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REVERSED PASSIVE ANAPHYLAXIS IN THE GUINEA-PIG

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(With 6 Figures in the Text)

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INTRODUCTION

IN a previous paper I (van den Ende, 1939) have suggested that the shock, resulting from the intravenous injection into normal guinea-pigs of antisera prepared in the rabbit against guinea-pig proteins, is probably dependent on an antigen-antibody union occurring in relation to the cells of the guinea-pig's tissues. In the light of the very restricted definition of anaphylaxis proposed by Wells (1925) the phenomenon could therefore be regarded as resembling anaphylactic shock in all its aspects, except in so far as it is independent of artificial sensitization.

In reversed passive anaphylaxis the sensitizing injection of an antigen foreign to the guinea-pig is followed within 48 hr. by a shock-eliciting dose of antibody. Theoretically, therefore, all the criteria required for Wells's definition can be satisfied even though the order of application of antigen and antibody are reversed.

Since the earliest attempts to prove that anaphylaxis depends on a humoral rather than a cellular mechanism, numerous reversed anaphylaxis experiments have been performed. Because the majority of these experiments were designed with the view to showing that a latent interval was not indispensable for passive sensitization, the injection of antigen preceded that of antibody at the most by a few minutes.

Symptoms resembling those of acute anaphylactic shock in guinea-pigs have indeed been obtained even when antigen and antibody were mixed *in vitro* before injection (Biedl & Kraus, 1910; Friedemann, 1909; Friedberger, 1910). In these circumstances, it is not possible to exclude intravascular

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aggregation phenomena as causes of shock. Another source of error in the interpretation of results of reversed anaphylaxis experiments seems to have been the use of antisera which are themselves toxic for guinea-pigs. Kellett (1930, 1935) has described reversed passive anaphylaxis in guinea-pigs when horse serum was used as antigen and a precipitating antiserum from rabbits as antibody. He found that a latent period of at least 45 min. appeared to be necessary between a sensitizing injection of antigen and the production of shock by injection of the corresponding antiserum, and from this concluded that a cellular mechanism was probably responsible, but most observers have found that the latent period required for the appearance of sensitiveness to the antigen (direct passive anaphylaxis) after injection of the sensitizing antibody is upwards of 6 hr. Kellett observed fatal reactions in not more than 50% of his test animals, and only slight and transient reactions in some of the small number of control guinea-pigs which received the antiserum alone. That these controls may have been inadequate is suggested by the experiments of Dean, Williamson & Taylor (1936) who found that six of sixteen batches of serum from rabbits immunized against horse serum were capable of eliciting fatal shock when injected alone into normal guinea-pigs. Such primary toxicity, for guinea-pigs, of antisera from rabbits immunized to horse serum, has been reported also by numerous other observers (Doerr & Weinfurter, 1912; Opie & Furth, 1926, etc.). Zinsser & Enders (1936), moreover, using similar reagents to those employed by Kellett, found that the latent interval was not necessary for the reversed passive sensitization of guinea-pigs. The reaction elicited by Kellett by the injection of antiserum which may itself have toxic properties for guinea-pigs, and one which Zinsser & Enders could elicit only when a very short latent interval was allowed, can only doubtfully be related to true anaphylaxis.

Reversed passive anaphylaxis in other species (rabbits and mice) has been described by Opie & Furth (1926) and Schiemann & Meyer (1926), but as the mechanism of the reaction regarded as indicating anaphylaxis in these species is incompletely known and not clearly related to the characteristic condition observed in the guinea-pig, the results of these observers have no direct bearing on the problem with which I am here concerned.

Numerous investigators (Weil, 1915; Doerr & Russ, 1909; Doerr & Bleyer, 1926) have been entirely unsuccessful in their attempts to demonstrate reversed passive anaphylaxis in guinea-pigs. They have drawn the general conclusion that ability to gain access to the tissue cells of guinea-pigs is a property peculiar to antibodies of certain species, and further that (direct) passive sensitization depends on activation of the fixed antibody by the tissue cells. These experiments, however, were made without the knowledge now available concerning the specific "acceptability" (Hartley, 1937, 1938) to the cells of the guinea-pig tissues of proteins from other species. In most cases horse serum was used as the antigen. It is now well known that antibodies produced in different species differ widely in their powers of producing direct

passive anaphylaxis in the guinea-pig to the corresponding antigens, in accordance with the varying degrees to which they are "acceptable" to the cells of the guinea-pig's tissues (Nissl, quoted from Doerr, 1929; Avery & Tillett, 1929; Brown, 1934*a*, *b*; Hartley, 1937, 1938). It seemed probable, accordingly, that normal proteins from different animal sources might similarly vary in their powers of producing reversed anaphylaxis when used as sensitizing antigens. Experiments on reversed passive anaphylaxis should therefore be performed with a variety of antigens, including proteins likely to be acceptable to guinea-pig's tissue cells and their homologous antisera.

Even when care is taken to use acceptable antigens several quantitative factors in immunology must be kept in mind. In the precipitation reaction which is probably concerned in the anaphylactic reaction, one molecule of antigen is usually capable of combining with many molecules of antibody globulin. It may well be that the extreme sensitivity of a guinea-pig to an antigen, to which it has been actively or passively sensitized, depends on the ability of a small amount of the antigen, when reinjected intravenously, to react with a relatively large amount of antibody widely distributed in the susceptible tissues, and fixed to the cells of the guinea-pig. In reversed passive anaphylaxis it can be expected that large shock-eliciting doses of antibody will have to be administered to ensure an equally widespread reaction with fixed antigen.

