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SOME OBSERVATIONS ON REVERSED ANAPHYLAXIS

BY M. VAN DEN ENDE, M.B.

John Lucas Walker Student

From the Department of Pathology, University of Cambridge

(With Plate VII and 12 Text-figures)

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INTRODUCTION

RICHET (1902) described the altered state of reactivity of dogs to reinjection of toxic substances obtained from the tentacles of certain marine animals. This increased susceptibility to toxins he called anaphylaxis. Since Richet's work, a very large number of papers have been published, dealing with phenomena to which this name has been applied. The use of the term has been loose and varied. A primary cause of confusion has been the use of the word anaphylaxis to describe, on the one hand, the condition of acquired, specific sensitiveness, and, on the other, the group of symptoms constituting the reaction shown by the animal when it receives an injection of the substance to which it has thus been rendered specifically sensitive. There can be no doubt that the term "l'anaphylaxie" was originally applied by Richet to the process or condition of abnormal sensitization, and not to the symptoms produced by reinjection of the sensitizing antigen. Those who first studied the "Theobald Smith phenomenon" in the guinea-pig (Otto, 1906; Rosenau & Anderson, 1906), and identified it with Richet's anaphylaxis produced in a different species and with normally innocuous proteins, certainly applied the term to the condition of abnormal sensitiveness and not to the train of symptoms which reinjection of the sensitizing antigen evoked. A striking and constant train of symptoms is

shown by guinea-pigs in acute anaphylactic shock. The common use of guinea-pigs for experimental work on anaphylaxis has, therefore, resulted in the frequent application of the term anaphylaxis to the syndrome rather than to the state of increased sensitivity. It has, however, been abundantly demonstrated that more or less closely similar syndromes, with characteristic differences in different species, can be produced by injecting into the blood stream of normal animals a wide variety of substances, including suspensions and colloidal dispersions of materials which are chemically inert, and devoid of any intrinsic pharmacodynamic action. This has led to confusion of thought and the use of such terms as "anaphylactoid" (Hanzlik & Karsner, 1920) which seem rather to emphasize than to avoid the suggestion that the syndrome, as such, is characteristic of the response of the anaphylactic animal to its antigen. The fact that the reactions of animals of different kinds to peptone (Biedl & Kraus, 1909) and to histamine (Dale & Laidlaw, 1910) closely resemble the reactions of anaphylactic individuals of the same species to injections of sensitizing antigen, has attracted much interest, on account of the origin of such substances from proteins. There is evidence that substances of this kind, normal constituents of the living cells of most tissues, are liberated by any process producing a widespread cellular injury and become direct agents in the syndrome which such injury produces (Dale, 1929; Feldberg & Kellaway, 1938). Such a conception makes the action of histamine and other natural cellular constituents responsible for the common features of syndromes produced by so many unrelated agents of cell injury. On this conception the action of the sensitizing antigen, reinjected into the anaphylactic animal, takes its place as an agent of widespread cell injury, characterized by its immunological specificity and not by the symptoms which it evokes. On the other hand, the derivation of peptone from protein by cleavage, and of histamine from a constituent amino-acid, contributed to the development of a different conception of the anaphylactic reaction, in which the union in the blood stream of the antibody with the reinjected antigen initiated an enzymatic action, leading to the production of an "anaphylatoxin" (Friedberger, 1910). There was, however, no convincing evidence that such a substance could be produced by interaction between the serum of an anaphylactic animal and the sensitizing antigen. The appearance of the familiar and unspecific syndrome in the guinea-pig when complexes produced by digesting serum with various inert colloids, or with bacteria, as in the experiments of Novy and his co-workers (1917), was doubtfully related to the phenomenon of anaphylaxis.

It now seems to be generally agreed that the term anaphylaxis ought to be restricted to conditions in which acute symptoms follow the interaction of antigen and antibody somewhere in the living animal. Views vary as to the exact nature of the reaction, and as to whether further restrictions should be placed on the use of the term. The definition proposed by Wells (1925) restricts the name anaphylaxis to the condition of specific sensitiveness caused by a previous injection of a soluble foreign protein, or by passive transfer of the

antibody responsible for the actively anaphylactic condition. This definition has the advantage of confining the term to the condition to which it was applied by those who first studied it in the dog and the guinea-pig.

If such a closely restricted definition were accepted, there would probably be a general agreement that anaphylaxis, in this sense, is due to the fixation of a precipitating antibody to the cell protoplasm, and that excess of the same antibody in the circulation protects the sensitized cells from reaction with the specific antigen, as shown by experiments of Dale (1913), Weil (1913 et seq.), Coca (1914), Dale and Kellaway (1921) and many others. On the other hand, such a mechanism would not explain a number of phenomena for which the name anaphylaxis is widely used, such as the sensitization of an animal by a single injection of intact foreign red corpuscles to a reinjection of red corpuscles from the same species.

If the definition is extended to include these, however, there is no logical reason for refusing to extend it to any specific agglutinating or aggregating reaction occurring in the blood, and provoking a reaction in which symptoms similar to those of the classical anaphylactic shock occur. The effects produced by foreign sera naturally causing agglutination or lysis of the red cells would then be included. The reaction observed by Dean, Williamson & Taylor (1936) to the simultaneous injection of a precipitating serum and the corresponding antigen in optimal precipitating proportion, would then be described as passive anaphylaxis. The application, indeed, of the name anaphylaxis to these phenomena is merely a question of definition, provided that it is not used to support a contention that all anaphylaxis, including the effects produced by various artificial serum-colloid digests, are due to an immunological reaction occurring in the blood.

The position in regard to the different types of so-called "reversed anaphylaxis" is somewhat different.

In the Forssmann reaction an antibody is injected which combines with an antigen naturally present in the cells of various organs of the test animal (Doerr & Pick, 1913; Redfern, 1926; Taniguchi, 1922; Amako, 1914; and others); so that the conditions are, indeed, similar to those causing the classical anaphylactic reaction, though the relation and application of antibody and antigen are reversed.

There is another phenomenon described as reversed anaphylaxis, in which symptoms are elicited by injecting into the vein of a guinea-pig the serum from a rabbit which has been immunized against guinea-pig serum, and accordingly gives a precipitate with guinea-pig serum. This is the phenomenon which forms the subject of the investigation here described. The object of my experiments has been to discover whether the shock-syndrome evoked under such conditions is due to the occurrence of this precipitating reaction in the blood of the test animal, or whether the antiserum reacts with antigenic constituents of the tissue cells; and, if both types of reaction occur, in what proportion they contribute to the production of the shock-like reaction.

The toxicity for the guinea-pig of precipitating antiserum against guinea-pig serum, prepared in the rabbit, has been studied by Doerr & Moldovan (1910, 1912), Uhlenhuth & Haendel, and others. Doerr and Moldovan found that, even with strongly precipitating antisera, relatively large doses were necessary to give rise to acutely fatal reactions. He draws attention also to the presence of Forssmann antibody in these sera. In spite of Sacharoff's (1926) claim that intravascular occlusion by precipitate or thrombi is not responsible, Doerr stresses the possibility of such mechanical causes.

Turro & Gonzalez (1912) gave a young rabbit a single injection of guinea-pig blood. Fifty-two days later the citrated or defibrinated blood of this rabbit was tested for toxicity to normal guinea-pigs. Several fatal reactions were recorded which the authors attributed to the presence of an anaphylactic poison, though it is possible that under such conditions a number of reactions, including intravascular agglutination or haemolysis, occurred. Similar experiments, using precipitating or haemolytic antisera, were performed by Smadel & Swift (1937), using the rat, and by Kraus (1909), using dogs and rabbits as test animals.

My own experiments have included:

(1) Observations on the symptomatology, morbid anatomy, and morbid histology in cases of fatal reactions following the intravenous injection into guinea-pigs of rabbit serum containing specific precipitins for guinea-pig serum.

(2) Observations on the mode of action of the antisera by studying their effects on the systemic blood pressure, pulmonary arterial pressure, bronchial resistance, and isolated heart preparations.

(3) Observations on the action of the antisera on the isolated guinea-pig uterus.

(4) The relation of the ability of the sera to produce symptoms to their content of (*a*) precipitin for guinea-pig serum, (*b*) Forssmann antibody, and (*c*) agglutinins for guinea-pig red blood corpuscles.

1. Effects of intravenous injections into guinea-pigs of antiserum prepared in rabbits against guinea-pig serum

The antisera were produced in a group of rabbits, each given two or more courses of six injections of guinea-pig serum. Doses of 2 c.c. of guinea-pig serum, heated to 56° C. for 30 min., were given at 4–5 day intervals, and the rabbits bled from the marginal ear vein on the 8th or 9th day after the last injection. The serum, after standing in contact with the clot for 18 hr. at room temperature, was separated from red cells by centrifugalization.

Before the injection of the guinea-pigs the antisera were sealed in glass ampoules and heated to 56° C. for 30 min. on three successive days, in order to destroy any non-specific, heat-labile toxic factors such as were found in normal goat serum by Aronson (1928). According to Doerr & Moldovan (1910, 1912), the power of anti-guinea-pig sera to affect guinea-pigs is not affected by such heating. In view of Redfern's (1926) findings that normal rabbit serum

has the power of stimulating contraction of the smooth muscle of the guinea-pig if used within the first 12 days after bleeding, the antisera were then kept for a further period of at least 2 weeks before use. Sera developing opalescence after storage were discarded.

All the guinea-pigs used were of the same stock and of approximately uniform weight (300–400 g.).

Injections were made into the jugular vein, which was exposed under local anaesthesia. Immediately after injection the guinea-pigs were released and the symptoms observed. When acutely fatal reactions occurred, immediate autopsies were performed. When delayed reactions occurred, some of the animals were killed at intervals after injection, and the others were allowed to survive.

Dosage. In the earlier experiments attempts were made to establish the minimal lethal dose for each serum. Owing to the great individual differences amongst guinea-pigs, however, this was found impracticable with the small amounts of serum available. A constant dose, 2·0 c.c., was therefore used throughout.

