PASSIVE ANAPHYLAXIS FOLLOWING THE IMMEDIATE INJECTION OF ANTIGEN AFTER ANTISERUM

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CONTENTS

I. Introduction

In his first paper on passive anaphylaxis Otto (1907) laid stress on the fact that the injection of antiserum must be made some time before the injection of the antigen. "We have come to the conclusion", he says, "that to produce hypersusceptibility a certain distribution of these bodies in the organism and an anchoring of the same to the body cells of the animal must have taken place before the capacity of the latter to react with the normal serum is altered." Doerr & Russ (1909) stated that the injection of antiserum followed after a short interval by the antigen injection produced no symptoms in the guinea-pig. If an interval of 1 hour between the two injections was allowed slight symptoms occurred; an interval of 2 hours resulted in severe symptoms and a 4-hour interval was necessary to produce the "maximum effect", acute shock and death. R. Weil (1912–13) reviewed earlier publications and concluded "that in no single instance in the literature has it been satisfactorily shown that passive sensitization can occur without the lapse of an incubation period". Weil introduced antiserum into one of the jugular veins of nine normal guinea-pigs and immediately afterwards injected antigen into the corresponding veins on the other side. In none of these nine guinea-pigs were anaphylactic symptoms observed, and Weil stated that "for sensitization to occur an interval of time must elapse between the two injections".

On the other hand, Friedemann (1909) had killed guinea-pigs by intravenous injections of mixtures of antiserum and antigen which had been incubated for a short period. After Friedemann's paper was published much

work was done and many papers written on the supposed production of a poisonous substance (sometimes called anaphylatoxin) by the interaction of antigen and antibody.

Gurd (1914) performed a series of experiments "in the hope of proving the possibility of immediate symptoms of intoxication following the introduction of transferred serum and antigenic protein without an intervening period of time", and concluded that his experiments prove "that a period of incubation is not necessary in order that animals may become passively hypersensitive".

Weil (1917) again returned to this question and criticized Gurd's experiments on the grounds of lack of necessary controls, and the possibility that he used antiserum which was itself toxic.

Doerr (1913) and Biedl & Kraus (1910) had suggested that although as a rule it was not possible to produce shock if antigen and antibody were separately and simultaneously injected it might occasionally take place under those conditions, in which event the symptoms were mild. Zinsser (1914) wrote that: "It is extremely difficult to obtain responses in guinea-pigs or rabbits when the interval between the injections of the two is not observed, personally I have had distinct symptoms when injecting antigen and antibody together." Weil dismissed these statements as based on uncontrolled experiments and said that they should always be controlled by the injection of two alien sera not related as antigen and antibody. Omitting his controls he details:

(1) Four experiments in which he gave simultaneous intravenous injections of an antiserum (three, rabbit anti-milk, and one, rabbit anti-ox bile serum) and sheep serum. There were no immediate symptoms; weakness and prostration followed 1–3 hours after injection, and two of the animals died.

(2) Three experiments in which he gave guinea-pigs simultaneous intravenous injections of different normal sera, one had no symptoms, the others prostration, 15 min. and $3\frac{1}{2}$ hours after the experiment respectively, both recovered.

Weil gives no description of any post-mortem appearances, and from the above seven experiments he concluded that "anaphylactiform symptoms and death may be produced in guinea-pigs by the simultaneous separate injection of two foreign sera or proteins not related as antigen and antibody. This result is evidently due to the generalized non-specific effect of foreign proteins on the organism; it explains the exceptional instances of the reaction mentioned under 1 (i.e. anaphylactiform symptoms by the simultaneous but separate injection of antigen and antibody by Gurd, and Thiele & Embleton), which are therefore erroneously attributed to an immunological mechanism." On the strength of nine experiments in which he gave varying combinations of antibody and antigen and failed to obtain a positive result, Weil believed that it was impossible to produce anaphylaxis by the simultaneous but separate injection of antibody and antigen, and that all others who had performed the same type of experiment and obtained a positive result had used toxic sera.

Many of the discrepancies in the early history of this subject are doubtless due to certain difficulties inherent to research of this type. It is generally believed that it is easier to produce anaphylaxis in some guinea-pigs than in others, consequently a factor is introduced which is uncontrollable. Further, the strength of antisera produced by different rabbits varies greatly, and in many papers there is no indication of the strength of sera used; and in some cases rabbits produce antisera which in itself is toxic.

We believe that we are the first to perform experiments on the production of passive anaphylaxis by the immediate injection of antigen after antiserum on anything approaching an adequate scale. We have encountered certain difficulties and untoward results and we have reported them fully.

II. Object of this research

When we planned the series of experiments herein described we were impressed by the statements of those authors who had claimed to have produced passive anaphylaxis without an interval between the injections of antiserum and antigen. We hesitated to conclude that all the authors who had observed signs of passive anaphylaxis without the latent interval were guilty of either experimental or observational errors. We thought it probable that the products of the vigorous reaction, which takes place when a strong precipitating antiserum is mixed with its antigen in a test-tube, would produce symptoms if such antiserum and antigen were allowed to meet in the circulating blood of an animal.

It is well known that both the rate of the reaction and the amount of the resulting precipitate depend on the proportions in which antiserum and antigen are mixed *in vitro*. We thought that any reaction which might result *in vivo* from the simultaneous presence of antiserum and antigen in the blood might produce its best effect if care were taken to inject antiserum and antigen in the proportions favourable to rapid precipitation.