In planning the following series of experiments the attempt has been made to avoid conditions which favour the occurrence of significant intravascular precipitation by allowing a long latent interval (18 hr.) between the sensitizing injection of antigen and the shock-eliciting dose of antibody. In order to remove as completely as possible from the antisera used to elicit the shock substances having non-specific toxic properties, only the purified globulin fractions from antisera were used in intravenous injections made for this purpose. Moreover, the proteins selected as sensitizing antigens had to be such as can reasonably be expected to be taken up and held by the tissues of the test animal. The numerous observations, that antibodies produced in rabbits are capable of inducing sensitivity in guinea-pigs whereas the corresponding antibodies produced in the horse are not, have suggested to me the use of rabbit serum globulins as the antigens conferring passive sensitiveness in the reversed anaphylactic experiments here reported. Not only the globulin from the serum of normal rabbits but also purified antibody globulin from immune rabbit sera were so used. The use of the latter as sensitizing antigen has the additional advantage that the occurrence of cellular fixation can be proved by the direct anaphylactic reaction produced by injecting the antigen against which the rabbit was immunized. For comparison, the sensitizing properties of several other antigens were investigated. These included globulins from horse and human sera and crystalline egg albumin, with all of which the results obtained were completely negative or doubtful. The negative experiments with egg albumin are described in detail as an example of this group.

(1) Experiments using egg albumin as sensitizing antigen

Material and methods

Egg albumin was crystallized from fresh egg white and five times recrystallized according to the method used by Taylor, Adair & Adair (1932). The final solution after dialysis was stored in the frozen state at $-10°$ C.

Antibody.

To 120 c.c. of the pooled antiserum from rabbits immunized to crystalline egg albumin 60 c.c. of saturated ammonium sulphate was added. The precipitate of euglobulin was removed by filtration and the concentration of ammonium sulphate in the filtrate increased to 50% saturation to precipitate the pseudoglobulin. Both globulin fractions were once reprecipitated from saline solution, dissolved and dialysed against saline at refrigerator temperature. The concentrations determined refractometrically were: euglobulin 3·2%, pseudoglobulin 1·7%. The precipitin contents of these globulin preparations were determined by the Dean & Webb (1926) optimal proportions method. In agreement with Adair & Taylor (1936), the antibody content of the euglobulin solution (optimal ratio 1 : 27) was found to be higher than that of the pseudoglobulin (1 : 60). (Optimal ratios of these globulin solutions in 1% strength calculated from these figures are: euglobulin 1 : 86·4, pseudoglobulin 1 : 102.)

It was decided therefore to use the euglobulin solution in reversed anaphylaxis experiments.

The sensitizing power of the euglobulin solution.

This was determined in three groups of five or six guinea-pigs. The sensitizing dose of euglobulin given intraperitoneally was 0·2, 0·1 and 0·05 c.c. for the three groups respectively. After a latent interval of 18 hr. the guinea-pigs received varying doses of egg albumin intravenously to determine the minimal shock-eliciting dose for each group. The results of a typical experiment are summarized in Table 1.

Table 1. *Showing the result of intravenous injection of egg albumin in guinea-pigs sensitized with* 0·05 *c.c. anti-egg albumin euglobulin solution*

Weight of guinea-pig g.	Dose of egg albumin intravenously mg.	Result
230	1·0	D 3½ m.
240	0·1	D 4½ m.
260	0·05	Near kill
240	0·04	+ + +
240	0·01	+

D 3½ m. Typical acute reaction terminating fatally in 3½ min.
+ to + + + Mild to very severe symptoms.

The minimal shock-eliciting dose of antigen for the group sensitized with 0·2 c.c. of euglobulin solution was 0·5 mg., for the group sensitized with 0·1 c.c.

0·05 mg., and for the group sensitized with 0·05 c.c. also 0·05 mg. The greater sensitizing power of the smaller doses of euglobulin is probably dependent on the presence after a latent interval of only 18 hr. of free or circulating antibody in those guinea-pigs which had received the largest sensitizing dose.

Reversed anaphylaxis experiments.

Groups of four to seven guinea-pigs of 200–250 g. weight were given varying doses of crystalline egg albumin intraperitoneally, and after a latent interval of 18 hr. they received at least five or ten times the minimal sensitizing dose of the antibody containing rabbit euglobulin solution intravenously. Intravenous injections were made into the right jugular vein, which was exposed under local anaesthesia.

After a further latent period of 48 hr. some of the survivors were tested for their sensitivity to intravenously injected egg albumin. The results of a typical experiment are shown in Table 2.

Table 2. *Showing the result of intravenous injection of anti-egg albumin euglobulin into guinea-pigs which had previously received varying amounts of egg albumin intraperitoneally*

Weight of guinea-pig g.	Egg albumin intraperitoneally mg.	Euglobulin Latent interval hr.	Euglobulin intravenously c.c.	Symptoms	Egg albumin Latent interval hr.	Egg albumin intravenously mg.	Result
200	0·1	18	0·25	—	—	—	—
210	0·5	18	0·25	—	—	—	—
200	1·0	18	0·25	—	—	—	—
230	5·0	18	0·25	—	—	—	—
240	Nil	—	0·25	—	—	—	—
240	0·1	18	0·5	—	48	0·15	D 4½ m.
210	0·25	18	0·5	—	48	0·15	D 4 m.
230	0·5	18	0·5	—	48	0·15	D 4½ m.
220	1·0	18	0·5	—	48	0·15	D 4 m.
240	2·5	18	0·5	—	48	0·15	D 3 m.
250	5·0	18	0·5	—	48	0·15	D 4½ m.
200	10·0	18	0·5	*	48	0·15	D 4 m.
230	Nil	—	0·5	—	48	0·15	D 4½ m.

D 4½ m. Typical acute anaphylactic shock terminating fatally in 4½ min.

* Slight symptoms were shown, but these were atypical, consisting only of weakness and ataxia of the hindlimbs.

From Table 2 it will be seen that although large amounts of antibody euglobulin were given, in not a single case did its injection evoke symptoms typical of anaphylactic shock. When tested 48 hr. after the injection of antibody, however, the test animals, which had previously received egg albumin intraperitoneally, were as sensitive to intravenous injections of egg albumin as control animals which had not received the initial egg albumin injection.

It is well known that egg albumin is rapidly eliminated from the animal body, and from the results recorded in Table 2 it might be supposed that, 18 hr. after its intraperitoneal injection, no egg albumin would remain to combine with the intravenously injected antibody. In subsequent experiments, there-

fore, a latent interval of only 4 hr. was allowed, and the effects of increasing dosage of both antigen and antibody were also investigated.