The precipitin content of the antisera was determined by the optimal proportions method of Dean & Webb (1926).

Table I. *Showing the result of intravenous injection of six samples of antiserum into normal guinea-pigs*

Serum*	Date	Wt. of guinea-pig g.	No.	Dose c.c.	Precipitin optimal ratio	Symptoms	Result
2604 A	15. ii	290	338	2·0	Not determined	+ + +	Death 4 min.
	15. ii	300	339	2·0	,, ,,	+ + +	,, ,,
	15. ii	340	346	2·0	,, ,,	+ + +	,, ,,
2604 C	18. ii	320	352	2·0	1 : 10	+ +	Death 25 min.
	25. ii	340	353	2·0	1 : 10	+	Recovery
	25. ii	320	354	2·0	1 : 10	−	—
	25. ii	300	355	2·0	1 : 10	±	Recovery
	25. ii	300	356	2·0	1 : 10	−	—
2605 C	2. iii	300	358	1·5	1 : 45	+ + +	Death 5 min.
	2. iii	320	359	1·5	1 : 45	+ + +	,, ,,
	2. iii	360	360	1·5	1 : 45	+ + +	Death 4½ min.
2605 D	20. vii	380	433	1·5	(Nil in 20 hr.)	−	—
	20. vii	360	434	2·0	,,	+ +	Recovery
	20. vii	350	435	2·0	,,	+ + +	Death 5 min.
2606 D	26. vii	390	454	2·0	1 : 44	+ +	Recovery
	26. vii	390	455	2·0	1 : 44	+ +	,,
	26. vii	390	456	2·0	1 : 44	+	,,
	26. vii	390	457	2·0	1 : 44	−	—
	26. vii	370	458	2·0	1 : 44	±	Recovery
	26. vii	370	459	2·0	1 : 44	±	,,
	26. vii	310	460	2·0	1 : 44	−	—
3055 A	28. vii	370	466	2·0	±1 : 16	+ + +	Death 6 min.
	28. vii	390	467	2·0	1 : 16	+ + +	Recovery
	28. vii	340	468	2·0	1 : 16	+ +	,,
	28. vii	360	469	2·0	1 : 16	+ + +	Death 6 min.
	28. vii	360	470	2·0	1 : 16	+	Recovery

+ and ± mild or very mild respiratory, and + + very marked respiratory symptoms.
+ + + convulsions. − no symptoms.

* The figure refers to the rabbit supplying the antiserum, the letter (A, C, D) to the number of he bleeding. Thus, A is serum obtained after the first course of injections, B the second, etc.

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Results

Table I shows some characteristic results obtained with six samples of serum. Comparison of 2604 C and A demonstrates that more severe reactions frequently occurred with the earlier bleedings. Although having a higher antibody content, 2604 C gave rise to only one fatal reaction (delayed) out of five, whereas 2604 A was rapidly fatal for all three guinea-pigs injected. It is interesting also to observe in this connexion that 2605 D, a serum which was collected a month after the last injection of the rabbit, and in which the optimal ratio could not be determined (very slight opalescence only developed in the tubes after 20 hr.), produced characteristic severe or fatal reactions in the two guinea-pigs injected. Nevertheless, the fact that toxicity is not characteristic of early bleedings only is shown by the uniformly fatal results obtained in three tests with 2605 C. Striking also is the fact that the two sera 2605 C and 2606 D, in spite of almost identical precipitin contents (optimal proportions 1 : 45 and 1 : 44), were widely different in their power of producing reactions in guinea-pigs.

Reactions in the guinea-pig.

These may be divided into the *Acute*, in which death occurred, after a constant train of symptoms, within 10 min. of the injection, and *Delayed*.

(*a*) *Acute*. The symptomatology resembles very closely that of acute anaphylactic shock. Signs of respiratory obstruction set in, usually after a latent interval of 1–3 min. At first there is coughing, slight restlessness, and vigorous rubbing of the nose, the last being an indication of skin and mucous membrane irritation. Later the guinea-pig huddles in a corner with its coat ruffled, and its breathing rapidly becomes more difficult, as evidenced by the overactivity of all the accessory muscles of respiration. Frequently, marked lachrymation occurs. Cyanosis then develops and is followed by a violent convulsive stage, during which urine and faeces are often passed. The convulsions are followed by complete flaccidity and gradual weakening of the respiratory efforts. Death occurs with complete cessation of all movements of respiration, the heart continuing to beat for several minutes.

Autopsy findings. In all cases autopsies were performed immediately after cessation of respiration and while the heart was still beating.

The right side of the heart was invariably engorged, but blood clot was never found in the heart or great vessels. (Doerr & Moldovan (1912), on the other hand, reported clots in the heart in some of their cases when large doses of antiserum were given.)

The lungs were in all cases emphysematous. In contrast to true anaphylactic shock, the amount of pulmonary distension was not always maximal. Petechial haemorrhages were numerous and extensive, apparently more so than in ordinary anaphylactic shock. In many instances apart from the petechiae, the surface of the lung had a uniform pinkish colour owing to general capillary distension. In no case was there obvious pulmonary oedema.

The gastro-intestinal tract showed no obvious abnormality.

Histology. The most characteristic lesions, consisting of gross alveolar overdistension with thinning and frequently rupture of the alveolar septa (Pl. VII, fig 1), were seen in the lungs.

In all cases haemorrhages were prominent, either in the form of capillary haemorrhages in the interstitial tissues and alveoli (Pl. VII, fig. 2), or as perivascular or peribronchial haemorrhages related to larger vessels.

Bronchi so constricted that their lumina were occluded by folded mucous membrane were common (Pl. VII, fig. 1).

The only frequent finding in other tissues was vascular engorgement associated with capillary haemorrhages. In the acute cases this was most commonly seen in the myocardium and diaphragm. Congestion of the liver, when present, was slight.

(*b*) *Delayed reactions.* The delayed manifestations were seen usually after recovery from mild acute symptoms, but occasionally without the preliminary respiratory distress. The animal remained listless, quiet, huddled and usually shivering in the corner of its cage. When disturbed it attempted to walk but with obvious weakness of the limbs, so that frequently the abdomen dragged along the floor. In severer cases weakness was extreme, so that the animal sagged down on to its abdomen, with its legs stretched out. In some cases, apart from overactivity of the *alae nasi*, there was no evidence of increased respiratory effort. Handling of the abdomen at this stage was resented. In a few cases the animals passed blood-stained faeces a short time before death. Death occurred after various time intervals, from 10 min. to several hours (see Table II).

Autopsy findings. Absence of gross emphysema was in striking contrast to the finding in the acute deaths. Haemorrhages in the lungs, however, were at least as prevalent as after acute death.

When death occurred after an interval of more than 30 min. intense splanchnic congestion was the most constant feature. The congestion involved not only the gastro-intestinal tract, where it produced cyanosis and extensive haemorrhages, but also the liver. In several cases the hepatic congestion was so intense that intraperitoneal haemorrhage was caused through the rupture of a large superficial vessel of the liver.

Different degrees of congestion of the spleen and kidneys occurred.

The findings in eight cases of delayed death are summarized in Table II.

Histology. Even in those cases in which no emphysema was seen on naked-eye examination, there was frequently microscopic evidence of patchy alveolar overdistension. The severity of the emphysema became progressively less, however, as the interval between injection and death increased. On the other hand, especially when death was delayed for several hours, numerous areas in the lung appeared to be solid. This solid appearance was found to be due to collapse and infiltration of the interstitial tissues by mononuclear cells (Pl. VII,

Table II. *Showing post-mortem findings in cases of delayed death*

Guinea-pig no.	Ephysema	Lung haemorrhage	Liver congestion	Intestinal congestion	Interval between injection and death
142	+ +	+	+	+	11½ min.
118	+	–	+	+	16 min.
352	+	+ +	+ +	+	25 min.
344	–	+ + +	+	+	1½ hr.
166	–	+ + +	+ + + (rupture)	+ + (haemorrhage)	2 hr.
465	–	– (oedema +)	+	+ + (*)	3 hr.
345	–	+	+	+ +	6 hr.
261	–	+ + +	+ + +	+ +	6 hr.

+ to + + + indicates degrees of severity of lesion. – none.

(*) Rupture of the stomach found at autopsy.

fig. 3). In perivascular sites there was, in addition, infiltration by polymorphonuclear leucocytes, both eosinophil and neutrophil (Pl. VII, fig. 4).

Histological examination of the liver revealed intense congestion with numerous petechial haemorrhages, usually placed round the central veins of the lobules.

In a series of animals killed at various intervals after severe late manifestations, areas of necrosis in the liver were seen as well as haemorrhages. Within three days blood and necrotic liver cells were absorbed and regeneration commenced. Necrosis was found only in the immediate vicinity of the haemorrhages.

(*c*) *Exclusion of non-specific factors as causes of the reaction.* Before undertaking further experiments it was considered advisable to exclude as far as possible the possibility that non-specific toxic factors might be responsible for the shock phenomena in the guinea-pig, such as: (i) The early toxicity of freshly clotted blood, which is due to a labile factor that rapidly disappears on standing, and causes intravascular clotting on injection (De Kruif, 1917). Presumably this factor is of the nature of a thrombokinase. As the sera used in these experiments were heated to 56° C. and stored for 2 weeks before use, the possibility that the reactions here described were due to this clotting factor can be excluded. To confirm its absence from the anti-guinea-pig sera, a series of mice were given intravenous injections of 0·5 c.c. of those sera which produced reactions in guinea-pigs. None of the mice showed any effects from the injection. On the other hand, normal rabbit blood, freshly clotted according to the method described by de Kruif (1917), was found to cause massive, acutely fatal intravascular coagulation within 1–2 min. if given in amounts of 0·25–0·5 c.c. This toxic power of normal rabbit serum passes off within 1–2 hr. at room temperature. (ii) A toxic constituent which is a heat stable substance associated with the albumin fraction of serum, and which has a relatively slow and persistent stimulating action on smooth muscle may persist after the disappearance of the clotting factor (Friedberger & Siedenberg, 1927). To exclude the possibility of the presence of this constituent in these antisera the following experiments were performed:

An antiserum prepared against guinea-pig globulins, which was found to be

fatal to guinea-pigs in a dose of 1·0 c.c. per 100 g., induced precisely similar symptoms to those elicited by rabbit antisera resulting from immunization with whole guinea-pig serum. Of this serum 30 c.c. were fractionated by half-saturation with ammonium sulphate. The globulin fraction was collected, reprecipitated by adding an equal volume of saturated ammonium sulphate solution, dissolved in saline and dialysed against normal saline at 4° C. The final solution of globulins was tested by the intravenous injection of three guinea-pigs in quantities corresponding to 1·0, 1·25 and 1·5 c.c. of original serum per 100 g. of guinea-pig. All died acutely with similar manifestations to those produced by the original whole serum.