III. Methods

(1) *Estimation of the strength of the sera used*

In all our experiments the substances injected were normal horse serum and its antiserum, the latter being obtained from rabbits which had received numerous injections of horse serum. The antisera were titrated by the optimal proportions method of Dean & Webb (1926, 1928), by which a constant volume of antiserum is titrated against falling volumes of horse serum and the proportions of the two ingredients most favourable to rapid particulation are determined. The optimal proportions for any particular specimen of antiserum may be expressed as the horse serum-antiserum ratio which in the case of a strong serum may be 1 to 6, in the case of a weak serum 1 to 100.

If the ratio figure depends on the antibody content of the serum it is possible to express the ratio figure in terms of units of antibody.

In this notation it is assumed that a unit of antibody is contained in that volume of the antiserum which particulates most rapidly with 0·00001 c.c. of horse serum. If the volume of antiserum which particulated most rapidly with 0·00001 c.c. of horse serum were 1 c.c., such an antiserum would contain 1 unit of antibody per c.c. For example, an antiserum which was found to have a horse serum-antiserum ratio of 1 in 20, that is to say 1 c.c. of horse serum reacted optimally with 20 c.c. of antiserum, would contain 5000 units per c.c. (Dean & Webb, 1928).

The majority of the antisera used in the following experiments have been "strong".

(2) *Details of the injections*

The skin and subcutaneous tissues of the anterior aspect of the neck of a guinea-pig were anaesthetized with 1 c.c. of a 1 in 4 solution of eudrenine. Three or four intracutaneous injections sufficed to produce complete local anaesthesia. A small incision was then made and antiserum was injected into the exposed jugular vein. In the earlier experiments of the series the volume of antiserum injected was 1 c.c., but in the majority of cases 2 c.c. were injected. For the injection of antiserum a syringe of 2 c.c. capacity was employed, and after the antiserum had been injected the syringe was disconnected from the needle which was held *in situ* in the vein. Another syringe previously filled with the diluted horse serum was then fitted to the needle and the horse serum was injected. The needles were of a type manufactured, with a short bevelled point for intradermal injections. They were sharpened immediately before they were used. The interval between the end of the injection of antiserum and the commencement of the injection of horse serum was as a rule from 3 to 5 sec. In eight experiments an interval of 1 min. was allowed between the two injections. After the diluted horse serum had been injected the jugular vein was tied and the small incision closed.

After the injections had been given the animal was placed on the floor of the laboratory and kept under close observation for about 20 min. It was then placed in its cage which was kept in the laboratory for the remainder of the day, so that the state of the animal could be observed from time to time.

IV. EXPERIMENTS IN WHICH THE INJECTION OF ANTIGEN IMMEDIATELY FOLLOWED THAT OF ANTISERUM

The experiments consisted in the injection of antiserum followed by the horse serum after a few seconds' interval. For control purposes, in a series of preliminary experiments, each batch of antiserum was tested by injecting a guinea-pig with 2 c.c. In no case was any sign of illness or shock observed. After more than thirty experiments an antiserum produced an entirely unexpected result. In this experiment 2·5 c.c. of antiserum were injected into the jugular vein as an antiserum control and the guinea-pig died with signs resembling those of acute anaphylactic shock within 5 min. of the injection.

At autopsy the lungs were distended and contained haemorrhages. The animal was in an advanced stage of pregnancy. The typical signs and anatomical changes of acute anaphylactic shock had been produced by the injection of antiserum alone. Weil (1912–13) stated that it was well known that the injection of antiserum might produce symptoms like those of acute anaphylactic shock. After the death of this control animal it became clear to us that the number of antiserum controls should be increased. From that time we performed most of the experiments in duplicate; i.e. of a pair of guinea-pigs, one was injected with antiserum and horse serum, and one with antiserum only. In the case of a few specimens of antiserum the number of animals which were injected with both antiserum and horse serum has exceeded by one or two the number injected with antiserum alone.

We propose to discuss only the results obtained in this latter series of fully controlled experiments. It consists of seventy experiments and sixty-six controls and includes all deaths which occurred in control animals. Of the controls fifty-six received intravenous injections of antiserum, five received normal horse serum and five normal rabbit serum. In no case was any sign of shock or illness observed in the control animals which were injected with either normal horse or normal rabbit serum. Out of fifty-six controls receiving intravenous injections of antiserum eight died; out of seventy experiments where an injection of horse serum followed immediately on the injection of antiserum thirty-eight animals died.

Our experiments following the immediate injection of antigen after antiserum are grouped under two headings: (1) Immediate results and (2) Late results, according to the effect of the injections.

(1) *Immediate results*

(*a*) *Acute anaphylactic death occurring within* 12 *min., usually within* 5 *min., of the termination of the injections*

Within a few seconds of the completion of the injections, the guinea-pigs ceased to run about and began to pant. A little later the hair became ruffled and twitching movements occurred. Suddenly the animal fell on its side and had violent convulsions. Respiratory movements were forced and violent and ultimately consisted of spasmodic, irregular gasps, which became more and more infrequent until the animal died. In many cases urine and faeces were evacuated just before death occurred.

In these cases of acute anaphylactic death the lungs presented the well-known appearance of acute emphysema. In most cases haemorrhages were present in the lungs, heart, diaphragm and occasionally in the intercostal muscles. The behaviour of the animal after the injections, the signs of respiratory embarrassment, the convulsions, sudden death and post-mortem appearances were those which have often been described in connexion with acute anaphylactic shock in the guinea-pig.