The results are recorded in Table 3.

Table 3. *Showing the result of intravenous injection of anti-egg albumin euglobulin into guinea-pigs which had previously received varying amounts of egg albumin intraperitoneally*

No.	Weight of guinea-pig g.	Egg albumin intraperitoneally mg.	Latent interval hr.	Euglobulin intravenously c.c.	Symptoms	Latent interval hr.	Egg albumin intravenously mg.	Result
1	240	0·1	4	0·5	—	48	0·15	+ +
2	240	0·25	4	0·5	—	48	0·15	—
3	230	0·5	4	0·5	—	48	0·15	—
4	250	1·0	4	0·5	—	48	0·15	—
5	240	2·5	4	0·5	—	48	0·15	—
6	240	5·0	4	0·5	*	48	0·15	—
7	220	10·0	4	0·5	*	48	0·15	—
8	230	10·0	4	1·0	—	—	—	—
9	250	25·0	4	1·0	—	—	—	—
10	240	40·0	4	1·0	—	—	—	—
11	220	50·0	4	1·0	—	—	—	—

+ + Severe symptoms.

* Mild symptoms doubtfully of anaphylactic nature, consisting of staring coat, and irregular jumping movements.

Although mild symptoms occurred in guinea-pigs nos. 6 and 7, these could not be regarded as definitely anaphylactic in nature. Their occurrence in those animals, which in comparison with nos. 1–5, had received large amounts of egg albumin, suggested the necessity of increasing the dosage still further. That this increase of dosage had no effect is shown by the entirely negative results with guinea-pigs nos. 8–11. The results differ from those in Table 2, in that shortening of the latent period between antigen and antibody injection has resulted in interference with the sensitization which would otherwise have followed the injection of antibody (cf. Table 2). In spite of the presence of antigenic egg albumin in the guinea-pigs at the time of the test injections of antibody no anaphylactic symptoms were elicited by these injections. The conclusion can be reached, therefore, that egg albumin is not a suitable antigen for use in reversed passive anaphylaxis, probably because of its inacceptability to the guinea-pig's tissues.

(2) Experiments using globulin from normal or immune rabbit sera as antigen

Material and methods

Heidelberger & Kendall (1936) have shown that pure antibody can be recovered from the specific precipitate from anti-pneumococcal serum, by treatment with 10–15% NaCl. This simple and convenient method has so far not been found applicable to specific precipitates other than those formed as result of combination between the pneumococcal capsular polysaccharides and

their homologous antibodies. The capsular polysaccharide and anti-pneumococcal antibody seem best suited for experimental work on anaphylaxis, and for these reasons they were selected for use in the present investigation.

Pneumococcal polysaccharide.

Dr Wilson Smith kindly supplied 1·0 g. of a crude capsular polysaccharide (type 1) prepared according to the method described by Dudley & Smith. This on purification by the method described by these authors (1933) yielded 0·46 g. dry powder. Stock solutions of 1/1000 were made up as required and kept at 0° C.

The preparation of pure antibody gobulin.

Pure antibody globulin (Ab.) was prepared from 250 c.c. of type 1 rabbit anti-pneumococcal serum, which was partly supplied by the State Serum Institute of Copenhagen, through the kindness of Prof. Madsen, and partly by Dr E. T. C. Spooner of Cambridge. Optimal precipitating proportions of specific pneumococcal polysaccharide type 1 and the antiserum were determined by Smith's (1932) modification of the Dean & Webb (1926) optimal proportions method.

In extracting the antibody the method described by Heidelberger & Kendall (1936) and Heidelberger & Kabat (1938) was followed: optimal precipitating proportions of antiserum and specific polysaccharide (SSS 1) were mixed and allowed to stand in the refrigerator until the sedimentation of floccules was complete. The precipitate was then removed by centrifugalization, and washed in frequent changes of chilled 0·9% saline, until the washings were free from heat coagulable proteins. To the precipitate was then added a 15% sodium chloride solution, which after thorough breaking up of the precipitate, was warmed to 35° C. for 1½ hr. The residual precipitate was then removed by centrifugalization and the solution freed from excess of salt by prolonged dialysis against 0·9% saline at refrigerator temperature. The concentration of the dialysed solution, after filtration from the traces of precipitate which reformed on dialysis was refractometrically found to be 0·23%.

Globulins from normal rabbit serum.

These were precipitated from 150 c.c. of fresh normal rabbit serum by the addition of an equal volume of saturated ammonium sulphate. The precipitated globulins were redissolved and reprecipitated twice. They were finally dissolved in saline and dialysed against 0·9% salt solution at refrigerator temperature.

Preparation of antisera

(*a*) *Precipitins for antibody globulin.*

Each of six large guinea-pigs (500–600 g.) received over a period of 3 weeks daily intraperitoneal injections of a suspension of specific precipitate from rabbit anti-pneumococcal serum. The specific precipitate was obtained from 50 c.c. of anti-pneumococcal serum (type 1) by the addition of the "optimal"

amount of SSS 1. After repeated washings in saline the precipitate was suspended in 50 c.c. saline. Each guinea-pig received 0·5 c.c. of the well-shaken suspension daily.

The guinea-pigs were bled by cardiac puncture on the ninth day after the last injection. The sera after separation were tested for the presence of precipitins by the ring-test method. Two of the guinea-pigs yielded rapidly reacting antisera. These were bled out from the carotid after stunning. All the serum samples were pooled, yielding "anti-antibody globulin" serum 1 (A Ab G–1).

Three months later the four surviving guinea-pigs received a further course of immunizing injections, after which they were bled out, their sera tested individually for the presence of precipitins, and then pooled, yielding anti-antibody globulin serum 2 (A Ab G–2).