The remaining proteins of the serum, not precipitated by half-saturation with ammonium sulphate, were also dialysed against saline, and injected in doses corresponding to 1·5, 2·0 and 2·5 c.c. of original serum per 100 g. of guinea-pig. They gave rise to no reactions.

The absence of the toxic factor from the albumin fraction proves that it cannot be responsible for the symptoms under consideration, and the results with the globulin fraction are a strong argument in favour of the shock symptoms being produced by an antigen-antibody reaction.

2. Observations on the functional disturbances induced during the shock state

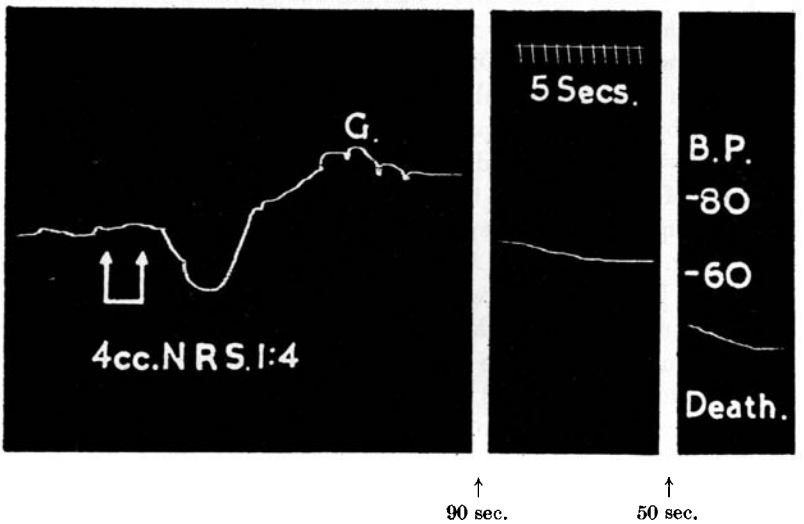
The choice of a suitable anaesthetic was difficult. Most workers agree that narcotics such as ether and urethane have an inhibitory effect on the anaphylactic reaction. Farmer (1938) has shown that inhibition of the contraction of the isolated guinea-pig uterus occurs if urethane is present, and Dale & Laidlaw have shown that anaesthesia inhibits the action of histamine in rodents (1910).

Light nembutal anaesthesia was found to be the most suitable, and only those experiments in which the animal showed characteristic signs of respiratory obstruction during anaesthesia were considered.

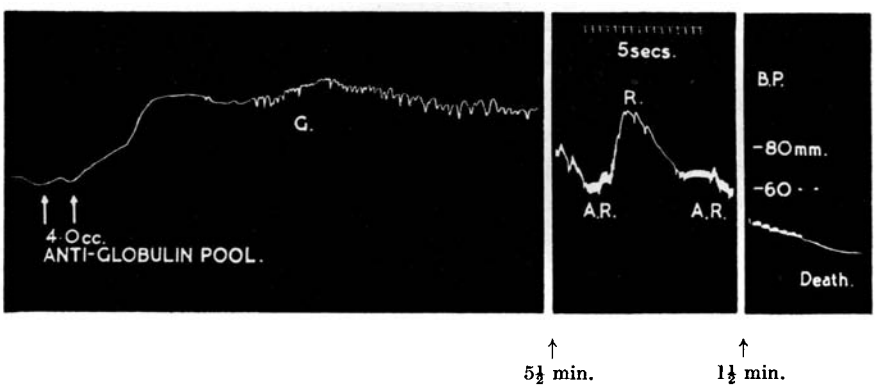
Anticoagulants, although it is doubtful whether they can inhibit anaphylaxis, were avoided, and as a consequence clotting in the artery cannula interrupted the observations in many experiments, and the results of these had to be discarded.

(*a*) *Systemic blood pressure*

This has been studied in anaphylaxis by numerous observers (Auer & Lewis, 1910; Biedl & Kraus, 1909; and others). Text-fig. 1 shows the blood-pressure changes during a true anaphylactic reaction in a guinea-pig previously sensitized to rabbit serum. The abrupt rise in pressure followed by a gradual fall is in agreement with the observations of previous workers. Text-fig. 2 shows the effect on the blood pressure of a normal guinea-pig of a shock-producing injection of anti-guinea-pig serum. The only difference from Text-fig. 1 lies in the more gradual secondary fall in pressure.



Text-fig. 1. Blood pressure recorded by mercury manometer from the carotid artery of a guinea-pig actively sensitized to rabbit serum. *G*, gasping. *NRS*, normal rabbit serum.



Text-fig. 2. Blood pressure response following injection of 4 c.c. of a pooled precipitating antiserum for guinea-pig globulin prepared in rabbits. *G*, gasping. *AR*, abnormal cardiac rhythm. *R*, respiratory movements.

(*b*) *Bronchial resistance*

This was measured by the increase in the amount of air passing through the side tube of an intratracheal cannula.

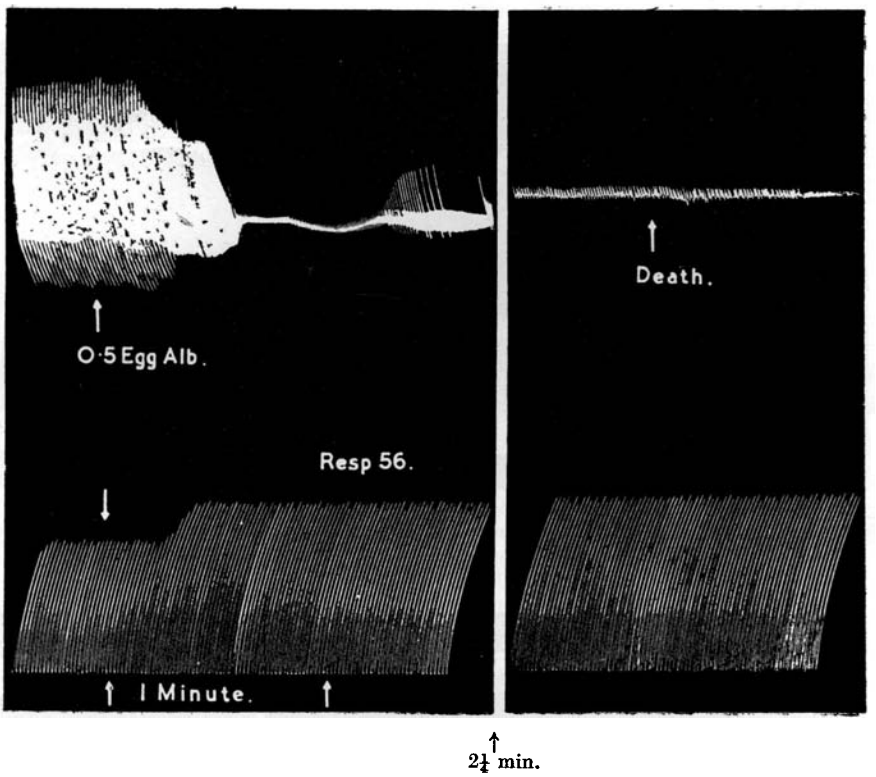
Auricular contractions were recorded at the same time (see upper tracings in Text-figs. 3–5).

Text-fig. 3 is a typical example of the response in anaphylaxis, showing diminution of amplitude of the auricular contractions coincident with the abrupt onset of bronchial obstruction.

Text-figs. 4 and 5, obtained by the injection into a normal guinea-pig of an

antiserum to guinea-pig serum, show that the response differs only in a slightly delayed and more gradual onset.

The results obtained with histamine were identical with those in true anaphylaxis.



Text-fig. 3. Auricular contractions (above) and bronchial resistance (below) during acute anaphylactic shock, following intravenous injection of 0·5 c.c. of 2% egg albumin into an actively sensitized guinea-pig.

(c) *Effects on the isolated heart of the guinea-pig*

Wilcox & Andrus (1938), using isolated heart preparations, claimed that an anaphylactic constriction of the coronary artery can be shown if the animal has previously been sensitized. The response was elicited by very small doses of the antigen (0·01 c.c. of horse serum). Normal serum will cause constriction of the coronaries of non-sensitized guinea-pigs only in relatively large doses (more than 0·1 c.c.). The effects of these antisera were therefore investigated.

The hearts were perfused with oxygenated Ringer's solution through a cannula tied into the aorta. The amount of perfusate escaping from the pulmonary artery was measured by an electric drop recorder and taken as an index of the coronary flow.

Ventricular contractions were recorded by direct traction on a writing lever. The whole preparation was kept at 37° C. throughout the experiment.

