S43 " " S60.
" S39 " " S23.

** Serum R264 was against Strain S39, *Type* S23.
" R321 " " " S43, " S60.

‡ Found dead next morning; blood culture sterile.

extract was titrated by giving the desensitized guinea pigs injections of 2 and 4 M.A.D. of this extract. The smaller dose produced no shock in L-3, and the larger dose produced moderately severe, but not fatal, shock in L-4. The latter

animal was killed by 5 M.A.D. of homologous HCl extract given intravenously the next day.

This experiment showed that NaOH extracts contained substances which could desensitize guinea pigs to a certain extent to shock with heterologous HCl extract containing the non-type-specific Y. It seems probable that this is due to the presence of the Y substance in both extracts. A similar experiment was performed with another *Type* S23 serum, as shown in Table VII.

Experiment 7.—This experiment differed from Experiment 6 in the use of *Type* S23 serum prepared against another strain. The first four animals sensitized with this serum served as controls on the activity of the extracts. M-5 was desensitized by subcutaneous and intraperitoneal injections of NaOH extract from an entirely heterologous strain by the same method as L 3 and L-4 in the last experiment. The following day it survived an injection of more than 2 M.A.D. of heterologous HCl extract but was killed on the next day by the homologous HCl extract. M-6 was desensitized by one intravenous injection of 10 mg. of heterologous NaOH extract, a usually fatal dose, which did not kill this animal. The following day it showed very slight symptoms on injection of heterologous HCl extract but died the next day after an injection of homologous HCl extract. See Table VII for details of the experiment.

In this experiment, which is similar to Experiment 6, desensitization with entirely heterologous NaOH extract given subcutaneously and intraperitoneally or intravenously, resulted in some degree of desensitization with respect to the heterologous HCl extract which previously had killed animals sensitized with this serum. These animals, however, were not desensitized to the homologous HCl extract, for both were killed by intravenous injections of homologous HCl extracts, which in the case of M-6 was in a dose comparable in size to the dose of heterologous HCl extract used on the preceding day. Here again the desensitization was probably due to Y contained in both NaOH and HCl extracts.

In the next experiment the animals were desensitized by preliminary injections of heterologous HCl extract instead of NaOH extract and the effect of desensitization tested for both these extracts as well as for the homologous HCl extract.

Experiment 8.—A series of guinea pigs was sensitized with 1 cc. of the same serum used in the last experiment. Two of these animals served as controls on

TABLE VII.

Antibacterial Serum, Q308, Rendered Type-Specific in Its Anaphylactic Reactions. Effect of Desensitizing with NaOH Extract, from a Strain Heterologous to the Serum and to All Test Extracts, on Subsequent Shock with Heterologous and with Homologous HCl Extracts.

Guinea pigs sensitized with 1 cc. of serum, Q308, from a rabbit immunized with Strain S23, *Type* S23.

Guinea pig	Desensitization of guinea pig prior to intravenous injections	Shocked by intravenous injections							
		Test No.	Days after sensitization	Extract from strain*	Kind of extract	Dose M.A.D. in terms of serum No.** Q308	Dose M.A.D. in terms of serum No.** R322	Result	Probable chief cause of reaction
M-1	None (control)	1	2	S43 (het.)	HCl	1	7.5	†4 min.	Y
M-2	" "	1	3	S3 "	NaOH	1±‡	1	†3.5 "	P
M-3	" "	1	2	" "	"	1±	1	†50 "	"
M-4	" "	1	1	S39 (hom.)	HCl	1	0	†4 "	M
M-5	5 subcutaneous and intraperitoneal injections, totaling 150 mg. (15 M.A.D.), of NaOH extract from heterologous Strain S3, 1 day after sensitization	1	2	S43 (het.)	"	2.7	20	—	—
		2	3	S39 (hom.)	"	10	0	†3.5 min.	M
M-6	None (desensitized by intravenous injections)	1	3	S3 (het.)	NaOH	1±‡	1	++	P
		2	4	S43 "	HCl	1.3	10	±	Y
		3	5	S39 (hom.)	"	1.5	0	†30 min.	M

* Strain S3 represents *Type* S3.
" S43 " " S60.
" S39 " " S23.