(*b*) *Precipitins for the globulin from normal rabbit serum.*

Ten large guinea-pigs were given daily intraperitoneal injections of 5·0 mg. of alum precipitated rabbit globulin. The period of immunization and method of collecting the serum was the same as for the previous group. Two batches of antiserum were therefore obtained, labelled "anti-globulin 1" and 2 (A G–1 and 2).

To approximately two-thirds of each batch of antiserum were added equal volumes of saturated ammonium sulphate to precipitate the globulins. The globulins were redissolved, and dialysed, and later used as shock-eliciting antibody preparations in the anaphylactic experiments to be described (A Ab G G, and A G G).

Testing of the reagents

Purified type 1 *anti-pneumococcal antibody* (*Ab*). This preparation was tested for its ability to induce passive sensitivity to the SSS 1. Guinea-pigs received 1·5–5·0 mg. of Ab intraperitoneally, and after 24 hr. 0·05–0·1 mg. SSS 1 intravenously. Acutely fatal anaphylactic shock occurred invariably even after the smallest sensitizing doses employed. The sensitivity induced by Ab has also been demonstrated by anaphylaxis *in vitro* (see Fig. 1).

Precipitins in the antisera from guinea-pigs. The small quantities of reagents available made the application of quantitative methods impossible. The precipitating titre of each antiserum was therefore determined by layering 0·1 c.c. amounts of antigen in increasing dilutions over an equal volume of undiluted antiserum in narrow test tubes. The tubes were examined for rings at $\frac{1}{2}$, 1 and 2 hr. intervals.

The results of a typical experiment are recorded in Table 4.

The table shows that striking immunological differences exist between the precipitins in anti-antibody (A Ab G) and antiglobulin (A G) sera. Whereas antiserum prepared by immunizing guinea-pigs to the specific precipitate from rabbit anti-pneumococcal antiserum contains precipitins capable of reacting as well with the purified antibody (Ab) as with the total globulins from norma

rabbit sera, antiserum from guinea-pigs immunized to the total normal globulins reacts only with the homologous antigen. It has also been possible to demonstrate this immunological difference between total normal and immune globulins from rabbit serum by anaphylactic methods. Both active and passive sensitization methods have been employed, and the anaphylactic responses have been elicited in the whole animal, as well as with uterine plain

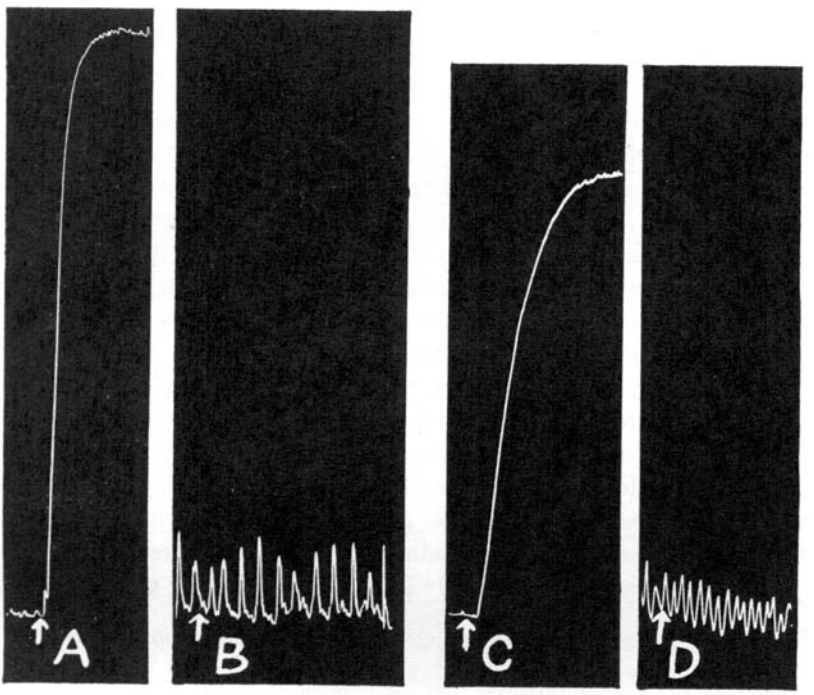

Fig. 1. Anaphylactic response *in vitro* of the uterus of a guinea-pig sensitized with 1·5 mg. eluted anti-pneumococcal antibody (Ab).
1st horn: at A, 0·02 mg. SSS 1; B, 0·02 mg. SSS 1. 2nd horn: C, 0·01 mg. SSS 1; D, 0·01 mg. SSS 1.

Table 4. *Showing the precipitins in A Ab and A G serum from guinea-pigs*

Antiserum ...	"Anti-antibody 2"												
Antigen ...	Purified antibody Ab 5 mg./c.c.							Total normal globulin 5 mg./c.c.					
	1/1	1/5	1/25	1/125	1/625	1/3100	Saline	1/1	1/5	1/25	1/125	1/625	1/3100
½ hr.	+ +	+ +	+ +	+	—	—	—	+ +	+ +	+ +	+	—	—
1 hr.	P	P	P	+	+	—	—	P	P	+	+	±	—
2 hr.	P	P	P	+	+	tr.	—	P	P	P	+	+	—
Antiserum ...	"Antiglobulin 2"												
½ hr.	—	—	—	—	—	—	—	+ +	tr.	—	—	—	—
1 hr.	tr.	—	—	—	—	—	—	P	+	tr.	tr.	—	—
2 hr.	+	—	—	—	—	—	—	P	P	+	+	—	—

tr., ±, +, + + represent increasing thickness and distinctness of the rings formed. P, sedimentation of the precipitate.

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muscle preparations *in vitro*. Typical examples of the *in vitro* reactions of uteri from guinea-pigs actively sensitized with Ab or normal globulins (G) are given in Figs. 2 and 3.