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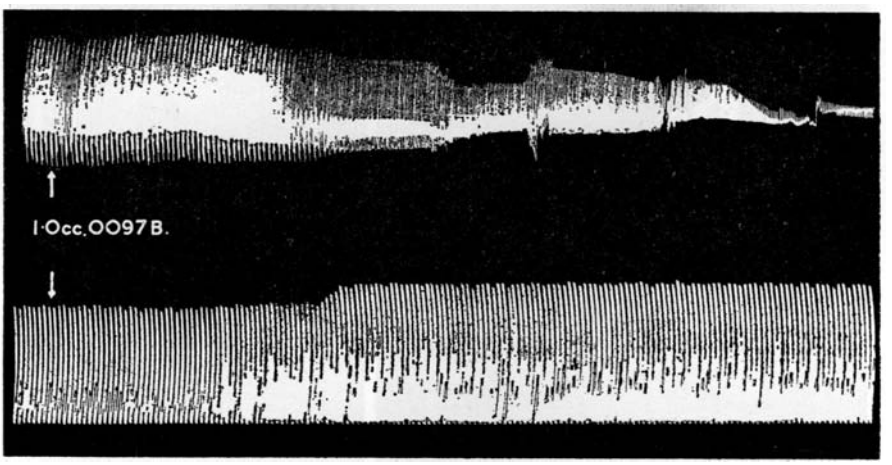
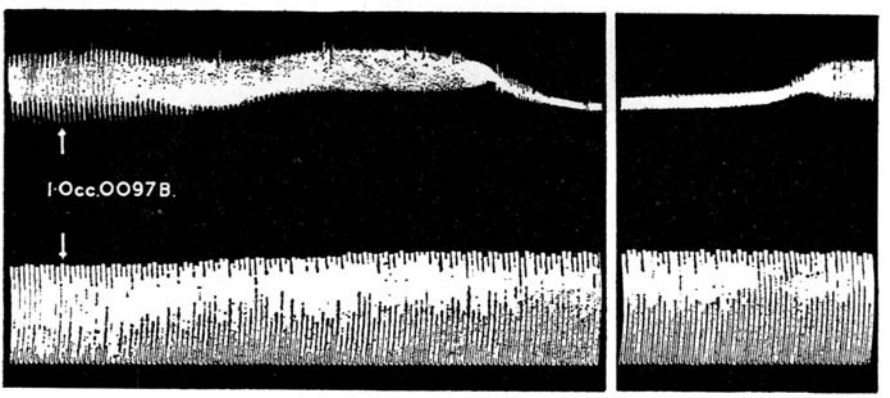
Normal rabbit serum was found to cause increase in the amplitude of ventricular contractions, and reduction in the amount of coronary flow, invariably when large doses were used, and infrequently when as little as 0·01–0·1 c.c. was injected into the perfusing fluid immediately above the aortic cannula. No desensitization to the effect of normal serum occurred.



Text-fig. 4. Auricular contractions and bronchial resistance during acute shock following injection of an antiserum for guinea-pig serum.



Text-fig. 5. Same as Text-fig. 4 but gradual in onset and followed by recovery.

Using small doses of antiserum (0·01 c.c.) the same response was obtained, but neither the same dose nor small multiples caused it after desensitization. When larger doses were used the effects of antiserum and normal rabbit serum were indistinguishable. (Such constriction of blood vessels by normal serum has been described by Friedberger & Siedenberg (1927), who pointed out also that the action was prolonged and not followed by desensitization.)

(d) Effect on the pulmonary artery pressure

Doerr (1929) has stressed the possibility that the reaction following the injection of antisera may depend on intravascular agglutination. If this were so, agglutinates would be expected to collect in the pulmonary vessels, leading to an increase of pressure in the main stem of the pulmonary artery. The regular presence of marked right-sided engorgement of the heart after acute deaths, as described later, made investigation of this suggestion necessary.

The pulmonary artery was exposed and a fine cannula, bent at right angles near the tip, was tied into the main stem according to the method described by Drinker & Went (1928). The pressure was recorded on a saline manometer and readings taken every 15–30 sec. The results of two typical experiments are shown in Text-figs. 6 and 7, where observed pressures have been plotted against time. Attempts at recording the changes in pressure directly failed owing to the insensitivity of the apparatus available.

Individual variations in the guinea-pigs were great, and cases of true anaphylaxis and also of reaction to antiserum have been observed, in which the initial rise in pressure reached 35 cm. or more. Normal rabbit serum had only a slight and transient action.

3. Experiments with the isolated uterus of the virgin guinea-pig

The results of all the experiments described so far have shown that anti-guinea-pig serum produces effects very similar to those observed in anaphylactic shock. Analogous results have also been recorded for such non-specific reactions as the shock produced by starch or agar "anaphylatoxins". There is, however, one striking difference from true anaphylaxis shown by these agar or starch anaphylatoxin reactions which is valuable in differentiation (Dale & Kellaway, 1922), namely, that anaphylatoxins have no important stimulating action on the guinea-pig's uterus *in vitro*. It was important, therefore, to determine the effect of the guinea-pig antisera on the isolated guinea-pig uterus.

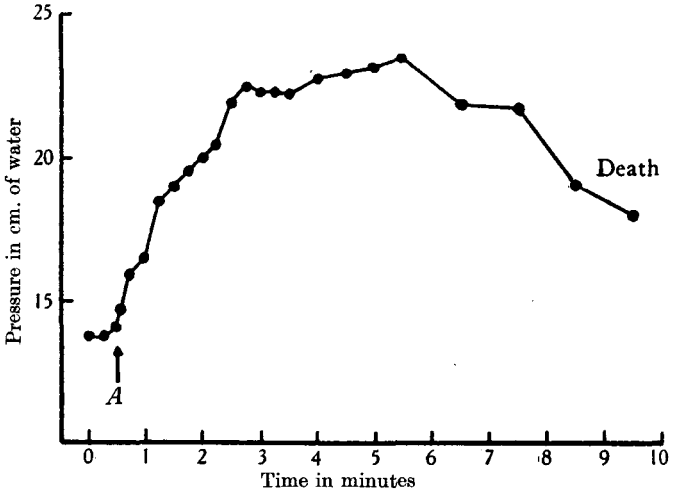
Methods and results

When possible the same uterus was used to test both the normal serum obtained by a control bleeding from a rabbit before the immunizing injections were begun, and the antiserum obtained subsequently from the same rabbit.

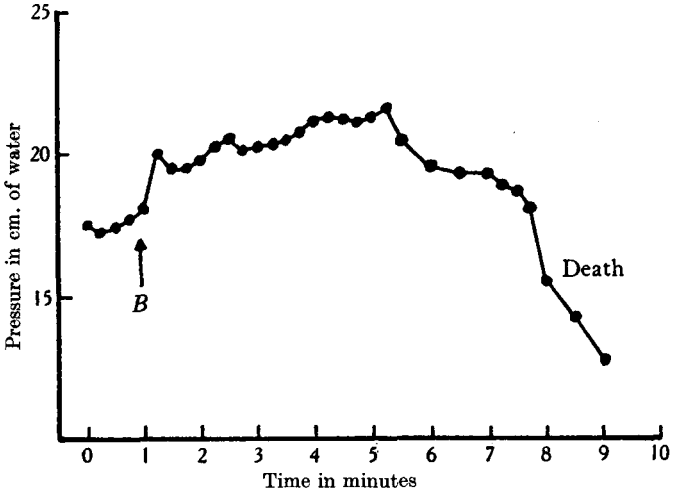
Redfern (1926) has shown that fresh normal rabbit serum, if used when less than 12 days old, causes non-specific contraction of the guinea-pig uterus, but in these experiments none of the sera was used unless heated to 56° C. for at least an hour and stored thereafter for 2 weeks. Characteristic results are shown in the accompanying tracings (see Text-figs. 10, 11).

Serum 0096 A, when given intravenously to two guinea-pigs, in doses of 2·0 and 3·0 c.c., caused no reaction. This serum in amounts of 0·5 c.c. gave rise to a very gradual and slight contraction only (Text-fig. 8).

Serum 0097 B proved fatal on intravenous injection in a dose as small as 0·6 c.c. Of this serum 0·5 c.c. in contact with the uterus gave rise to abrupt maximal contraction with subsequent desensitization. An earlier bleeding of the same rabbit, 0097 A, which produced no reaction on intravenous injection,



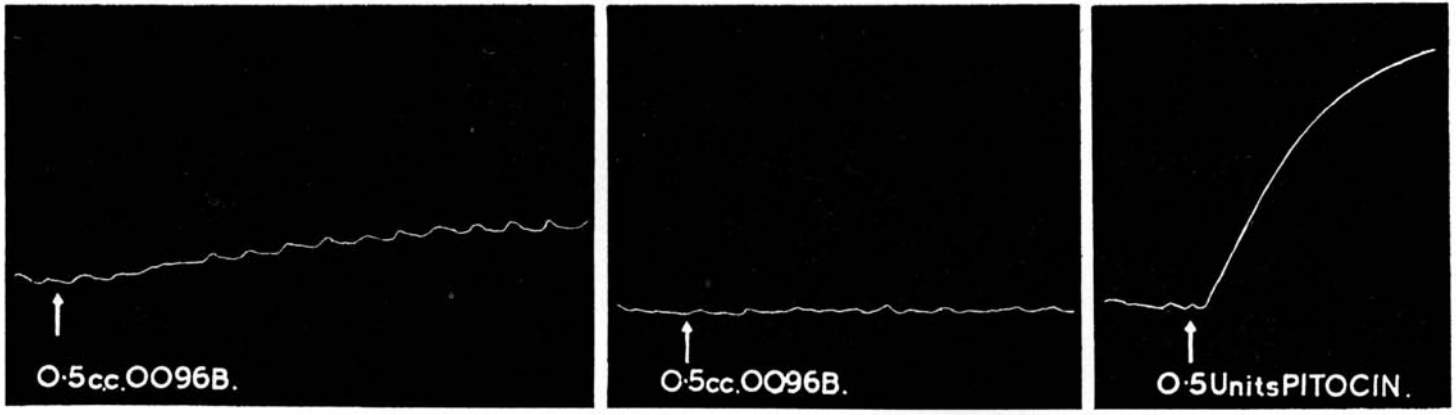
Text-fig. 6. Pulmonary artery pressure following injection at *A* of antigen into the jugular vein of an actively sensitized guinea-pig.