(*b*) *Early signs of illness after the injections without death from respiratory embarrassment*

Almost all the remaining guinea-pigs showed some signs of illness, either very mild and transitory, or so severe that death appeared imminent. In the mild cases the animals were still and did not run about the floor of the laboratory as did the majority of the control animals in which no sign of illness was apparent. The hair was ruffled and respiratory embarrassment was evidenced by panting. In more severe cases respiratory movements were more violent and twitching was observed. In a few cases the animal fell over on its side, gasped and almost died.

Of the animals which were ill, but did not die, some, the milder cases, recovered slowly but completely, within 2 or 3 hours; others developed the late signs of shock described below and either recovered more slowly, or died within 24 hours of the injections.

(2) *Late results*

The guinea-pigs which did not recover within 1 or 2 hours of the injections presented the following picture.

The animal lay prone and flaccid on the floor of the cage. The body was cold and the head was dropped forward on the front paws. The abdomen was distended, doughy and apparently tender to the touch. Respiratory movements consisted of short, sharp jerks, curiously reminiscent of the hiccough associated with certain cases of peritonitis in man. The condition was that of shock due to an acute abdominal lesion and resembled the anaphylactic state as seen in the dog (Dean & Webb, 1924).

Fawcett & Ryle (1923) have described a case of anaphylaxis in a man where abdominal symptoms were so marked that an intra-abdominal lesion with haemorrhage was suspected.

The animals with signs of late shock and abdominal distension may be divided into two groups: (*a*) those which died, and (*b*) those which recovered.

(*a*) These animals became gradually weaker and died without the development of any further signs. The interval between the injections and death varied considerably, and in several cases occurred during the night, so the hour of death is not definitely known. The shortest interval between the injections and death was 35 min., but in the majority of the cases, death, when it occurred, took place between the third and the eighth hour. In addition to haemorrhages in the lungs, heart and diaphragm, which we regarded as evidence of the early manifestations of anaphylactic shock, the post-mortem appearances in cases of late death consisted of congestion of the blood vessels of the peritoneum and abdominal viscera. The splanchnic vessels were dilated. In all cases it seemed that there was more than the normal amount of peritoneal fluid, and in well-marked cases 1–3 c.c. of fluid were collected in a pipette. In some cases the fluid was tinged with blood, in others there had been haemor-

rhage into the peritoneal cavity. In a few cases the stomach and small intestine contained relatively large amounts of blood. We looked in every case for evidence of the congestion of the liver which is so striking a feature of anaphylactic shock in the dog. In the majority we were doubtful whether the liver contained more than the normal amount of blood. In a few cases there was definite congestion, and in these the surface showed a characteristic pattern which probably corresponded to the distribution of the lobules. In three or four cases the liver had ruptured and the cracks in the surface were covered by soft and imperfect clots. Usually the blood in the heart and great vessels was fluid or contained imperfect clots. Blood collected at the post-mortem examination clotted very slowly.

(*b*) The cases of late shock, which did not die, recovered slowly and were quite well on the morning following the injections.

Table I

No. of anti-serum	No. of guinea-pig	Weight of guinea-pig g.	Antiserum injected: Volume in c.c.	Antiserum injected: Units	Horse serum injected volume in c.c.	Signs of illness: Early	Signs of illness: Late	Early or late death or survival
1308 I	54	505	2	8,000	0·16	None	.	S.
	55	580	2	8,000	0·16	+ + +	?	S.
	56	620	2	8,000	0·16	None	.	S.
	57*	580	3	12,000	None	Much air injected. Died immediately		
	58	420	3	12,000	0·24	+ + +	?	S.
	59	640	3	12,000	0·24	+ + +	+ + + +	L.
	60*	670	3·5	14,000	None	+ + + +	.	E. 5 min.
1308 J	61	580	2	8,695	0·173	+ + + +	.	E. 9 min.
	62	500	2	8,695	0·173	+ + + +	.	E. 6 min.
	63*	600	2	8,695	None	+ + + +	.	E. 3 min.
	64*	500	2	8,695	None	+ + + +	.	E. 3 min.
1601 C	75	460	2	7,142	0·1428	+ + + +	.	E. 7 min.
	76*	410	2	7,142	None	None	.	S.
	77	450	2	7,142	0·1428	+ + + +	.	E. 11 min.
	78*	420	2	7,142	None	None	.	S.
	79	420	2	7,142	0·1428	+ + + +	+ + + +	L.
	80*	400	2	7,142	None	None	.	S.
	81	405	2	7,142	(?)0·1428	+ +	.	S.
	82	420	2	7,142	0·1428	+ + + +	.	E. 7 min.
	83	430	2	7,142	0·1428	+ + +	+ + +	S.
	84*	400	2	7,142	None	None	.	S.
	85*	400	2	7,142	None	None	.	S.
1058 V	87	400	2	9,090	0·190	+ +	+ + + +	L. 2 hr.
	88	420	3	13,636	0·285	+ + +	+ + + +	L.
	90*	400	3	13,636	None	None	None	S.
1607 C	89	480	2	6,452	0·5	+ + + +	.	S.
	91	350	3	9,678	0·1935	+ + + +	.	E. 5 min.
	92*	350	3	9,678	None	None	.	S.
	93	400	3	9,678	0·1935	+ + +	+ + +	Killed 3½ hr.
	94	420	3	9,678	0·1935	+ + + +	.	S.
1596 C	98	360	2	20,000	0·4	+ + +	+ + + +	L. 4½ hr.
	99*	410	2	20,000	None	None	.	S.
	100	360	2	20,000	0·4	+ + +	+ + + +	L. 6 hr.
	101*	370	2	20,000	None	None	.	S.
	102	370	2	20,000	0·4	+ + +	+ + + +	L. 40 min.
	103*	360	2	20,000	None	None	.	S.
	104	380	3	30,000	0·6	+ + + +	.	E. 4 min.
	105*	400	3	30,000	None	None	.	S.