** Serum Q308 was against Strain S23, *Type* S23.
" R322 " " " S43, " S60.

‡ 10 mg.

the effectiveness of the antigens; N-1 was killed by an intravenous injection of 2 M.A.D. of heterologous S43 HCl extract, and M-4 by 1 M.A.D. of homologous S39 HCl extract.

Guinea Pig N-3 was desensitized 2 days after sensitization by subcutaneous injections of 15 M.A.D. (in terms of both homologous and of heterologous sera) of heterologous S43 HCl extract. The next day this animal gave practically no reaction to an intravenous injection of 4 M.A.D. of the same extract, while it

TABLE VIII.

Antibacterial Serum, Q308, Rendered Type-Specific in Its Anaphylactic Reactions. Effect of Desensitizing with Heterologous HCl Extract on Subsequent Shock with Heterologous NaOH Extract and with Heterologous and Homologous HCl Extracts.

Guinea pigs sensitized with 1 cc. of serum, Q308, from a rabbit immunized with Strain S23, *Type* S23.

Guinea pig	Desensitization of guinea pig prior to intravenous injections	Shocked by intravenous injections							
		Test No.	Days after sensitization	Extract from strain*	Kind of extract	Dose* M.A.D. in terms of serum No. Q308	Dose* M.A.D. in terms of serum No. R322	Result	Probable chief cause of reaction
N-1	None (control)	1	2	S43 (het.)	HCl	2	2	†4 min.	Y
M-4	" "	1	1	S39 (hom.)	"	1	0	†4 "	M
N-3	3 subcutaneous injections, totaling 15 M.A.D. of S43 HCl extract 2 days after sensitization	1	3	S43 (het.)	"	4	4	±	Y
		2	4	S39 (hom.)	"	5	0	†4 min.	M
N-4	None (desensitized by intravenous injections)	1	2	S43 (het.)	"	0.7	5	++	Y
		2	4	" "	"	4.7	35	—	—
		3	5	S3 "	NaOH	2±	2**	—	—
		4	6	S39 (hom.)	HCl	5	0	†22 min.	M

* See foot-notes * and **, Table VII.

** 20 mg. See Guinea Pigs M-2, M-3, and M-6, Table VII, for controls on the M.A.D. of this extract.

died in 4 minutes on the following day after an intravenous injection of 5 M.A.D. of homologous S39 HCl extract.

Guinea Pig N-4 was desensitized by intravenous injections of heterologous S43 HCl extract. 2 days after sensitization, 0.7 M.A.D. of this solution caused ++ shock; 2 days later 4.7 M.A.D. of the same solution produced no effect; and the next day, 2 M.A.D. of NaOH extract from an entirely different strain, S3, caused

no symptoms. On the following day, however, the animal died a typical anaphylactic death, though delayed to 22 minutes, following an intravenous injection of 5 M.A.D. of homologous S39 HCl extract. Table VIII gives these results.

This experiment was the reciprocal of Experiments 6 and 7. In the latter, heterologous NaOH extract was used to desensitize against HCl extract from a heterologous strain of still another type. In Experiment 8 the reverse procedure was employed: the heterologous HCl extract was used to desensitize against the NaOH extract. In both instances, a certain degree of reciprocal desensitization was obtained without as much desensitization for the homologous HCl extract. These sera, with which it had previously been impossible to demonstrate type-specific anaphylactic reactions, were, therefore, rendered type-specific in that the desensitized animals reacted slightly, or not at all, with several multiples of the M.A.D. of heterologous extracts and were killed by similar, or even smaller doses, of homologous extracts.

If no other evidence were available, the cross-desensitization between NaOH extracts and HCl extracts could be explained as due to the presence in both solutions of the non-type-specific substances, C and P, but it has already been shown in Experiment 2 that the cross-reactions of the HCl extracts cannot be due to C or to P. Since the desensitization does not prevent the reaction with the homologous extracts, it seems improbable that it could be accounted for as non-specific reduction of reactivity such as is occasioned by injection of peptones or other foreign proteins. The correct explanation, therefore, seems to be the assumption of this other non-type-specific substance, Y, in extracts of the hemolytic streptococcus.

DISCUSSION.