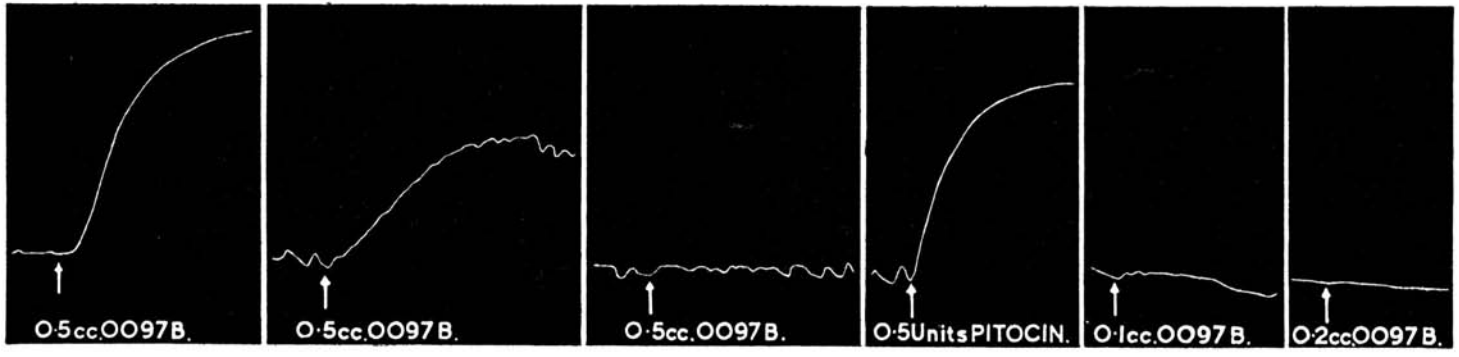
Text-fig. 7. Pulmonary artery pressure following injection at *B* of an antiserum to guinea-pig serum into the jugular vein of a normal guinea-pig.

caused no contraction of the second horn of the uterus, but partially desensitized it to 0097 B (Text-fig. 10).

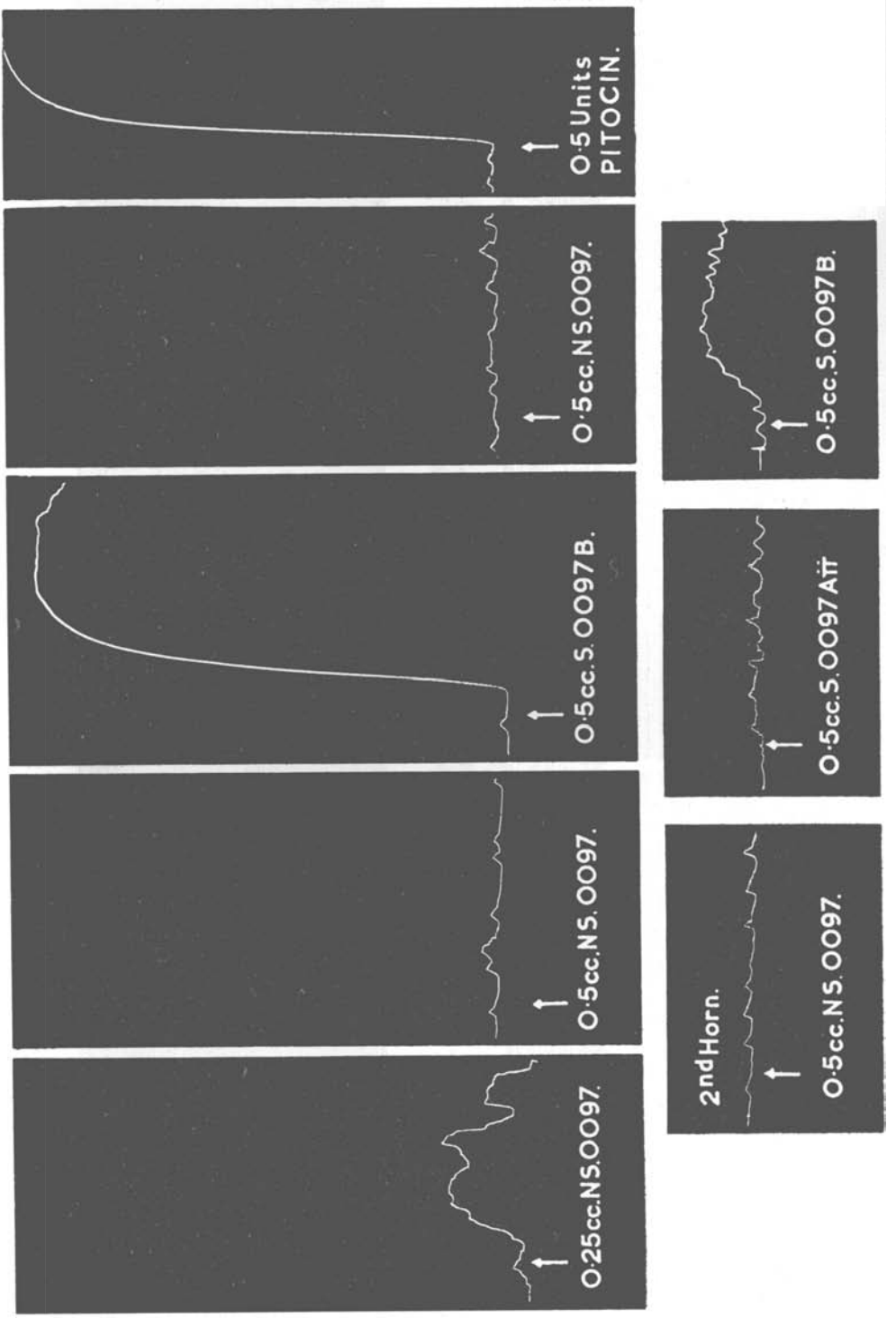
A guinea-pig was killed by a blow on the head, exsanguinated and then perfused through the aorta for 1½ hr. (that is, until white and commencing to show oedema). Its uterus still reacted to the same dose (0·5 c.c.) of 0097 B. Smaller doses were without effect (Text-fig. 9).



Text-fig. 8. The response of a guinea-pig's uterus to repeated contact with antiserum 0096B. Reactivity of the uterus at the termination of the experiment was demonstrated by its reaction to pitocin.



Text-fig. 9. The effect of repeated doses, and of small doses of serum 0097B, on the uterus of a perfused normal guinea-pig.



Text-fig. 10. The effect of an antiserum (0097B) compared with that of normal serum from the same rabbit (NS 0097).

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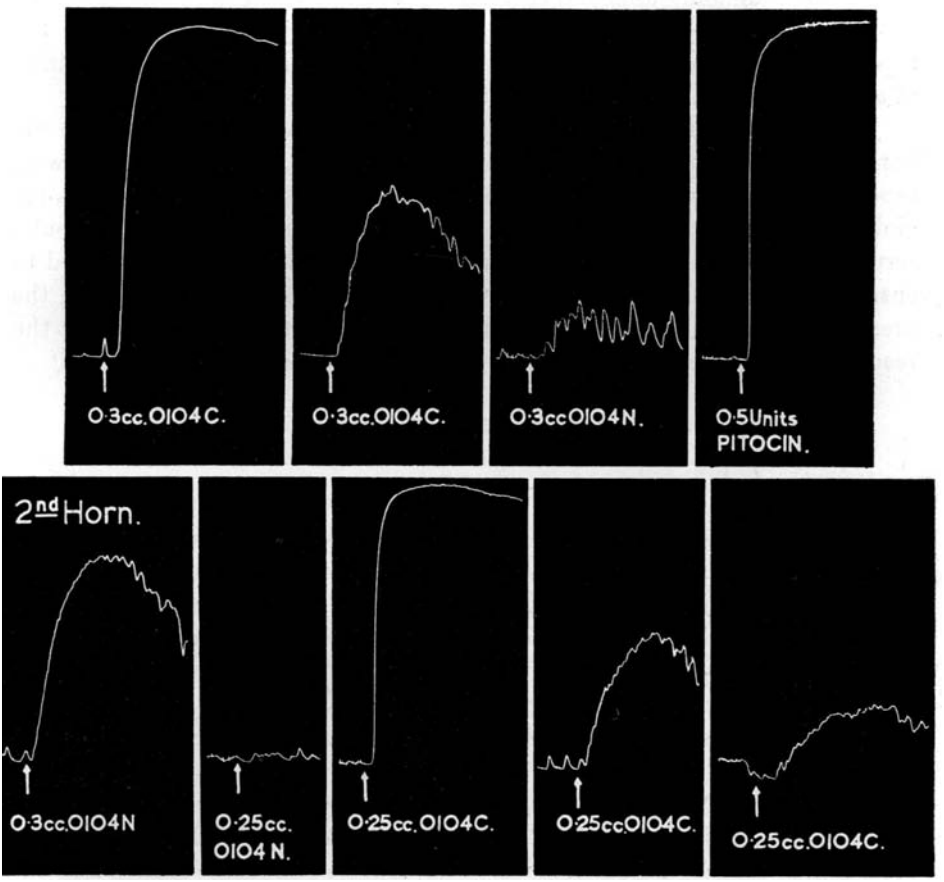
In subsequent experiments in which a bath of smaller volume was used, 15 c.c. instead of 75 c.c., 0·1 c.c. of 0097 B was found as effective as five times the dose in the larger one.

The same maximal contraction was obtained with 0·25–0·3 c.c. of 0104 C, an antiserum prepared against the globulin fraction of guinea-pig serum. Text-fig. 11 shows also the type of reaction sometimes encountered with normal



Text-fig. 11. Contact with a normal serum (0104 N) gave rise to a smaller and more transient contraction than the antiserum obtained from the same rabbit after immunization to guinea-pig globulin (0104 C). No desensitization to the effect of the antiserum resulted from the previous contact with normal serum.

serum. This is always submaximal, more gradual in development, and transient in duration.