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Table I (*continued*)

No. of anti-serum	No. of guinea-pig	Weight of guinea-pig g.	Antiserum injected: Volume in c.c.	Antiserum injected: Units	Horse serum injected volume in c.c.	Signs of illness: Early	Signs of illness: Late	Early or late death or survival
596 C	106	390	3	30,000	0·6	+ + +	+ + + +	L. 35 min.
	107	380	3	30,000	0·6	+ + +	+ + + +	L. 6 hr.
396 H	108	400	2	33,333	0·7	+ + + +	+ + + +	L. 30 min.
	109*	365	2	33,333	None	None	.	S.
	110	400	2	33,333	0·7	+ + + +	+ + + +	L. 30 min.
	111*	400	2	33,333	None	None	.	S.
	112	400	3	50,000	1·0	?	+ + + +	L.
	113*	Not weighed	3	50,000	None	+ + + +	.	E. 3 min.
597 C	114	420	2	13,333	0·5$\dot{3}$	+ + +	+ + + +	L. 40 min.
	115*	470	2	13,333	None	None	.	S.
	116	450	2	13,333	0·5$\dot{3}$	None	.	S.
	117*	450	2	13,333	None	None	.	S.
	118	470	2	13,333	0·5$\dot{3}$	+ + + +	.	E. 6 min.
	119*	450	2	13,333	None	None	.	S.
	120	500	2	13,333	0·5$\dot{3}$	+ + +	+ + + +	L. 50 min.
	121*	490	2	13,333	None	None	.	S.
	122	450	2	13,333	0·5$\dot{3}$	+ + +	+ +	S.
	123*	475	2	13,333	None	None	.	S.
	124	490	3	20,000	0·8	+ + +	+ +	S.
	125*	450	3	20,000	None	None	.	S.
	126	400	3	20,000	0·8	+ + +	+ + + +	L. 1 hr.
	127*	400	3	20,000	None	None	.	S.
308 K	128	390	2	4,761	0·095	None	.	S.
	129*	380	2	4,761	None	+ +	.	S.
	130	370	2	4,761	0·095	+ +	.	S.
	131*	360	2	4,761	None	None	.	S.
	132*	355	3	7,143	None	None	.	S.
	133	365	3	7,143	(?) 0·143	None	.	S.
	142	350	3	7,143	0·143	+ + +	.	S.
	143*	360	3	7,143	None	+ + + +	.	E. 5 min.
568 D	134	350	3	8,571	0·171	+ + +	+ + +	S.
	135	350	3	8,571	0·171	+ + +	+ + + +	L. 3 hr.
	136	360	3	8,571	0·171	+ + + +	.	E. 3 min.
	137*	350	3	8,571	None	None	.	S.
	138*	370	3	8,571	None	None	.	S.
	139*	350	3	8,571	None	None	.	S.
	140	350	3	8,571	0·171	+ + + +	.	E. 4 min.
	141	370	3	8,571	0·171	+ + + +	.	E. 6 min.
601 D	144	350	2	5,714	0·114	+ + +	+ + + +	L. 1 hr.
	145*	400	2	5,714	None	None	.	S.
	146	360	2	5,714	0·114	+ +	.	S.
	147*	370	2	5,714	None	None	.	S.
	148	360	2	5,714	0·114	+ +	.	S.
	149*	370	2	5,714	None	None	.	S.
	150	370	2	5,714	0·114	+ +	.	S.
	151*	370	2	5,714	None	Died within 24 hr.; not anaphylaxis		
334 I	152	350	2	5,555	0·111	+ +	.	S.
	152*a**	350	2	5,555	None	None	.	S.
	153	370	2	5,555	0·111	+ + + +	.	E. 3 min.
	154*	340	2	5,555	None	None	.	S.
	155	340	2	5,555	0·111	+ +	+ + + +	L.
	156*	340	2	5,555	None	None	.	S.
	157	355	2	5,555	0·111	+ +	.	S.
	158*	350	2	5,555	None	None	.	S.
	159	340	2	5,555	0·111	+ + + +	.	E. 3 min.
	160*	345	2	5,555	None	None	.	S.
193 L	161	350	2	5,555	0·111	+ +	.	S.
	162*	345	2	5,555	None	None	.	S.