Marrack & Duff (1938) have shown that antiserum prepared against the specific precipitate from horse anti-pneumococcal serum, will precipitate the globulins of normal horse serum. It was considered possible, in the light of this work that conversely, precipitins capable of reacting with antibody globulin could be prepared by immunizing against the total globulins from normal serum. The serum from guinea-pigs, immunized against normal globulin could then be used to elicit shock in guinea-pigs sensitized with antibody globulin. This would have been a great advantage, as normal rabbit globulins are more easily obtained in amounts sufficient for the immunization of guinea-pigs, than pure antibody globulin. The demonstration of striking immunological differences between the total globulin from normal rabbit serum, and rabbit antibody globulin, has shown however that it is necessary to use antiserum prepared by immunization against antibody globulin itself.

The observed immunological differences can most readily be explained on the basis of quantitative differences in the *y* globulin present: thus it is possible that the concentration of *y* globulin present in normal rabbit serum is insufficient to evoke appreciable amounts of antibody when injected into guinea-pigs, but sufficient to give rise to a precipitate when mixed with an antiserum prepared against *y* globulin. It is possible that in Marrack & Duff's experiments the reaction between normal horse globulin and antiserum from rabbits immunized against specific precipitates was due to the presence in normal horse serum of globulins antigenically identical to antibody globulin.

Sensitizing power. Antibody preparations from guinea-pigs (A Ab G G and A G G)

Direct passive sensitization

The small quantities of reagents available made the accurate determination of minimal sensitizing doses impracticable.

Each antibody preparation was given intraperitoneally to guinea-pigs in doses varying from 0·2 to 0·5 c.c. 18–24 hr. later each of these guinea-pigs received intravenously 2·0–5·0 mg., of Ab or total normal rabbit globulin, and the severity of shock shown by each animal was noted.

The sensitizing ability of A Ab G 1 and A G G 1 were approximately equal, 0·5 c.c. of either preparation being required to induce sensitivity of such a degree that fatal anaphylactic shock results from the shock-eliciting injection of homologous antigen. Of the second batches (A Ab G 2 and A G G 2) 0·25 c.c. was sufficient to induce the same degree of sensitiveness.

Reversed passive anaphylaxis.

(1) *Using purified antibody globulin.* Normal guinea-pigs 200–250 g. in weight were given varying amounts of Ab by intraperitoneal injection. 18 hr. later each received 1·5–3·0 c.c. of A Ab G intravenously. The symptoms

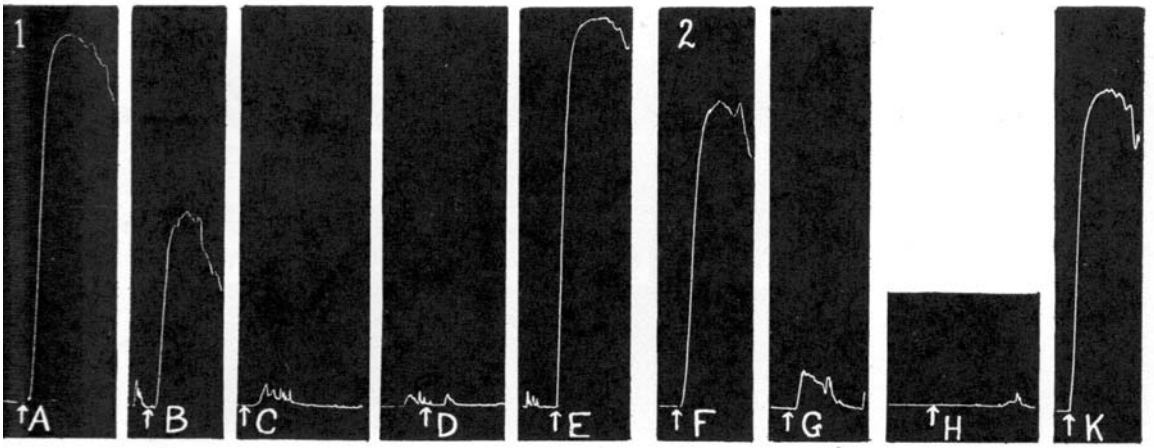

Fig. 2. Anaphylactic response *in vitro* of the uterus of a guinea-pig actively sensitized with anti-pneumococcal antibody globulin (Ab).
1st horn: at A, B and C, each 0·1 mg. Ab; D, 0·1 mg. normal rabbit globulin; E, 0·002 mg. histamine.
2nd horn: F, G, each 0·1 mg. normal rabbit globulin; H, 0·2 mg. Ab; K, 0·002 mg. histamine.

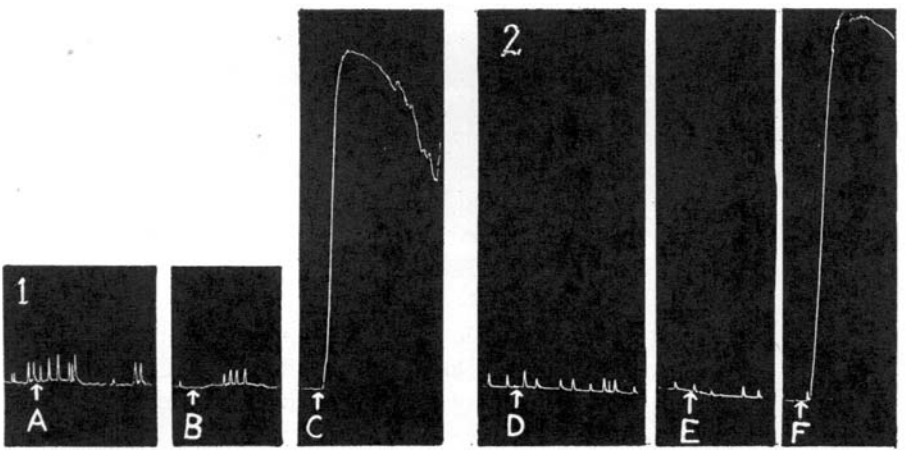

Fig. 3. Anaphylactic responses *in vitro* of uterine strips from two guinea-pigs: 1, actively sensitized with normal rabbit globulin; 2, passively sensitized with guinea-pig anti-rabbit globulin (A G G). 1: A, 0·1 mg. eluted anti-pneumococcal globulin (Ab); B, 0·2 mg. Ab; C, 0·1 mg. normal rabbit globulin. 2: D, 0·1 mg. Ab; E, 0·2 mg. Ab; F, 0·2 mg. normal rabbit globulin.