Excess of antibody added to the bath in which a sensitized uterus is suspended will prevent the contraction which invariably follows the addition of a suitable dose of antigen (Dale & Kellaway, 1921). The "reversed" experiment was here tried. Repeated small doses of normal guinea-pig serum (0·05 c.c. ten times) were added to the bath and the uterus was allowed to relax com-

pletely after the small contraction caused by each addition. The addition of 0·3 c.c. of the antiglobulin serum still gave rise to maximal contraction. This procedure was repeated and the second contraction was found to be less abrupt—desensitization was only slight. Next, normal rabbit serum was used instead of the antiserum, and the non-specific effect was much smaller (Text-fig. 12). This uterus was not well suited to the experiment, as it was thicker than those commonly used and therefore difficult to desensitize, and moreover showed marked spontaneous activity. This activity was well shown by the repeated contractions which could be elicited with the second horn by addition of antiserum alone.

In view of the fact that the high concentration of protein present in the bath may conceivably have some non-specific effect, the experiments were repeated, using a minimum of protein. This was done by using guinea-pig globulin as antigen, and the globulin fraction extracted from the antiglobulin serum as antibody. If sufficient quantities of antibody globulin were used to ensure maximal contraction of the uterus identical results were obtained, the presence of the antigenic guinea-pig globulin in the bath failing to prevent the reaction of the uterus in response to the addition of antibody.

4. Correlation of the abilities of the antisera to produce symptoms in guinea-pigs with their antibody content

(*a*) *Intravenous injection of precipitating antisera*

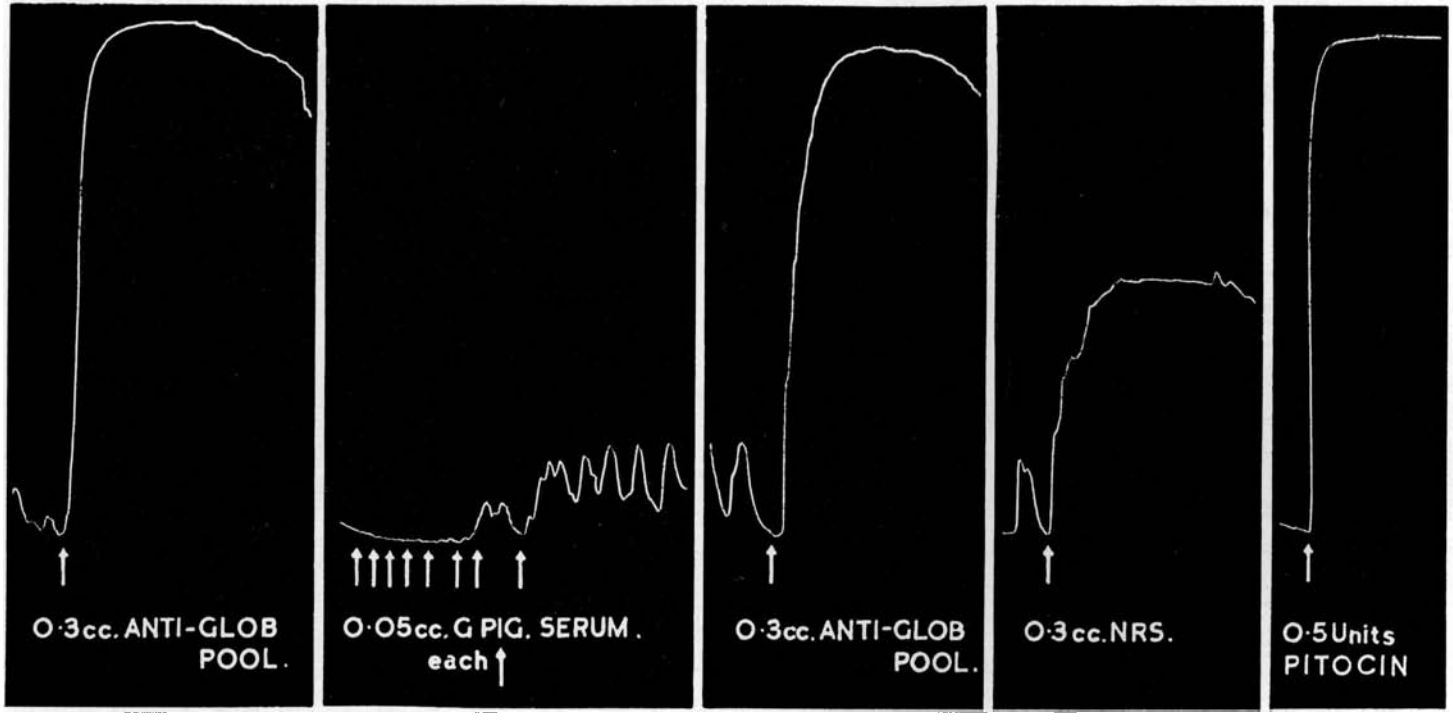
The results given in Table I indicate that no close correlation exists between the "potency" of sera, that is, power of producing reaction in guinea-pigs, and their precipitin content. The experiments were repeated, using antisera prepared against guinea-pig whole serum, as well as against serum albumin and globulin fractions.

Globulins were separated from guinea-pig serum by half-saturation with ammonium sulphate; the precipitate was dissolved in water and reprecipitated once. The globulin dissolved in distilled water was then dialysed against saline at 4° C. The filtrate after the first precipitation of globulin yielded the albumin fraction. An attempt was made to crystallize the albumin according to the method described by Adair & Robinson (1930) for horse serum albumin, but proved unsuccessful. The amorphous precipitate, produced by the further addition of acetic acid raising the *p*H to 4·7, was therefore dissolved and reprecipitated with ammonium sulphate, sodium acetate and acetic acid. Heavy losses of protein in the filtrates prohibited further attempts at purification.

Rabbits were given three courses, each course consisting of six injections, at daily intervals, of 5 mg. protein intravenously, and bled on the 8th or 9th day after the last injection.

The sera were heated to 56° C. for half an hour on three successive days, and stored in the frozen state (−10° C.).

Testing of the antisera. As yields from individual rabbits at each bleeding were small, minimal lethal doses could not be determined. The potency of each



Text-fig. 12. Before each addition of antiserum (pooled serum from a group of rabbits immunized to guinea-pig globulin) or normal rabbit serum (*NRS*) norma guinea-pig serum had been added to the bath in ten lots of 0·05 c.c. No inhibition of the response to the antiserum was seen. The addition of guinea-pig serum alone caused an increase in the spontaneous rhythm and tone of the uterus, as is shown by the second tracing in the figure.

sample was therefore roughly gauged by observing the symptoms produced in two guinea-pigs; the first being given 2·0 c.c., and the other 1·0 or 3·0 c.c., according to the fatal or non-fatal outcome of the first experiment.

Precipitin contents were determined by the optimal proportions method, using a fixed amount of antiserum diluted ten times, and the same batch of guinea-pig serum as antigen throughout. The amount of Forssmann antibody present was determined by finding the highest dilution of antiserum producing microscopic agglutination of a 1/50 suspension of washed sheep red cells in 3 hr. Agglutinins for guinea-pig red cells were measured in the same way. Different red cell suspensions were used for each of the three bleedings, so that agglutinin titres are only comparable between sera of the same bleeding.

Table III. *Showing the content in precipitins and agglutinins of second bleeding antisera, and the power of these antisera to cause reactions in normal guinea-pigs*

Serum no.	Precipitins for guinea-pig serum optimal ratio	Agglutinins for guinea-pig red cells	Agglutinins for sheep red cells	Result of intravenous injection into guinea-pigs		
				1·0 c.c.	2·0 c.c.	3·0 c.c.
Anti-albumin 0093 B	1 : 64–1 : 128 30 min.	1/2	1/2		No symptoms	No symptoms
Anti-albumin 0095 B	1 : 64–1 : 128 25 min.	1/2	1/2		Death 16 min.	Death 6 hr.
Anti-albumin 0096 B	1 : 64–1 : 128 50 min.	1/4	1/2		Very slight symptoms	Very slight symptoms
Anti-whole serum 0097 B	1 : 32–1 : 64 6 min.	1/64	1/4	Death* 4 min.	Death 5 min.	
Anti-whole serum 0098 B	1 : 64–1 : 128 5 hr.	1/128	1/4		Severe symptoms	No symptoms
Anti-whole serum 0099 B	1 : 32–1 : 64 5 min.	1/32	1/2		Very slight symptoms	Very slight symptoms
Anti-whole serum 0100 B	Nil in 20 hr.	1/64	1/4		Very slight symptoms	No symptoms
Anti-globulin 0101 B	1 : 64–1 : 128 19 hr.	1/16	1/1		No symptoms	No symptoms
Anti-globulin 0104 B	1 : 32–1 : 64 19 hr.	1/64	1/4		No symptoms	No symptoms
Anti-globulin 0105 B	±1 : 32 19 hr.	1/32	1/8		Death 4 min.	Severe symptoms

* Death occurred even after the injection of 0·6 c.c., but not with smaller doses.

The results of the tests of the second and third bleeding antisera are recorded in Tables III and IV. Of the first bleedings, only 0105 A produced fatal shock in a dose of 3 c.c., but other sera produced only very slight or no reaction in the guinea-pigs. Serum 0105 A, at 1/10 dilution, contained no precipitins demonstrable by optimal proportions. Of the second (B) bleedings, 0097 and 0099 had the same optimal ratio and flocculation occurred at the same rapid rate in both. Nevertheless, the former was acutely fatal to guinea-pigs in doses of 1·0 c.c. or less, whereas 3·0 c.c. of 0099 produced only very slight and transitory symptoms. Also 0104 B and 0105 B, in spite of similar precipitin content, differed widely in their potency for guinea-pigs. Of third bleeding antisera (C) 0104 and 0105 possessed the greatest precipitin content, and also had the greatest capacity to affect guinea-pigs. On the other hand,

0098C elicited reaction in spite of a precipitin content equal to or less than that of 0096C, 0097C and 0101C, which produced only moderate shock reactions.