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Table I (*continued*)

No. of anti-serum	No. of guinea-pig	Weight of guinea-pig g.	Antiserum injected: Volume in c.c.	Antiserum injected: Units	Horse serum injected volume in c.c.	Signs of illness: Early	Signs of illness: Late	Early or late death or survival
1193 L	163	350	2	5,555	0·111	+ + + +	.	E. 4 min.
	164*	345	2	5,555	None	None	.	S.
	165	340	2	5,555	0·111	+ +	.	S.
	166*	340	2	5,555	None	None	.	S.
	167	345	2	5,555	0·111	+ +	.	S.
	168*	350	2	5,555	None	None	.	S.
	169	365	2	5,555	0·111	+ + + +	.	E. 3 min.
1607 D	170	350	2	8,000	0·16	+ +	.	S.
	171*	350	2	8,000	None	None	.	S.
	172	340	2	8,000	0·16	+ +	.	S.
	173*	340	2	8,000	None	None	.	S.
	174	360	2	8,000	0·16	+ +	.	S.
	175*	360	2	8,000	None	None	.	S.
	176	330	2	8,000	0·16	+ +	+ + + +	L.
1596 D	177	340	2	8,000	0·16	+ + +	+ + + +	L. 40 min.
	178*	340	2	8,000	None	None	.	S.
	179	330	2	8,000	0·16	+ + +	+ + + +	L. 4½ hr.
	180*	320	2	8,000	None	+ + + +	.	E. 4 min.
	181	335	2	8,000	0·16	+ + +	+ + + +	L. 4½ hr.
	182*	320	2	8,000	None	None	.	S.
	183	350	2	8,000	0·16	+ + + +	+ + +	S.
	184*	350	2	8,000	None	+ + + +	.	E. 3 min.
	188	350	2	8,000	0·16	+ + +	+ + + +	L. 30 min.
	189*	350	2	8,000	None	None	.	S.
	190	355	2	8,000	0·16	+ +	+ + +	S.
	191*	360	2	8,000	None	None	.	S.
1614 B	192	320	2	4,166	0·083	None	.	S.
	193*	320	2	4,166	None	None	.	S.
	194	350	2	4,166	0·083	None	.	S.
	195*	350	2	4,166	None	None	.	S.

Notes on Table I. The number in the left-hand column is the number of the rabbit which provided the antiserum and the letter refers to the bleeding. In the last column but one we have made an attempt to summarize the signs of illness under the subheadings of early and late signs. By early signs we mean evidence of respiratory distress or in more severe cases asphyxia. By late signs we mean shock and collapse associated with abdominal symptoms. Antiserum controls are indicated by an asterisk. In addition five guinea-pigs which were injected with 2 c.c. horse serum only, and five guinea-pigs with normal rabbit serum showed no signs of illness. In the case of guinea-pigs 163, 165, 169, 170, 172, 174 and 176 an interval of 1 min. was allowed between the injection of antiserum and the injection of horse serum. In the case of guinea-pig 167 the interval was 2 min. In every other case the horse serum was given as soon after the antiserum as possible.

(3) *Analysis of the foregoing experiments*

The results in Table I are complicated and obscured by the death of eight control animals which received antiserum injections but no horse serum. Sixteen batches of antiserum were used in the experiments, and with five of these batches deaths occurred on the injection of antiserum only. We can thus make the results more clear by dividing the experiments into two groups:

Group 1. Experiments performed with the antisera of rabbits which at no bleeding produced symptoms or death when injected alone.

Group 2. Experiments performed with the antisera of rabbits which at some bleeding produced death when injected alone.

Table II. *Analysis of results using antisera which did not kill controls. Group* 1

Batch of antiserum used	No. of antiserum controls	No. of consecutive injections of antiserum and horse serum	Results		
			Early deaths	Late deaths	Total deaths
1601 C	5	6	3	1	4
1601 D	4	4	0	1	1
1058 V	1	2	0	2	2
1607 C	1	4	1	0	1
1607 D	3	4	0	1	1
1597 C	7	7	1	3	4
1568 D	3	5	3	1	4
1334 I	5	5	2	1	3
1193 L	4	5	2	0	2
1614 B	2	2	0	0	0
	35	44	12	10	22

Table III. *Analysis of results where deaths occurred among the controls. Group* 2

Batch of antiserum used	No. of antiserum controls	Results			No. of consecutive injections of antiserum and horse serum	Results		
		Early deaths	Late deaths	Total deaths		Early deaths	Late deaths	Total deaths
1308 I	2	2	0	2	5	0	1	1
1308 J	2	2	0	2	2	2	0	2
1308 K	4	1	0	1	4	0	0	0
1396 H	3	1	0	1	3	0	3	3
1396 C	4	0	0	0	6	1	5	6
1596 D	6	2	0	2	6	0	4	4
	21	8	0	8	26	3	13	16

In forty-four immediate injections of horse serum after antiserum, where there were thirty-five controls in which no death occurred, twelve guinea-pigs died of acute anaphylaxis and ten of late anaphylaxis. In twenty-six, where there were eight early deaths among twenty-one controls, three guinea-pigs died of acute anaphylaxis and thirteen of late anaphylaxis. Details of the deaths due to injection of antiserum only will be discussed later.

V. EXPERIMENTS WITH AN INTERVAL OF 24 HOURS BETWEEN THE INJECTIONS MADE ON ANTISERUM CONTROL ANIMALS

Fifty-eight control guinea-pigs which received antiserum only were kept under observation until the morning following the injection. Of these thirty-three were given an injection of horse serum about 24 hours after the antiserum injection. That is to say, the interval, customary in passive anaphylaxis experiments, was allowed. The results are given in Table IV.

All thirty-three animals died with signs of respiratory embarrassment and asphyxia. The lungs were distended and presented the picture which has been described as typical anaphylactic shock in the guinea-pig.