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following the intravenous injection were carefully observed for an hour. 24–48 hr. later the survivors were tested for their sensitivity to SSS 1 or Ab by the intravenous injection of one of these antigens.

The results are recorded in Table 5.

Table 5. *Showing the result of intravenous injection of A Ab G into guinea-pigs which had been given Ab* 18 *hr. previously*

Weight of guinea-pig g.	Ab intraperitoneally mg.	Latent interval hr.	Antibody intravenously c.c.	Symptoms	Latent interval hr.	Antigen intravenously mg.	Result
220	2·0	18	1·5 A Ab G 1	±	24	2·0 Ab	—
230	5·0	18	1·5 A Ab G 1	±	24	2·0 Ab	—
210	5·0	18	2·0 A Ab G 1	+	—	—	—
210	8·0	18	2·0 A Ab G 1	+	24	0·2 SSS 1	D 4 m.
220	Nil	—	2·0 A Ab G 1	—	24	2·0 Ab	D 4½ m.
200	5·0	18	3·0 A Ab G 2	+	48	2·5 Ab	—
240	10·0	18	3·0 A Ab G 2	+ + +*	48	0·5 SSS 1	D 3 m.
210	20·0	18	3·0 A Ab G 2	±	48	0·5 SSS 1	D 3½ m.
230	Nil	—	3·0 A Ab G 2	—	—	—	—

± Very mild symptoms. + Mild but definite symptoms.

* The animals showed typical acute anaphylactic shock, and after 4 min. it was thought to be dead, but recovery commenced after 10 min.

It was found that very severe anaphylactic shock occurred in one guinea-pig, while in the remainder symptoms were mild, but occurred in a high percentage of the test animals and resembled those usually encountered in mild degrees of true anaphylactic shock.

Anaphylaxis in vitro. Virgin female guinea-pigs weighing approximately 220g. were sensitized by a single intraperitoneal injection of Ab in amounts varying from 2 to 10 mg. They were killed 24 hr. later, and the excised uterine strips suspended in Locke's solution. By the use of a double bath the two uterine strips of each guinea-pig could be tested at the same time. One strip was tested for its sensitiveness to SSS 1 (direct anaphylaxis) and the other to A Ab G (reversed anaphylaxis). The result of a typical experiment is shown in Fig. 4. The reactions of the first strip to SSS 1 proves not only that cellular fixation of Ab had occurred but also that although sensitiveness induced by Ab had been almost completely abolished by a single contact with SSS 1, the plain muscle was then still capable of responding with nearly maximal contraction to contact with A Ab G. The reactions of the second horn of the same uterus show that a single contact of A Ab G resulted in maximal contraction, with resultant almost complete desensitization to A Ab G, while the uterus remained sensitive to SSS 1.

The A Ab G or SSS 1 alone have repeatedly been found incapable of stimulating a normal, non-sensitized uterus to contraction.

It must be concluded that, with the reagents employed, it has been possible to demonstrate both direct and reversed passive anaphylaxis in the same individual, whether the test method has involved the use of the whole guinea-

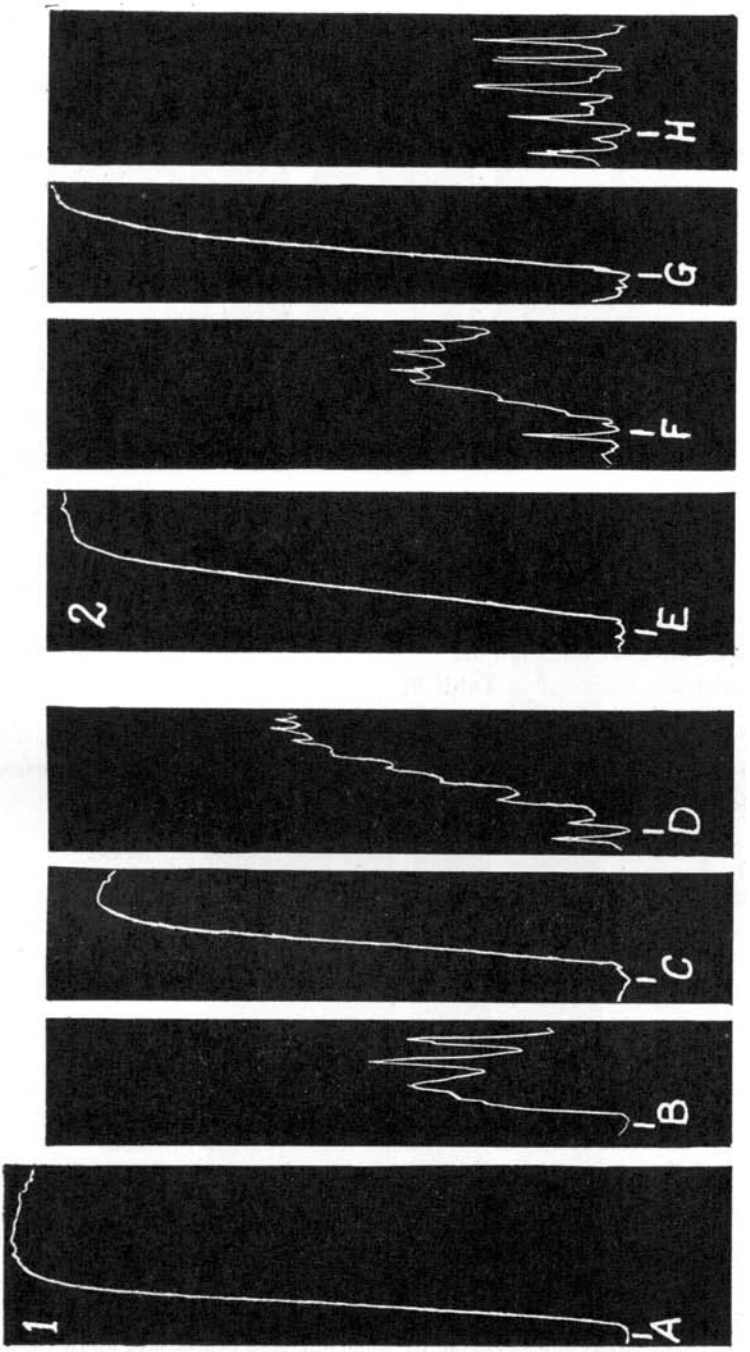

Fig. 4. Anaphylactic responses *in vitro* (reversed and direct) of the uterine strips of a guinea-pig which had received 6·0 mg. of purified anti-pneumococcal antibody globulin (Ab) 18 hr. previously.
1st horn: A, B, each 0·2 mg. SSS 1; C, D, each 1·0 c.c. A Ab G 2. 2nd horn: E, F, each 1·0 c.c. A Ab G 2; G, H, each 0·2 mg. SSS 1.