In comparing the three different bleedings from each rabbit, it was found that, with the exception of 0095, the most "potent" was that which had the smallest optimal ratio.

The tests with 0095, owing to an insufficient amount of serum, were not completed.

It is evident that anti-guinea-pig sera from different rabbits differ in their power to produce reversed anaphylaxis in guinea-pigs, but, for different bleedings

Table IV. *Showing the content in precipitins and agglutinins of third bleeding antisera, and the power of these antisera to cause reactions in normal guinea-pigs*

Serum no.	Precipitins for guinea-pig serum optimal ratio	Agglutinins for guinea-pig red cells	Agglutinins for sheep red cells	Result of intravenous injection into guinea-pigs		
				1·0 cc.	2·0 c.c.	3·0 c.c.
Anti-albumin 0093C	1 : 51–1 : 102 45 min.	1/4	1/2		No symptoms	No symptoms
Anti-albumin 0095C	1 : 25–1 : 51 9 min.	1/2	1/16	No symptoms	Mild symptoms	*
Anti-albumin 0096C	1 : 51–1 : 102 35 min.	1/8	1/8		No symptoms	No symptoms
Anti-whole serum 0097C	1 : 51–1 : 102 30 min.	1/128	1/8		Mild symptoms	Severe symptoms
Anti-whole serum 0098C	1 : 51–1 : 102 90 min.	1/32	1/16	Very slight symptoms	Death 6 min.	
Anti-whole serum 0099C	1 : 102–1 : 204 23 min.	1/32	1/8		No symptoms	Slight atypical symptoms
Anti-whole serum 0100C	1 : 2 20 hr.	1/64	1/16		Very slight symptoms	Very slight symptoms
Anti-globulin 0101C	1 : 25–1 : 51 5 hr.	1/16	1/4		Slight symptoms	Slight symptoms
Anti-globulin 0104C	1 : 12–1 : 25 20 hr.	1/128	1/8	Mild symptoms	Death 6 min.	
Anti-globulin 0105C	1 : 25–1 : 51 4 hr.	1/64	1/16	Death 6 min.	Death 8 min.	

* Insufficient serum for the injection.

from the same rabbit, the greatest shock-eliciting power is associated with the sample which has the highest precipitin content.

The lack of correlation between potency and Forssmann antibody content is shown best in Table III. Sera 0098B, 0100B, 0104B and 0105B all have sheep cell agglutinating titres equal to or greater than 0097B; the latter, however, possesses far greater shock-producing properties. Similarly, 0098B, 0100B and 0104B agglutinate guinea-pig red cells to the same or greater titre than 0097B, indicating that the mechanism of the reaction is independent of the guinea-pig red cell agglutinins.

Also the results obtained with third bleeding antisera (C) showed that the highest agglutinating titre for guinea-pig or sheep red cells was not always associated with the greatest potency, as tested on the whole animal.

(*b*) *Adsorption experiments*

Aronson (1927) has shown that toxicity of normal goat serum for guinea-pigs can be removed by contact with a washed suspension of guinea-pig tissues. This can be explained by supposing that a natural antibody in goat serum combines specifically with an antigen present in guinea-pig tissues.

Investigation of the shock-producing power of anti-guinea-pig sera, after selective adsorption of the various antibodies, may therefore be of assistance in determining which of these antibodies is responsible for the smooth muscle stimulating action of the antisera.

One hundred c.c. of a pooled serum from eight rabbits immunized against guinea-pig globulin were divided into samples of 20 c.c. each. One was kept as a control and the remainder used for adsorption. Precipitins for guinea-pig serum were removed by the addition of guinea-pig globulin or whole serum to the undiluted antiserum at optimal ratio and centrifuging off the precipitate after standing 1 hr. at 37° and a further 18 hr. at 4° C. Contact with four-times washed sheep red cells for 2 hr. at 37° removed the Forssmann antibody.[1]

For adsorption on a guinea-pig tissue, 5 g. of thoroughly perfused and washed intestine were finely ground in a mortar. Twenty c.c. of the anti-guinea-pig globulin serum were added to the sediment after it had been washed three times in saline. After contact for 3 hr. at 37° C., the serum was freed of tissue suspension by centrifugalization.

All five samples were then tested for precipitins to guinea-pig serum, Forssmann antibody and guinea-pig red cell agglutinins, as well as for the power of

Table V. *Showing the effect of adsorption on the antibody content of an anti-guinea-pig serum, and on its power to cause reaction on intravenous injection into normal guinea-pigs*

Serum	Precipitins for guinea-pig serum optimal ratio	Agglutinin titre for guinea-pig red cells	Agglutinins for sheep red cells	Result of intravenous injection of guinea-pigs		
				1·25 c.c. per 100 g.	1·5 c.c. per 100 g.	1·75 c.c. per 100 g.
Unadsorbed	1:12·5–1:25 (30–40 min.)	1/32	1/4	Death 9 min.	Death 3 hr.	Death 4½ min.
Adsorbed on guinea-pig intestine	1:12·5–1:25 (35 min.)	1/32	Nil	No symptoms	Death 6 hr.	Mild symptoms
Adsorbed on sheep red cells	1:12·5–1:25 (36 min.)	1/32	Nil	Severe symptoms	Death 5 min.	Death 30 min.
Adsorbed on guinea-pig globulins	Nil	1/32	1/4	Slight symptoms	Death* 6 hr. +	No symptoms
Adsorbed on guinea-pig serum	Nil	1/32	1/2	Very slight symptoms	No symptoms	No symptoms

* Post-mortem findings not typical of delayed death.

[1] Two hours' contact of an antiserum prepared against guinea-pig kidney suspension, with washed sheep cells at 37° C., effectively removes all sheep cell agglutinins, as well as the power to produce shock in guinea-pigs. The fatal reaction following injection of anti-guinea-pig kidney serum is apparently dependent entirely on the presence of Forssmann antibody.

producing the manifestations of reversed anaphylaxis when injected in doses of 1·25, 1·5 and 1·75 c.c. per 100 g. of guinea-pig. The results are recorded in Table V.

It was found, therefore, that the removal of precipitins for guinea-pig serum globulins, either by guinea-pig globulin or whole serum, resulted in the loss of shock-eliciting power of the antiserum. Removal of Forssmann antibody, on the other hand, did not significantly reduce this power.

The intestine suspension significantly reduced the potency without greatly altering the precipitin content of the serum. It is, of course, possible that with repeated adsorptions on intestine the removal of the shock-eliciting property from the serum might be complete, but as it is impossible to free the intestinal suspension completely from serum proteins, there would at the same time be a substantial loss of precipitin from the antiserum.

Discussion

The symptoms of acute respiratory obstruction followed by convulsions and death with maximal pulmonary emphysema were for long regarded as characteristic of anaphylaxis. It has since, however, been shown that this picture can be produced in the guinea-pig by a large variety of substances, such as fresh normal serum (goat, ox, etc.; see Aronson, 1928; Hyde, 1927; Doerr & Weinfurter, 1913; Doerr & Moldovan, 1910, etc.) and numerous so-called "anaphylatoxins" or "serotoxins". These observations show that a study of the symptomatology and post-mortem findings alone are insufficient to establish the anaphylactic nature of a reaction. Detailed analysis of the method of causation as well as of the onset and course of every shock state is required in order to establish the relationship of the shock state to that of classical anaphylaxis. When this analysis is done, it is found that in some instances the resemblance is superficial only and in others the mechanism of the reaction resembles true anaphylactic shock more or less closely.

The symptoms and autopsy findings of the acutely fatal cases of "reversed anaphylaxis" observed in this work reveal striking similarities to those found in the condition generally known as true anaphylactic shock. Thus there is an abrupt onset of respiratory obstruction with its attendant symptoms, asphyxial death and pulmonary emphysema, while the heart continues to beat for some minutes. The only differences to be observed in the lung are its pinker colour and more numerous capillary haemorrhages than are usual in anaphylaxis.

Severity of haemorrhage and oedema of the lung are described by Redfern (1926), Taniguchi (1922), and Doerr & Pick (1913) in the differentiation of the Forssmann type of anaphylaxis from other forms. The validity of differentiation on these grounds has been questioned by Kumagai (1913), who found that oedema and haemorrhage of the lungs in acute anaphylaxis could be produced by increasing the total volume of the shock-eliciting dose (by dilution of the antigen). Presence of oedema and severity of haemorrhage therefore do not assist in the differential diagnosis of anaphylaxis.

One manifestation of non-specific primary serum toxicity is intravascular coagulation, produced by a substance present in fresh serum which is destroyed by heating at 56° C. for one hour or by storage. This substance was not responsible for the reaction observed in my experiments because the sera were heated to 56° C. and stored before use. The absence of clot at autopsy and the negative results obtained with the experiments on mice are confirmatory.

The "Spätgift" referred to by Doerr (1929), present in the albumin fraction of some normal sera, cannot be responsible for the reactions under study, as they were shown to be dependent entirely on a substance present in the globulin fraction of the antisera.

In reversed anaphylaxis the blood pressure disturbances, both systemic and pulmonary, observed during the shock state as well as the increase in bronchial resistance, are similar in type to those observed in direct anaphylaxis, and differ only in the more gradual course. These observations suggest a similarity to true anaphylaxis in the mechanism of production of symptoms, but cannot be regarded as proof of the true anaphylactic nature of "reversed anaphylaxis". Dale & Kellaway (1922) describe similar functional disturbances in shock, following injection of anaphylatoxins.

The positive results obtained with the isolated uterus in this work differentiate not only non-specific reactions such as those associated with injection of anaphylatoxin, but also Forssmann anaphylaxis. The failure of Forssmann antibody to cause contraction of the isolated uterus is not easy to explain. It may be due, as Redfern (1926) suggests, to the inaccessibility of the Forssmann antigen, situated within the cells of the uterus.