Table IV. *Experiments with an interval of 24 hours between the injection of antiserum and antigen*

No. of guinea-pig	Antiserum injected: Volume in c.c.	Antiserum injected: Units	Horse serum 24 hours later volume in c.c.	Result showing time of death in min.
99	2	2,000	0·4	3
101	2	2,000	0·4	3
103	2	2,000	0·4	3
109	2	33,333	0·4	2
111	2	33,333	0·4	4
115	2	13,333	0·53	4½
117	2	13.333	0·53	4
119	2	13,333	0·53	3
121	2	13,333	0·53	3½
123	2	13,333	0·53	3
125	3	20,000	0·8	4
127	3	20,000	0·8	7
129	2	4,761	0·095	10
131	2	4,761	0·095	4
132	3	7,143	0·143	4
137	3	8,571	0·171	3
138	3	8,571	0·171	6
139	3	8,571	0·171	3
145	2	5,714	0·114	3
147	2	5,714	0·114	3
149	2	5,714	0·114	3
152*a*	2	5,555	0·111	6
154	2	5,555	0·111	4
156	2	5,555	0·111	4
158	2	5,555	0·111	4
160	2	5,555	0·111	3
162	2	5,555	0·111	3
164	2	5,555	0·111	3
166	2	5,555	0·111	3½
168	2	5,555	0·111	3
171	2	8,000	0·16	3
173	2	8,000	0·16	4
175	2	8,000	0·16	3

VI. Discussion

(1) *Review of experiments in relation to mortality*

Testing our results solely by the mortality that occurred, we find that of fifty-six control animals forty-eight lived and eight died, of seventy experimental animals thirty-two lived and thirty-eight died.

Treated by the appropriate analytical method the odds are very heavy against the divergence being a mere chance fluctuation.

If we consider late deaths only there were twenty-three out of seventy experiments and no late deaths among the controls.

Considering as critical early deaths only we find that of fifty-six control animals eight died early, and of seventy experimental animals fifteen died early. Taken as a whole there is no significant difference between the rates of mortality of the experimental animals and the controls; but we can subdivide these results according to whether the sera used killed or did not kill controls. In forty-four experiments there were twelve early deaths and no deaths among the thirty-five guinea-pigs which served as controls for the serum used in

these experiments. It seems reasonable to attribute the death of these animals to the consecutive injection of antiserum and antigen. Details are given in Table V.

Later we shall discuss fully the deaths which occurred among the other controls on the injection of certain batches of serum.

Table V

	Survived	Early deaths	Late deaths	Total death-rate %
Group 1:				
Controls	35	0	0	0
Experimental	44	12	10	50
Group 2:				
Controls	21	8	0	38
Experimental	26	3	13	62

It is noteworthy that the antisera innocuous to controls had a high early to late ratio of mortality when antiserum and antigen were injected, viz. 12 to 10. But the antisera immediately fatal to controls gave a low ratio of early to late deaths when antiserum and antigen were injected, viz. 3 to 13. The significance of this we do not know.

(2) *Review of experiments in relation to symptoms*

Our observations on the symptoms which occurred in the control and experimental guinea-pigs are important, and should be taken into consideration in discussing the early deaths. Slight and transitory symptoms are neither easy to observe nor to describe, but there was a striking difference between the behaviour of the animals which received a single injection of antiserum (the controls) and those which received antiserum and antigen. The injection of antiserum alone seemed to have no effect except on the eight animals that died. The injection of antiserum and antigen produced symptoms in almost every case whether the animal died or not. Some of the animals showed only the early signs, others showed signs of dyspnoea and subsequently prolonged shock with signs of abdominal discomfort. The majority of the guinea-pigs which died of delayed shock, accompanied by abdominal lesions, had presented signs of dyspnoea and respiratory embarrassment during the first 15 min. following the injections. At autopsy small petechial haemorrhages, similar to those found in death from acute anaphylaxis, were sometimes found in the lungs and muscles of the chest wall.

(3) *Latent interval not necessary for the production of passive anaphylaxis*

Taking into consideration all the above facts we are of the opinion that passive anaphylaxis can be produced in guinea-pigs by the immediate injection of antigen after antiserum. Of seventy guinea-pigs which received such injections, fifteen died of acute shock, twenty-three of deferred shock and of the surviving thirty-two the majority showed signs of illness. The volumes of

antiserum and horse serum which we have injected have been larger than those employed by other workers and in excess of those necessary for the production of passive anaphylaxis when an interval is allowed between the two injections.

(4) *Passive anaphylaxis is more easily produced if there is a latent period*

The generally accepted belief that it is easier to produce passive anaphylaxis when an interval is allowed between the injections of antiserum and antigen is confirmed by our experiments, but of thirty-three guinea-pigs where an interval of 24 hours was allowed between the injection of antiserum and antigen none survived; out of seventy guinea-pigs where the injection of antiserum was followed immediately by that of antigen thirty-two survived.

Details of our results where an interval of 24 hours was observed are given in Table IV. All the animals died and presented the signs and post-mortem appearances of acute anaphylactic shock.

(5) *Comparison of the signs of anaphylaxis in the guinea-pig and in other animals*

Anaphylaxis is a name which has been given to a complex of symptoms, signs and lesions which may occur when there is a reaction in the body between antigen and antibody. In the guinea-pig the usual reaction is spasm of the bronchial muscles with resulting expiratory emphysema and death within a few minutes. Of seventy guinea-pigs which received injections of antigen immediately after antiserum fifteen died in this way but twenty-three died of deferred shock between the end of the third and the end of the eighth hour. We claim that these were genuine anaphylactic manifestations produced in a normal animal by the injection of antigen immediately after that of antiserum. Such deaths did not occur with injections of antiserum alone.