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pig or its uterine muscle *in vitro*. More importance is attached to the demonstration of the reaction *in vitro* than to the results of the small number of experiments performed with the whole animal. An adequate number of experiments in the whole guinea-pig would require larger amounts of the reagents than were available for this investigation. With the *in vitro* technique which avoids excess of free antigen positive results can be obtained more easily, and with greater regularity than in the whole animal, where excess of unbound antigen is not so easily avoided.

Dale & Kellaway (1921) have shown that an excess of antibody, in the medium in which sensitized plain muscle is suspended, will inhibit the contraction which otherwise invariably follows the addition of antigen. The reversed experiment was here tried, and it was found that an excess of Ab, added to the bath in which a uterus from an Ab sensitized guinea-pig was suspended, prevented a contraction when subsequently A Ab G was added. Subsequently when the uterine muscle had been washed in frequent changes of Locke's solution, the single addition of A Ab G was found to elicit contraction (see Fig. 5).

(2) *Total globulin (G) from normal rabbit serum used as antigen.* Normal guinea-pigs of 200–250 g. in weight were given varying amounts of G by intraperitoneal injection. 18 hr. later each received an intravenous injection of A G G. Some of the guinea-pigs were given antigen (G) intravenously after a further latent interval of 24–48 hr.

The results are recorded in Table 6.

Table 6. *Showing the results of intravenous injection of A G G (globulin fraction of antiglobulin serum) into guinea-pigs sensitized by the intraperitoneal injection of rabbit globulin (G)*

Weight of guinea-pig g.	Rabbit globulin (G) intraperitoneally mg.	Latent interval hr.	AG G intravenously c.c.	Symptoms	Latent interval hr.	Rabbit G intravenously c.c.	Result
220	20·0	18	1·5 AG G 1	?±	—	—	—
220	5·0	18	1·5 ,,	?±	—	—	—
210	8·0	18	2·0 ,,	?±	24	2·0	—
210	5·0	18	2·0 ,,	?±	—	—	—
210	50·0	18	3·0 AG G 2	?±	48	5·0	—
250	25·0	18	3·0 ,,	?±	—	—	—
220	10·0	18	3·0 ,,	?±	48	5·0	—
210	5·0	18	3·0 ,,	+	48	5·0	±
220	Nil	—	3·0 ,,	—	—	—	—

?± Very mild symptoms of skin and mucous membrane irritation.
\+ Definite but mild symptoms of respiratory obstruction.

It was found therefore that only very mild symptoms, which could doubtfully be regarded as anaphylactic in nature, were observed in the majority. Only one guinea-pig reacted with mild symptoms definitely resembling those of true anaphylactic shock.

In *in vitro* tests also the severity of reaction was much less than in corresponding experiments with purified antibody globulin (see Fig. 6).

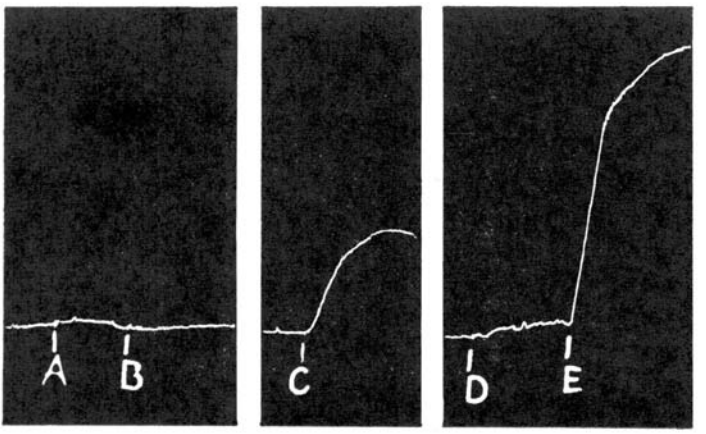

Fig. 5. Response of the uterus from a guinea-pig which had received 10·0 mg. Ab 18 hr. previously. At A 5 mg. of Ab added to the bath in which the uterus was suspended; at B, 1·0 c.c. A Ab G 2. After B the Locke's solution in the bath was changed several times. C, 1·0 c.c. A Ab G 2; D, 1·0 c.c. A Ab G 2; E, 0·002 mg. histamine.

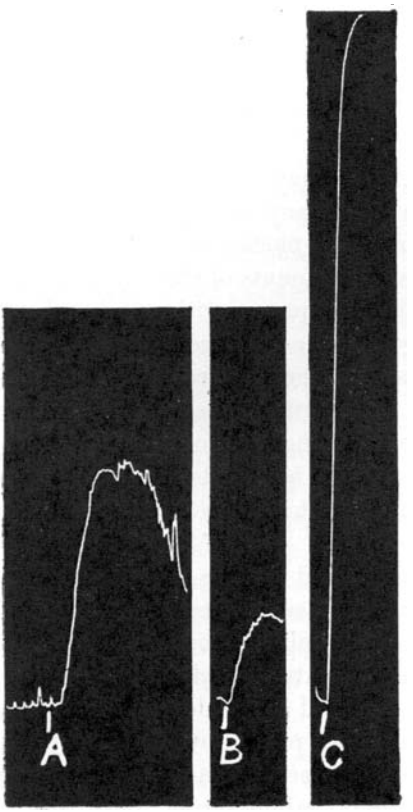

Fig. 6. Response of the uterus of a guinea-pig which had received 6·0 mg. of normal rabbit globulin 18 hr. previously. At A, B, each 1·0 c.c. A G G 2; C, 0·002 mg. histamine.