That anti-guinea-pig sera are able to stimulate contraction of the guinea-pig's uterus is, therefore, not only in support of the anaphylactic nature of the reaction produced by these sera, but is of value in distinguishing it from Forssmann anaphylaxis. It seems probable that here the antigen is more easily accessible to the antibody *in vitro* than is Forssmann antigen. That the shock phenomenon here described is not Forssmann anaphylaxis is further substantiated by the fact that removal of sheep cell agglutinins from the antisera leaves their power to produce shock in guinea-pigs unaltered.

Adsorption experiments show that the precipitins present in the antisera are responsible, at least in part, for the reactions observed in guinea-pigs. The fact, however, that shock is not brought about merely by the intravascular union of precipitins and their homologous antigens, viz. the serum proteins, is made clear by the results obtained with the uterus *in vitro*. A uterus freed as much as possible from serum protein by prolonged perfusion responds as well to the addition of antiserum as does a uterus suspended in Ringer containing an excess of guinea-pig serum proteins.

The explanation of this is not easy. Either the precipitins for serum proteins are not directly responsible, or the fixed antigen has a greater avidity for the antibody than that free in the circulation. In this connexion it is of interest to observe that Doerr & Pick (1913), in experiments on heterophile

anaphylaxis, were not always able to inhibit the response to Forssmann antibody completely by the previous intravenous injection of a suspension of tissues rich in Forssmann antigen. They explained this failure of inhibition on the basis of "filtration" in the lung capillaries of the particulate antigen, as well as the greater affinity of the heterophile antigen contained in the tissues of the test animal (guinea-pig).

The delayed reactions described here have an added interest in the similarity they display to the usual form of anaphylaxis in the dog. Similar changes follow the intraperitoneal or intravenous injection of a fraction of the smallest fatal dose of the antigen into sensitized animals (see Doerr, 1914; Weil, 1917; Williamson, 1936). Weil (1917) regards the delayed reaction as being due simply to the more gradual access of the antigen. Doerr (1914), on the other hand, favours the view that it indicates a dual mechanism in anaphylaxis in the guinea-pig. The post-mortem findings in the cases here reported are in favour of the latter view. It is striking that intestinal and hepatic vascular changes are greater in animals which die several hours after the injection, and in which early respiratory symptoms may have been mild or absent. At autopsy emphysema has been minimal. The vascular reaction (congestion and haemorrhages) is more extensive than can reasonably be attributed to vascular spasm in the guinea-pig. (It is also of interest that the hepatic veins of the guinea-pig are not as abundantly supplied with smooth muscle as those of the dog.) The decreased coagulability of the blood in anaphylaxis has recently been shown to be due to liberation of heparin. It may be that the liver lesions, which in the guinea-pig have been found to go on to necrosis, bear some relation to the liberation of the anticoagulant factor.

The results obtained, after the intravenous injection into normal guinea-pigs, of antisera to guinea-pig serum or serum fractions, appear therefore to be dependent on the combination of an antibody with a corresponding antigen present in the cells of the guinea-pig. Probably the similarity in all the aspects studied indicates that the same intermediate products such as, for example, histamine, are liberated from the cells so injured in the animal as in the usual form of anaphylaxis.

Reversed anaphylaxis differs from direct anaphylaxis, therefore, only in its independence of previous sensitization to a foreign protein. The mechanism by which the reaction is produced is the same in both, and the reversed reaction can be differentiated from true anaphylaxis on a basis of definition only if Wells's restrictions for anaphylaxis are accepted.

If, as apparently is the case, reversed anaphylaxis depends on the presence in the tissue cells of a natural antigen easily accessible to injected antibody, then it should be possible to demonstrate a true reversed passive anaphylaxis by using a foreign antigen, which can be taken up and held by the tissues of the test animal. It has been shown that the ability of antisera to give rise to passive sensitization depends on the species of animal producing the antiserum, and it seems reasonable to suppose that similarly produced antigens from different

species will be found to differ similarly in their acceptability to the cells of the guinea-pig's tissues. Experiments on reversed passive anaphylaxis must, therefore, be performed with a variety of antigens, and their homologous antisera. Several experiments on these lines have already been undertaken. Attempts to sensitize guinea-pigs passively with crystalline egg albumin and to elicit an anaphylactic reaction by intravenous injection of rabbit anti-egg-albumin serum have hitherto given entirely negative results. Further experiments with other antigens are being carried out.

Summary

1. The symptoms and autopsy findings in guinea-pigs following intravenous injection of antisera prepared against guinea-pig serum or serum fractions are described. Two types of reaction were observed, acute and delayed, similar to those described in direct anaphylaxis.

2. The alterations in systemic blood pressure, pulmonary arterial pressure, and bronchial resistance, were investigated and found to simulate closely those observable in ordinary anaphylactic shock.

3. The antisera have the power of stimulating contraction of the isolated uterus of the guinea-pig, either in the presence or absence of excess guinea-pig serum. The reaction, like that observed in direct anaphylaxis, is therefore cellular.

4. Antisera prepared against guinea-pig serum proteins contain, in addition to precipitins, agglutinins for the red cells of that species, and Forssmann antibody. Neither of the last two antibodies, however, is responsible for the shock phenomena here described. It appears that the potency of a serum to produce shock in guinea-pigs is dependent on several factors, of which the most important is the content in precipitins reacting with the guinea-pig serum proteins. These precipitins give rise to the reactions following intravenous injection into guinea-pigs, not merely as a result of humoral combination with homologous antigens, but largely, if not wholly, as the result of an immune reaction with antigens in the protoplasm of the tissue cells.

I wish to express my thanks to Dr R. Williamson, under whose supervision the first part of this work was undertaken, and to Dr A. N. Drury and Mrs M. Adair for their invaluable advice, help and criticism. Mr W. Collison has kindly helped me in the preparation of the figures.

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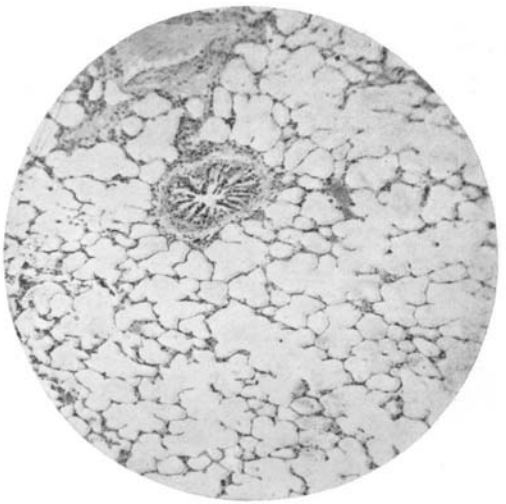

Fig. 1. Appearance of the lung after an acutely fatal injection of antiserum, showing marked emphysema and obstruction of a bronchus by folded mucous membrane. ×60.

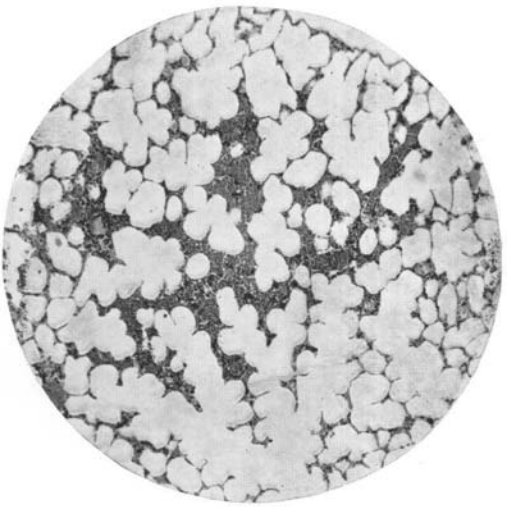

Fig. 2. Lung after acutely fatal reaction, showing emphysema and extreme capillary haemorrhages. ×60.

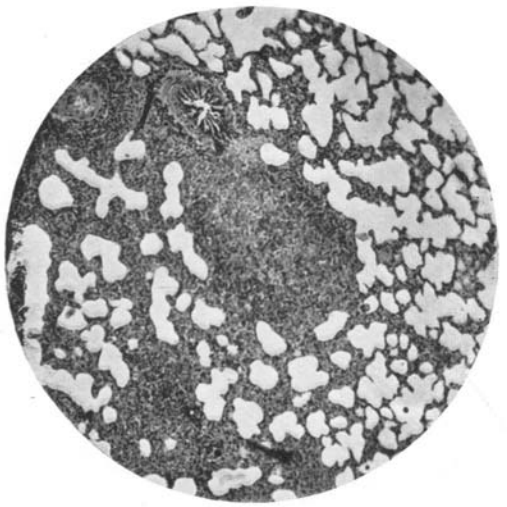

Fig. 3. Lung after delayed reaction, showing slight emphysema and areas of consolidation. ×60.

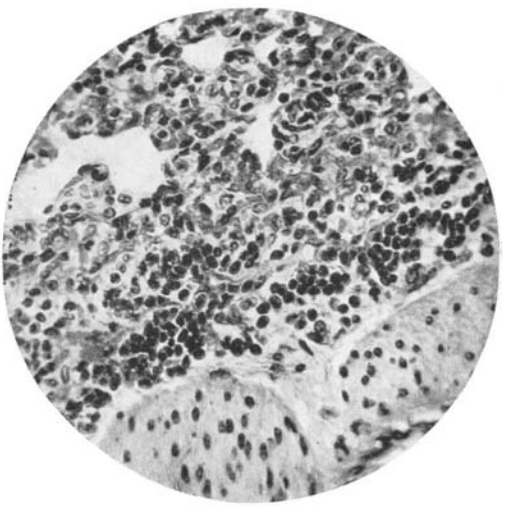

Fig. 4. Perivascular accumulation of polymorphonuclear leucocytes in the lung after delayed reaction. ×250.

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