In the cases of late death there was vascular engorgement and often haemorrhage in the submucosa of the stomach, small intestine and caecum. There was excess of fluid in the peritoneal cavity, sometimes tinged with blood, in some cases there had been intraperitoneal haemorrhage. In a few cases the liver was engorged. The condition of the stomach and bowel was very like that seen in dogs which die or are killed some hours after anaphylactic shock. The changes in the dog have been described by Richet (1913), Weil (1917) and Dean & Webb (1924).

A very common statement is that the signs and anatomical changes associated with anaphylactic shock differ in different animals. The condition observed in our cases of late death recalls the condition found in dogs and the condition found in anaphylactic shock in man by Gurd *et al.* (1920) and by Dean (1922).

Cases of anaphylactic shock in man with abdominal symptoms have been recorded recently by J. A. Ryle (1935), and Dr A. F. Hurst has been kind

enough to let us see a short unpublished account of a case, at first taken for ulcerative colitis, which may have been one of abdominal anaphylaxis.

On the other hand, respiratory embarrassment is known to accompany the anaphylactic state in man, and respiratory distress has been described in dogs by Dean & Webb (1924). In dogs killed during the early stages of anaphylactic shock the lungs may be distended and do not collapse when the thorax is opened. The appearance presented is just like that seen in the anaphylactic guinea-pig. Parker & Parker (1923–4) showed that anaphylactic shock in the white rat may cause death from acute respiratory distress or that death may occur a few hours after the injection when marked abdominal lesions are found. They also refer to similar lesions which they have seen in anaphylaxis in guinea-pigs.

Although the guinea-pig is peculiarly susceptible to death from bronchial spasm the conditions of our experiments were such that they often recovered from the early respiratory symptoms and so gave time for the development of abdominal lesions and late or deferred shock.

(6) *Relation between strength of antiserum and passive anaphylaxis*

At the outset of our investigations it was our intention to correlate the occurrence of anaphylactic shock with the proportions of horse serum and antiserum injected. In all cases the antiserum was titrated with horse serum by the precipitation method, and the proportions of the two ingredients most favourable to rapid flocculation were determined. In the earlier experiments antiserum and horse serum were injected in proportions optimal for rapid particulation, but in the majority of our experiments we injected a volume of horse serum which was twice the volume required for optimal particulation with the volume of antiserum used. In view of the irregularity of the results which we obtained with different specimens of antiserum and of the fact that the number of experiments which could be performed with any one specimen of antiserum was limited, we decided to abandon, for the time being, an attempt to compare the results which might be obtained by varying the proportions of the horse serum and antiserum injected. The factors involved in the production of passive anaphylaxis appear to be too varied and complex to permit, at present, the experimental determination of the proportions of horse serum and antiserum most favourable to the production of shock. In all except the first few experiments the volume of antiserum injected has been either 2 c.c. or occasionally 3 c.c. The volume of horse serum has been twice the optimal volume for rapid particulation with the volume of antiserum injected.

While the volume of antiserum injected has been nearly always 2 c.c., the number of units of antibody has varied from 4000 to 50,000. The relation of the number of units of antibody injected to death from anaphylaxis is shown in Table VI.

The number of experiments carried out with very strong antisera containing over 6000 units per c.c. is not large, but our figures appear to show that the number of deaths bears a relation to the number of units injected.

Table VI. *Relation of strength of antisera to death from passive anaphylaxis*

Number of units injected	No. of experiments	Died within 12 min.	Died within 24 hr.	Total deaths	% of deaths
4,000 to 5,999	18	4	2	6	33
6,000 to 7,999	9	3	1	4	44
8,000 to 9,999	24	6	7	13	54
10,000 to 11,999	No experiments				
12,000 to 13,999	8	1	4	5	63
14,000 to 20,000	No experiments				
20,000 to 50,000	11	1	9	10	91

(7) *Deaths due to the injection of antiserum only*

Details of deaths due to the injection of antiserum only are given in Table VII.

Table VII. *Details of deaths due to injection of antiserum only*

	Antiserum					
No.	Age in days	Quantity injected c.c.	No. of guinea-pig	Signs of illness	Post-mortem appearances	Remarks
1308 I	35	3	57	Died at once	Lungs distended	Air injected with antiserum
	40	3·5	60	Died in 5 min., asphyxia	,,	Apparently typical acute anaphylactic death
1308 J	5	2	63	Died in 3 min., asphyxia	,,	,,
	5	2	64	,,	,,	,,
1308 K	27	3	143	Died in 5 min., asphyxia	,,	,,
1396 H	6	3	113	Died in 3 min., asphyxia	,,	,,
1596 D	12	2	180	Died in 4 min.	Lungs not distended	Mode of death like acute anaphylactic shock, but post-mortem not typical
	13	2	184	Died in 3 min.	,,	,,
1601 D	6	2	151	Found dead next morning	Enteritis	Death not due to anaphylaxis

Death with signs of anaphylaxis occurring after the injection of antiserum has been observed by other workers and requires consideration. The antiserum used in our experiments was derived from sixteen bleedings of eleven rabbits. The fifty-six guinea-pigs which served as controls received injections of antiserum only, and, excluding No. 151, eight of the animals died as a result of the injections. Guinea-pig 151 died of enteritis the day following the injection of antiserum, and is not counted as a control death due to the injection of antiserum. Certain factors which might conceivably play a part in these accidents require consideration.