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It can be concluded, therefore, that when the purified antibody globulin from rabbit anti-pneumococcal serum was used as sensitizing antigen reversed passive anaphylaxis could be demonstrated, both in the whole animal, and *in vitro*. As in direct anaphylaxis, the reaction is dependent on an antigen antibody reaction occurring in relation to the cells of the guinea-pig's tissues.

Although positive results of a similar type have been obtained when the globulins from the serum of normal rabbits were used as sensitizing antigen, the responses elicited were less constant, and less marked. The significance of this difference will be dealt with in the discussion.

Discussion

There can be no doubt that the phenomenon of reversed passive anaphylaxis has been demonstrated. The success of the reaction was found, however, to be dependent on the use of a particular sensitizing antigen, namely, one which is acceptable to the cells of the guinea-pig's tissues.

Like direct anaphylaxis, the phenomenon of reversed passive anaphylaxis is dependent on an immunological reaction occurring in relation to the guinea-pig's tissues. Excess of free antigen in the circulation will, therefore, inhibit the reaction which would follow the administration of a suitable dose of antibody to an animal in which no such excess was present. It has long been established that in direct anaphylaxis great excess of antibody in the circulating blood is necessary to inhibit the shock-eliciting power of antigen. This is probably dependent on the ability of single molecules of the majority of soluble antigens to combine specifically with many molecules of antibody. It is highly probable, therefore, that in the reversed phenomenon inhibition of shock will result from the presence of even small amounts of circulating antigen. This may account, at least in part, for the infrequency with which it has been possible to elicit severe degrees of shock in the reversed passive anaphylaxis experiments *in vivo*, and it is likely that with the use of carefully determined optimal sensitizing doses of antigen fatal shock will be elicited with greater regularity, and with the use of smaller shock-eliciting doses of antibody. With the *in vitro* technique an excess of free antigen can be avoided and this probably accounts for the comparative ease with which it has here been possible to demonstrate reversed passive anaphylaxis *in vitro*.

The success of the reversed passive anaphylaxis experiments when rabbit antibody globulin was used as sensitizing antigen confirms the well-established fact that rabbit antibody globulin is acceptable to the cells of the guinea-pig's tissues. The properties of antibody globulin, on which tissue accessibility depend, are not clear. That all antigenic substances are not acceptable to the tissue cells of the guinea-pig is proved by the entirely negative results obtained with egg albumin. Great interest attaches to the results obtained with the total globulins from normal rabbit serum. With this complex antigenic substance only mild reversed anaphylactic reactions were elicited, both in the whole animal and in experiments *in vitro*. Either, therefore, the normal

globulins are but moderately acceptable to the cells of the guinea-pig's tissues, or the antigen responsible for the mild reactions forms only a very small fraction of the complex antigen used. It is of interest in this connexion that Tiselius & Kabat (1939) and Kabat (1939) in electrophoresis experiments have shown that y globulin in the serum of rabbits increases with immunization. In animals such as the horse immunization results in the production of globulins, different from those in normal horse serum and, unlike antibody or y globulins of rabbits, incapable of sensitizing guinea-pigs. It may well be, therefore, that acceptability to the guinea-pig tissue cells depends on the properties of the y globulin in rabbit serum.

The demonstration of both direct and reversed anaphylaxis in the same individual has an important bearing on the phenomenon of specific sensitization in general. The prevention of direct passive sensitization with antiserum from rabbits, by the previous injection of normal rabbit serum, has been looked upon as depending on the development of anti-antibodies. The results described in this paper prove conclusively that the neutralization of antibody by its specific precipitin does not abolish the state of specific sensitiveness which depends on the presence of antibody.

It is obvious that further experiments on reversed anaphylaxis should be performed, using not only the purified antibody globulin from rabbit serum as sensitizing antigen, but also the individual globulin fractions from the normal serum of rabbits, and more especially the y globulin. From a study of this kind, and from a comparison of the sensitizing powers of globulins from the rabbit and other species, much light may be thrown on the problem of tissue acceptability. In view of the marked inhibitory power of excess circulating antigen on the phenomenon of reversed anaphylactic shock, the *in vitro* technique will probably be best suited to a study of this kind.

The results reported in this paper have an added interest, in demonstrating that immunological differences exist between antibody globulin and the total serum globulins of rabbits. That antisera prepared by immunization of guinea-pigs with the total globulins of normal rabbit serum are incapable of precipitating antibody globulin, is in support of the conception that antibodies are newly formed globulins, resulting from the stimulus of immunization, and not modifications of serum globulins already present. Antigenic differences between the sera of normal and immunized animals has previously been show by Ando and his co-workers (1937, etc.). It seems likely that these immunological differences between the total normal globulins of the rabbit, and antibody globulin from rabbit anti-pneumococcal serum depend on quantitative differences in y globulin present.

Summary

Attempts to demonstrate reversed passive anaphylaxis in the guinea-pig with crystalline egg albumin as sensitizing antigen have been uniformly negative.

When purified anti-pneumococcal antibody globulin was used as sensitizing antigen, reversed anaphylactic shock could be elicited in guinea-pigs by the intravenous injection of precipitins for the antibody globulin.

The mild reactions which could be elicited when the total globulins from the serum of normal rabbits were used as sensitizing antigen are probably dependent on the presence of small amounts of γ globulin.

Reversed passive anaphylaxis, like direct anaphylaxis, is dependent on a cellular mechanism, and the success of experiments in which rabbit antibody globulin was used as sensitizing antigen depends on the acceptability of the antibody to the cells of the guinea-pig's tissues.

Antigenic differences between antibody globulins and total normal globulins from rabbit serum are noted.

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