Type-specific anaphylactic reactions were easily obtained with the protein, M, (HCl extract) when injected into guinea pigs passively sensitized with most of the antibacterial sera used in these experiments. Four sera produced by immunizing rabbits with strains of one type were, however, encountered, which caused non-type-specific anaphylactic death in passively sensitized guinea pigs when HCl extract from a strain of another type was used for the intoxicating injection. In order to obtain type-specific anaphylactic shock in guinea pigs pas-

sively sensitized with these sera, it was necessary either (1) to absorb the serum with heterologous bacteria or (2) to desensitize the passively sensitized guinea pigs with heterologous extracts. Since the HCl extract responsible for these atypical reactions did not cause reactions in guinea pigs sensitized with potent anti-P sera or with sera potent in C antibody and, conversely, since the C substance did not cause reactions with these atypical antibacterial sera, it was necessary to assume that another non-type-specific substance was present in HCl extracts and in the hemolytic streptococcus itself. The additional fact that this so called Y substance was digested by trypsin, while C was not, eliminated the possibility that C and Y were the same substance. Reciprocal desensitization experiments with heterologous NaOH extracts and with the HCl extract which gave the atypical crossing, showed some cross-desensitization for these heterologous extracts though not for homologous, a fact probably explainable as due to the presence of varying mixtures of all the non-type-specific substances in these extracts.

Active sensitization was not accomplished with M although numerous attempts were made under varying conditions, a result which agrees with another negative result, namely, the failure so far to immunize rabbits with this substance. In both instances, however, the failure must be taken with reserve, since it is possible that some change in technique might reverse the result. Active sensitization with Y (as contained in HCl extracts) was not observed.

A certain amount of additional information as to the nature and relationships of some of the reactive substances contained in the hemolytic streptococcus has been obtained by the use of the anaphylactic reaction. It is certain that the type-specific M produced typical anaphylactic shock in passively sensitized guinea pigs. *A priori*, this fact might be considered an indication that the M substance could function as an antigen in inducing antibody production: actually, all immunization experiments with rabbits and active sensitization experiments with guinea pigs yielded negative results. But in view of the additional evidence that C, which seems to be carbohydrate in nature, also shocked passively sensitized guinea pigs and never gave evidence of antibody stimulation, it seems probably that these two substances are haptens which react with antibodies produced by the

intact bacteria, when tested by means of the precipitin reaction and the passive anaphylactic reaction, but that they have no power to elicit antibodies themselves.

The facility with which acute anaphylactic death was produced by injecting bacterial extracts into passively sensitized guinea pigs was striking. Several factors are probably involved. Many investigators have found it necessary to resort to the Dale method with the excised uterus on account of the primary toxicity of the extracts to be tested. Zinsser and Parker (3) found this true in working with extracts of typhoid bacilli, and later Zinsser and Mallory (4) had the same experience with pneumococcus extracts and even with that method found that the margin between the dose which caused reactions in the normal uterus and that which caused it in the sensitive uterus was not so great as in anaphylaxis with egg albumin or with horse serum. With the hemolytic streptococcus, however, such difficulties fortunately were not encountered, and normal guinea pigs did not react to many multiples of the test doses. The fact that relatively large amounts of concentrated extracts were available and that the serological activity, as well as the number and kind of substances present, was capable of titration in most instances contributed to the success of these experiments. An analysis of the antibodies present in the immune sera used for passive sensitization was also made by means of the precipitin test, with the result that sera with known antibody content were used in the anaphylactic experiments and the control of the results was, therefore, increased.

SUMMARY.

1. Type-specific anaphylactic shock was produced with HCl extracts of *Streptococcus hæmolyticus* in guinea pigs passively sensitized with antibacterial sera.

2. With occasional sera and certain HCl extracts, type-specific shock was not produced unless the serum was first absorbed with heterologous bacteria or unless the guinea pigs were desensitized with heterologous extracts before testing with the homologous extract. The findings indicated that this was due to the presence of the non-type-specific substance which has been provisionally designated as Y.

3. Tryptic digestion destroyed the ability of HCl extracts containing

the type-specific M substance and the non-type-specific Y substance to produce anaphylactic shock in passively sensitized guinea pigs.

4. Active sensitization was not accomplished with the type-specific M. It seems probable, therefore, that this substance is a hapten, reacting with antibodies but not stimulating their production after separation from the bacterial cell.

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