The quantity of antiserum which was injected varied from 2 to 3·5 c.c., in the majority of cases only 2 c.c. were given. The incidence of death in our control animals in relation to the quantity of serum injected was as follows:

Quantity of serum injected (c.c.)	2	3	3·5
Total number of animals injected	43	12	1
Number of deaths in injected animals	4	3	1

Field (1931) found that the toxicity of human and rabbit serum persisted for at least 72 hours, the toxicity decreasing with age and completely disappearing by the end of a week. The age of the antisera we used varied from 4 to 40 days. The following table shows the relationship of the deaths occurring in both control and experimental animals to the age of the serum.

Table VIII

	Antiserum and antigen				Antiserum only	
	Total No.	Died early	Died late	Total deaths	Total No.	Died
Antiserum 4–6 days old	15	3	9	12	13	3
Antiserum more than 11 days old	55	15	14	29	43	5

No antiserum between 6 and 11 days old was used.

It is interesting to note that five of the eight deaths following injections of antiserum only were caused by antiserum obtained from three separate bleedings, I, J and K of rabbit 1308. Thus:

Antiserum 1308 I (35–40 days old) killed 2 out of 2 guinea-pigs
Antiserum 1308 J (5 days old) killed 2 out of 2 guinea-pigs
Antiserum 1308 K (27 days old) killed 1 out of 1 guinea-pig.

It seemed possible that these deaths were due to some toxicity or peculiarity of the serum of this animal. To test this hypothesis another bleeding—1308 L—was taken from this rabbit. The results, using the serum the twelfth day after bleeding, were as follows:

Table IX

No. of guinea-pig	Weight g.	Volume of antiserum c.c.	Results
185	330	2	No signs of illness
186	320	2	Ill after 4 min., rolled over and struggled: ill for 45 min. but recovered
187	350	2	Very ill immediately after injection. Seemed likely to die, but began to recover in 3 min. There was violent panting. Recovery after 1 hour

The remaining three antisera which caused death when injected alone were used at 6–13 days after bleeding. It would appear that other factors than the age and quantity of the serum injected are concerned in the death of guinea-pigs on the injection of antiserum only.

Forssman (1911) has shown that the injection of organs of certain animals into rabbits produces heterophile antibodies in the serum, and the presence of heterophile antibodies in the normal serum or in antisera of certain animals has been suggested as an explanation for the death of guinea-pigs following the injection of normal sera or antisera. These deaths have been attributed to reversed anaphylaxis. Nearly all experiments with immune heterophile serum have been made with sera prepared by the injection of organ extracts.

Taniguchi (1922) states that: "The primary toxicity of antisheep immune sera from the rabbit appeared also to be peculiar, because the majority of

antisera from the rabbit obtained by the injection of blood corpuscles or sera from other animals have been shown to be practically non-toxic for guinea-pigs. A certain degree of toxicity has, however, been recognized in some specimens of antisera after multiple injections of large amounts of antigen. It has been shown by Friedberger and Mita and others that those obtained after the injection of *Horse serum*, etc., were strongly toxic." As may be seen from the tables only a few of our anti-horse sera were toxic for the guinea-pig.

Redfern (1926) states that the autopsy in the case of deaths due to injection of heterophile serum differs from anaphylactic shock in that there is froth in the larynx and trachea and the cut surface shows oedema and massive haemorrhages. The lungs were 24·5 per cent greater in weight than anaphylactic lungs. We saw nothing comparable to this description in our series of cases.

In the majority of cases no signs of illness followed the intravenous injection of 2 c.c. of antiserum; in a few cases transient signs of illness occurred. It is worthy of note that if signs of illness followed an injection of antiserum only, such signs of illness were immediate, and if serious, death resulted within about 5 min. of the injection. Prolonged shock, and abdominal lesions such as we have described as characteristic of deferred anaphylactic death, were never observed. On the other hand, in eight guinea-pigs which had received antiserum only, death occurred a few minutes after the injection, with signs which we could not distinguish from those of typical acute anaphylactic shock. The post-mortem appearances also were in most cases indistinguishable from those of acute anaphylaxis.

VII. Summary

1. Our experiments show that an intravenous injection of antigen immediately after an intravenous injection of antiserum in the guinea-pig were followed by:

(*a*) Acute shock and death within 5 min. The signs and post-mortem appearances were indistinguishable from acute anaphylactic shock as typically seen in the guinea-pig. Some control animals injected with antiserum only died in a way similar to those which received both antiserum and antigen. These are discussed in detail in the body of the paper.

(*b*) Delayed shock and death some hours later. The post-mortem appearances were those of gastro-intestinal congestion and haemorrhage resembling the changes seen in dogs dying of anaphylactic shock. Such changes were never seen in the control animals.

(*c*) Recovery. Practically all the animals which recovered had symptoms of respiratory embarrassment immediately following the injections of antiserum and antigen and many had later symptoms of abdominal shock. The animals which were given an injection of antiserum only rarely had any symptoms and never abdominal symptoms.

2. It is necessary to test the antisera used by control inoculation since some antisera are *toxic*.

3. Passive anaphylaxis can be produced by the intravenous injection of antigen immediately following that of antiserum, but it is easier to produce it by allowing an interval between the two injections.

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